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Gene-Targeting Therapeutics for Neurological Disease: Lessons Learned from Spinal Muscular Atrophy

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Abstract

The last few decades have seen an explosion in identification of genes that cause monogenetic neurological diseases, as well as advances in gene-targeting therapeutics. Neurological conditions that were once considered incurable are now increasingly tractable. At the forefront is the motor neuron disease spinal muscular atrophy (SMA), historically the leading inherited cause of infant mortality. In the last 5 years, three SMA treatments have been approved by the US Food and Drug Administration (FDA): intrathecally delivered splice-switching antisense oligonucleotide (nusinersen), systemically delivered AAV9-based gene replacement therapy (onasemnogene abeparvovec), and an orally bioavailable, small-molecule, splice-switching drug (risdiplam). Despite this remarkable progress, clinical outcomes in patients are variable. Therapeutic optimization will require improved understanding of drug pharmacokinetics and target engagement in neurons, potential toxicities, and long-term effects. We review current progress in SMA therapeutics, clinical trials, shortcomings of current treatments, and implications for the treatment of other neurogenetic diseases.

INTRODUCTION

Neurogenetic disorders are diseases affecting the brain, spinal cord, peripheral nerves, and/or muscles caused by a defect in one or more genes. During the 1980s and 1990s, gene mutations underlying the more common monogenetic neurological disorders, such as Duchenne muscular dystrophy (DMD), Huntington's disease, and Charcot Marie Tooth disease type IA, were identified (1), but recent advances in sequencing technologies have led to the accelerated discovery of highly penetrant, single-gene mutations underlying a multitude of rare disorders (2). In aggregate, these diseases are surprisingly common, though not frequently encountered in a primary care setting (2, 3). An epidemiological study in northern England found that ~1 in 1,100 individuals is affected by a monogenic neurological disorder (3). Despite the advances in disease gene discovery, novel treatment for these disorders has lagged, partly because of the difficulties of penetrating the blood–brain barrier as well as delivering to anatomically complex tissues. Remarkably, in the last 5 years, three novel and distinct gene-targeting treatments have shown clinical efficacy in infants and children with the inherited motor neuron disease spinal muscular atrophy (SMA). The success and continued challenges facing these treatments provide insights relevant to the development of similar therapeutics for multiple neurogenetic disorders.

SPINAL MUSCULAR ATROPHY: AN UNUSUAL OPPORTUNITY FOR GENE-TARGETING THERAPEUTICS

Affecting approximately 1 in 10,000 individuals, SMA is the most common inherited cause of infant and early childhood death (4). SMA is caused by recessive, loss-of-function mutations of *SMN1* (the survival motor neuron 1 gene) (5), but disease severity correlates inversely with the number of copies of the paralogous gene *SMN2* (6, 7). Loss of α motor neurons and muscle atrophy cause severe muscle weakness, particularly of the proximal limb, truncal, and bulbar muscles (8). Patients show a wide range of severity from infantile-onset (type I) disease (60% of cases), without achievement of early motor milestones such as sitting, to adult-onset (type IV) disease which is characterized by mild to moderate weakness of limb-girdle muscles (9). *SMN1* and *SMN2* are constitutively transcribed, but a single base pair difference in *SMN2* (C→T) in exon 7 alters its precursor messenger RNA (pre-mRNA) processing such that exon 7 is most often excluded in the mature mRNA (10–13). When translated, this shortened mRNA produces a truncated SMN protein that is rapidly degraded. A small percentage (~20%) of *SMN2* transcripts retain exon 7 and produce a low amount of functional protein (9). In the absence of *SMN1*, this is sufficient to rescue the embryonic lethality caused by complete loss of SMN, but it is insufficient to prevent motor neuron degeneration. This unique genetic cascade has led to two distinct strategies to increase SMN levels: (*a*) modification of *SMN2* pre-mRNA splicing to facilitate exon 7 inclusion and (*b*) *SMN1* gene replacement (**Figure 1**).

GENE-TARGETING THERAPEUTICS FOR NEUROGENETIC DISEASES

Gene-directed therapeutics include those targeting DNA by gene-replacement or gene-editing strategies and those targeting RNA using antisense oligonucleotides (ASOs), short interfering RNAs, or micro RNAs (miRNAs) (14). Here, we focus on those platforms that have shown recent clinical success in SMA, but the reader is directed to other reviews for more information about gene-editing strategies including clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 systems (15) and short interfering RNA and miRNA therapeutics (14).

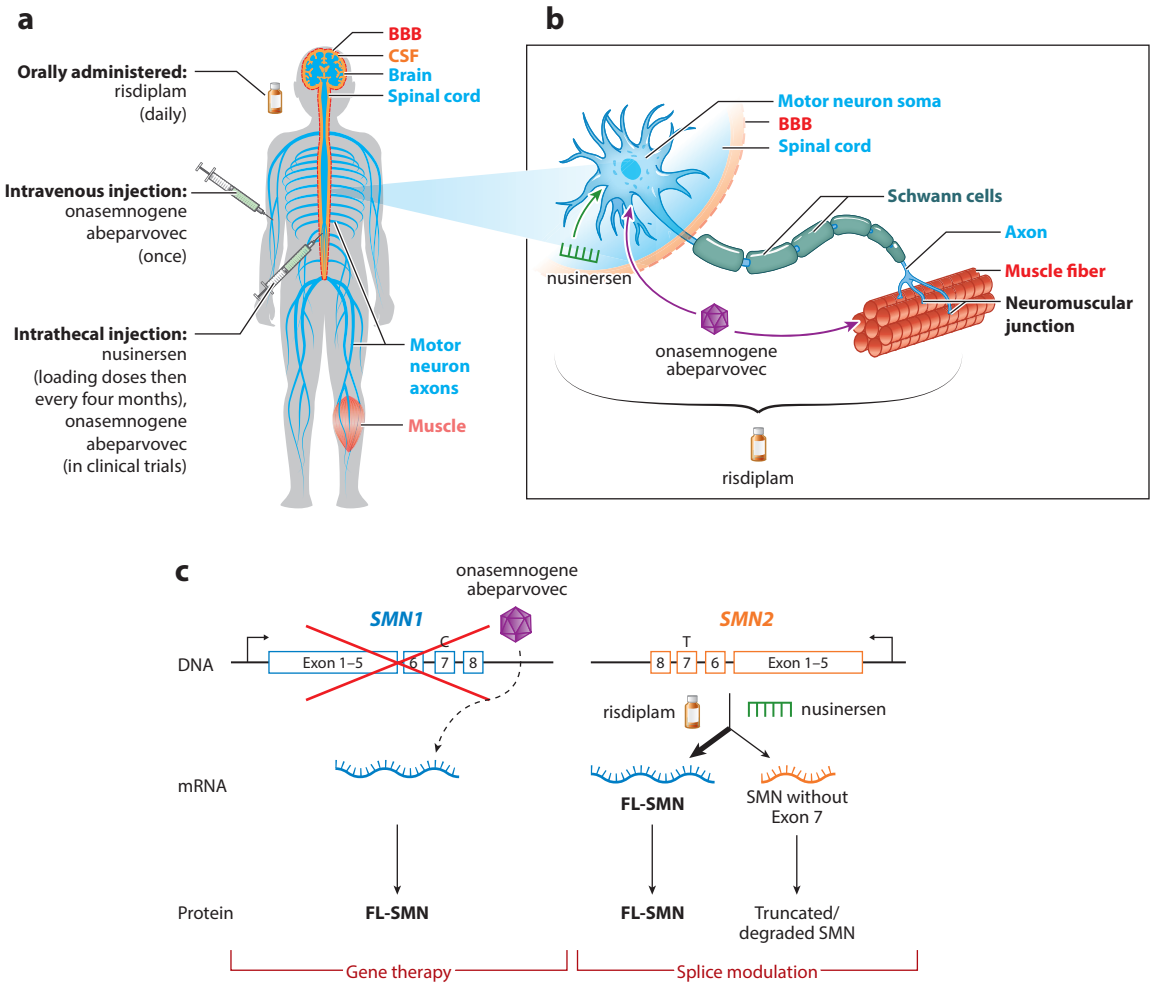

Figure 1

SMA gene-targeting therapeutics. (*a*) Routes of administration: intrathecal, intravenous, and oral. (*b*) Cell targets: α motor neurons (cell bodies in ventral horn of spinal cord) and, in some cases, myofibers and other cell types. (*c*) Therapeutic mechanisms of scAAV9-SMN cDNA (onasemnogene abeparvovec), the splice-switching ASO nusinersen, and the splice-switching small molecules risdiplam and branaplam. Panel *c* adapted from Reference 19, figure 1. Abbreviations: ASO, antisense oligonucleotide; BBB, blood–brain barrier; CSF, cerebrospinal fluid; FL-SMN, full-length survival motor neuron protein; SMA, spinal muscular atrophy; SMN, survival motor neuron (gene or protein).

Antisense Oligonucleotides

ASOs are synthetic single-stranded nucleic acids that bind to specific pre-mRNA or mRNA sequences by Watson-Crick base pairing and affect downstream protein synthesis either by selecting mRNAs for RNase-H-mediated degradation or by modulating pre-mRNA stability or splicing by functioning as a steric block (16). Chemical modifications to ASOs enhance target specificity, resistance to nucleases, plasma half-lives, and tissue uptake (17). Cells take up ASOs by endocytosis after receptor binding and clear them using endo- and exonuclease pathways (18). The mechanisms that mediate intracellular distribution of ASOs to determine "productive

uptake" are poorly understood. In addition, cell type–specific variability in ASO uptake can occur due to differences in receptor binding ability and the physiological state of the cell (18). ASOs are currently being developed for a large number of neurogenetic disorders (17, 19). Because they do not cross the blood–brain barrier, they must be delivered by intrathecal (IT) or intracerebroventricular routes when targeting the central nervous system (CNS) (20). In 2016, the *SMN2* splice-switching ASO nusinersen was approved by the US Food and Drug Administration for all patients with SMA. Nusinersen, a 2′-*O*-methyoxyethyl-modified ASO, is delivered to the cerebrospinal fluid (CSF) by lumbar IT injection four times during the first 2 months (loading phase) and every 4 months thereafter. Following uptake in motor neurons localized in the ventral horn of the spinal cord, nusinersen binds *SMN2* pre-mRNAs at the intronic splicing silencer N1 (ISS-N1) motif, thereby sterically hindering splicing factors, promoting exon 7 inclusion, and increasing expression of full-length SMN protein (21, 22).

Other ASOs now commercially available include inotersen for familial amyloid neuropathy, an autosomal dominant disease caused by *TTR* (transthyretin gene) mutations and characterized by progressive neuropathy and cardiomyopathy due to amyloid deposition. Systemically delivered inotersen targets hepatocytes and reduces transthyretin synthesis. It was FDA approved in 2018 coincident with approval of patisiran, a systemically delivered RNAi therapeutic also targeting *TTR* (23, 24). Eteplirsen is an exon-skipping ASO that was FDA approved for the treatment of patients with DMD in 2016. Administered by intramuscular injection, it promotes skipping of a single exon 51 of *Dystrophin* mRNA, thereby restoring the reading frame and converting the DMD phenotype to a milder Becker muscular dystrophy phenotype (25). While this is effective for ∼14% of DMD cases, ASOs that support multi-exon skipping in DMD can target up to 70% of cases (26). Two more ASOs, golodirsen and viltolarsen, that both target exon 53 are also FDA approved (approval for viltolarsen is conditional pending results of an ongoing phase III clinical trial). The ASO tominersen is currently in phase III clinical trials in patients with Huntington's disease. This drug is delivered by lumbar IT injection and targets the *HTT* mRNA to reduce mutant HTT expression (27). Tofersen, now in clinical trials, is delivered by lumbar IT injection in familial amyotrophic lateral sclerosis (ALS) patients with *SOD1* mutations. To date, the drug has been well tolerated and reduces SOD1 levels in the CSF (28).

Gene Therapy

Although several viral vectors, including retroviruses, lentiviruses, adenoviruses, and herpesviruses, have been considered for neurological disease indications, there has been a recent coalescence around adeno-associated viruses (AAVs) because they are nonpathogenic and can transduce neurons (29, 30). AAVs establish themselves as persistently expressing episomes with little incorporation into the host genome and can theoretically persist indefinitely in nondividing cells such as neurons. They have demonstrated safety and long-lasting expression in clinical studies for many neurological diseases (31). Different AAV serotypes vary in their efficacy in transducing specific neuronal populations, CNS distribution, and ability to transduce the CNS by the intravenous route (31). Despite AAVs' limited payload capacity (∼4.7 kB), replacement of the native AAV genome with desired cDNA driven by a promoter is tractable for many diseases. To enable rapid transgene expression by circumventing the requirement of second-strand synthesis, double-stranded, self-complementary recombinant AAVs (scAAVs) have been designed (32). Voretigene neparvovec, administered by subretinal injection for patients with retinal dystrophy caused by biallelic mutations of *RPE65* (33), was the first AAV-based gene therapy approved by the FDA, in 2017. The breakthrough that led to the use of AAV gene transfer in SMA was the discovery of successful transduction of spinal motor neurons by AAV9 delivered intravenously in preclinical models (34–36). Subsequent clinical trials using scAAV9-delivered *SMN* cDNA

driven by the chicken β-actin promoter resulted in the 2019 FDA approval of onasemnogene abeparvovec delivered by a single intravenous injection for SMA infants <2 years of age. As systemic delivery is not feasible in older individuals, clinical trials of onasemnogene abeparvovec delivered by lumbar IT injection are ongoing in older children with SMA.

Currently, in addition to ongoing trials in SMA, multiple clinical trials are ongoing or planned in loss-of-function neurogenetic disorders (31). For example, scAAV9/JeT-GAN delivered by IT injection to replace gigaxonin is in a phase I clinical trial for patients with the pediatric, autosomal recessive neurodegenerative disorder giant axonal neuropathy (NCT02362438). AAV1-mediated gene replacement of α-glucosidase (GAA) delivered by intramuscular injection is in a phase I/II clinical trial in Pompe disease (NCT02240407). In disorders characterized by a toxic gain of function, AAVs have been used as in vivo delivery tools for mRNA targeting therapeutics such as miRNAs and short hairpin RNAs. For example, in Huntington's disease, the gene therapy candidate AMT-130, which uses AAV5 to deliver a miRNA to inhibit toxic mHtt protein expression, was recently granted Fast Track designation by the FDA. Gene therapy approaches are currently in clinical trials for 15 rare neurological disorders with a monogenetic etiology (see ClinicalTrials.gov).

Small Molecules

Because of their low molecular weight, small molecules have obvious advantages as pharmaceuticals due to their potential wide tissue biodistribution and ease of administration (often oral). Most available small-molecule therapeutics target proteins, and developing small molecules that target RNA or DNA with sufficient specificity has been a major challenge (37). Identification of and focus on RNA structural motifs that are sufficiently sophisticated to allow for high affinity and specificity during binding are key to successfully target RNA using small molecules (37). Chemical screening and optimization efforts led to the identification of orally available SMN-C class small molecules (close analogs of risdiplam) and branaplam (LMI070) that promoted improved clinical outcomes in preclinical models of SMA (38, 39). Mechanistic studies of these molecules showed that they bind the RNA duplex formed between U1 RNA and the *SMN2* pre-mRNA and are possibly stabilized further by components of U1 small nuclear ribonucleoprotein particle proteins (40, 41). The FDA approved risdiplam in 2020, and clinical trials for branaplam are ongoing. Risdiplam is the first and only at-home, orally administered treatment for SMA. It is a liquid solution administered orally. Daily dosing regimen for infants, children, and adults is dependent on age and weight (2 months to 2 years: 0.2 mg/kg weight; ≥2 years to <20 years: 0.25 mg/kg weight; ≥20 years: 5 mg) (42).

Attempts to develop gene-targeting small-molecule therapeutics are ongoing in other neurogenetic and muscle diseases. Ataluren, a small-molecule drug in clinical trials for DMD, promotes ribosomal read-through of premature stop codon mutations in *Dystrophin* mRNA (43). In myotonic dystrophy, a designer small molecule, Cugamycin, was shown to selectively cleave disease-causing CUG repeat expansions in vivo (44). Other neurogenetic diseases for which promising small-molecule therapeutic candidates have been found include familial dysautonomia (45), Huntington's disease (46), and ALS (47).

KEY CLINICAL TRIALS IN SPINAL MUSCULAR ATROPHY AND IMPLICATIONS FOR OTHER NEUROGENETIC DISEASES

Central to successful development of nusinersen, onasemnogene abeparvovec, and risdiplam have been well-designed and -executed clinical trials. The principal approval-enabling trials for each of these treatments are summarized in **Table 1** [long-term efficacy and safety trials are not

Table 1 Recent and ongoing clinical trials involving SMN-inducing therapeutics

Treatment	Tissue distribution	Administration and dosage	Disease severity	Clinical trials	Design	Clinical outcomes
ASO (nusinersen)	CNS	Intrathecal 4 loading doses on days 0, 14, 28, 63; maintenance doses every 4–6 months	Type I	ENDEAR (symptomatic infants ≤6 months)	Blinded, placebo controlled	47% decrease in death or need for permanent assisted ventilation Motor milestone improvement in 51%: 22% head control, 8% sit, and 1% stand with assistance (59)
				NURTURE (presymptomatic infants ≤6 weeks at first dose with 2–3 copies of *SMN2*)	Open label	100% survival At 27.1 months, 100% sit without support, 88% walk with assistance, 68% walk independently (56, 93)
			Types II, III	CHERISH (children 2–12 years)	Blinded, placebo controlled	Motor function (HFMSE score) improved by 57% in treated versus 26% in sham cohort 20% achieved new motor milestone compared to 6% in sham cohort (58)
Gene therapy (onasemnogene abeparvovec)	Systemic (but lost from dividing cells)	Intravenous or intrathecal Single dose	Type I	CL-101 (infants ≤6 months)	Open label, intravenous	At ~24 months, all alive and event free 92% head control, 17% walk independently (94)
				STRIVE (infants ≤6 months)	Open label, intravenous	21 of 22 survived Improved motor function scores (95)
			Types I, II	SPR1NT (presymptomatic infants ≤6 weeks)	Open label, intravenous	All 18 alive and event free 8 patients with 2 copies of *SMN2* achieved age-appropriate motor milestones; 50% sit independently, 1 child stands with support (96)
			Types II, III	STRONG (children 6–60 months who could sit but not stand/walk)	Open label, intrathecal	Treatment well-tolerated Patients ≥24–<60 months: mean 5.9-point increase in HFMSE Patients ≥6–<24 months: 2 stand independently, 1 child walks without assistance Patients ≥24–<60 months: 25% gain motor milestones, 1 child walks with assistance (97)
Splice switching small molecule (risdiplam)	Systemic	Oral, daily	Type I	FIREFISH (infants 1–7 months)	Open label, two dose cohorts	At 12 months, 90% alive without permanent ventilation (all-patients cohort) In recommended dose cohort, 77% show motor milestone improvements (HINE-2), 41% sit without support for ≥5 sec (BSID-III scale), 88% feed orally (98, 99)
			All types	RAINBOWFISH (presymptomatic infants ≤6 weeks)	Open label, single arm	Ongoing
			Types II, III	SUNFISH (children and adults 2–25 years)	2 part, placebo controlled	Full-length/Δ7 *SMN2* mRNA ratio increased by as much as 400% in blood At 12 months, significant improvements in motor function compared to placebo (MFM-32 and RULM) (100)

Abbreviations: ASO, antisense oligonucleotide; BSID-III, Bayley Scales of Infant and Toddler Development Third Edition; CNS, central nervous system; HFMSE, Hammersmith Functional Motor Scale Expanded; HINE-2, Hammersmith Infant Neurological Examination Module 2; MFM-32, Motor Function Measure 32; RULM, Revised Upper Limb Module; SMN, survival motor neuron protein.

included but can be found in other reviews (9)]. Because of the variable clinical severity of SMA, trials were designed to test therapeutic efficacy in separate but parallel studies of infants and older children using age-appropriate outcome measures, including well-validated motor functional scales (48–53). In addition, because preclinical data in SMA mouse models had repeatedly demonstrated that earlier intervention was significantly more efficacious, each drug development program included a study to test drug efficacy in at least a small number of very young, presymptomatic patients. Because older patients progress very slowly, they were not included in these controlled trials, although observational studies of nusinersen efficacy in adults are now being reported (54).

Interestingly, despite the distinctions in type, administration route, and biodistribution of the three therapeutics, the trial results across the drugs are more similar than discrepant. In each case, presymptomatic or very early initiation of the drug resulted in marked reductions in mortality as well as near normal achievement of early motor milestones, including sitting and walking in some cases (55–58). In contrast, postsymptomatic initiation of treatment in infants or children resulted in more modest improvements in motor function (58–60). Long-term outcome data remain fairly limited, but at least for nusinersen-treated patients, slow improvement in motor function may continue for many years (61). While the success of these clinical trials remains a breakthrough, perhaps their most significant lesson is that SMA is far from being deemed cured. Further, the observed variability in clinical outcomes is poorly understood. In the following section, we delineate some of the variables that may be limiting efficacy and their relevance to other neurogenetic disease drug development programs.

CHALLENGES OF GENE-TARGETING THERAPIES

Drug Biodistribution and Toxicities

Optimization of the pharmacokinetics (PK) of gene-targeting therapeutics can be particularly challenging for neurological diseases because neural tissues are not readily sampled in living patients. Although lumbar IT delivery is being used for many ASOs and gene therapies, including nusinersen and onasemnogene abeparvovec, PK in the IT compartment is poorly understood. Drug biodistribution along the rostro-caudal axis of the spinal cord and in the brain likely depends on several parameters, including drug injection site, injection rate, dose amount and volume, protein binding properties, tissue affinity, and CSF clearance dynamics (62). CSF measures of HTT and SOD1 levels are being used as surrogate markers of CNS drug delivery in Huntington's disease and ALS, respectively (63, 64), but unfortunately SMN protein quantification in CSF has not proven feasible. Assessments of human tissues at the time of expedited autopsy is critical to fully understand drug PK in patients. In nine SMA patients analyzed to date, nusinersen concentrations and full-length *SMN2* mRNA induction were highest in lumbar and thoracic spinal cord regions and much lower in brainstem and brain regions (59, 65). This caudal-to-rostral gradient raises concerns about insufficient drug delivery to cervical and brainstem motor neurons in SMA patients. Currently, all patients regardless of age or weight receive 12 mg of nusinersen in a volume of 5 ml. The DEVOTE clinical trial is evaluating higher doses of nusinersen (two induction doses of 50 mg followed by maintenance doses of 28 mg) in SMA patients (NCT04089566). Furthermore, ongoing studies in ALS and Huntington's disease are using higher doses of ASO (100 or 120 mg, respectively) in order to target brain neurons, including those in deep brain nuclei (NCT02623699 and NCT02519036). These and other studies will establish the tolerability of higher-dose ASOs delivered intrathecally. Reported neuroinflammatory events following IT delivery of AAVs also require further study (66).

Another consideration to improve rostral delivery is positioning during injection. Rats inverted in the Trendelenburg position or continuously rotated while receiving AAV9 IT infusions showed improved neuronal transduction, greater animal-to-animal consistency of gene expression, and improved delivery to cortical regions (67). Other delivery routes, such as cervical (68) or intraparenchymal injections, despite being more invasive, may be suitable in some cases. Advanced multimodal imaging has enabled the tracking of ASO movement in rats to define ASO regional distribution, regional uptake, and CNS penetration (62). Such techniques are also being applied in human clinical trials to evaluate biodistribution of ASO tofersen targeting SOD1 when coadministered with radiolabeled 99mTc-MAG3-BIIB067 (NCT03764488).

Variations in therapeutic uptake by individual cell types involved in disease pathogenesis, such as neurons and glia, may also determine drug efficacy. Moreover, it is not known if degenerating neurons' ability to take up gene-targeting therapeutics is comparable to that of healthy neurons. In SMA, although motor neurons are particularly affected by the loss of SMN, other cell types including myofibers have also been shown to contribute to disease pathogenesis (69, 70), and clinical outcomes may be enhanced if therapeutics can be targeted to muscle in addition to neurons.

Systemically delivered gene-targeting therapeutics have the potential advantage of more widespread tissue targeting, including in the CNS. Analysis of tissues from two infants who died following intravenous treatment with onasemnogene abeparvovec demonstrated increased SMN protein expression in spinal motor neurons, in neuronal and glial cells of the brain, and in the heart, liver, skeletal muscles, and other tissues (71). This delivery route requires high titers of virus and thus is associated with very high initial exposure in particular tissues such as liver and dorsal root ganglion, which may cause immediate inflammatory reactions in some cases (72). In the long term, low rates of viral host DNA integration could trigger oncogenesis (73). Unlike IT delivery, systemic delivery of ASOs can be associated with thrombocytopenia and renal insufficiency (74). Based on preclinical data, risdiplam is freely distributed from the blood into the CNS and other tissues owing to its high passive permeability and resistance to the multi-drug-resistance gene (MDR1) (75). Although studies indicate robust drug levels and SMN induction in blood, further studies are needed to verify efficiency of motor neuron targeting in SMA patients. In animal studies, administration of risdiplam during pregnancy or throughout pregnancy and lactation resulted in adverse effects on development. Risdiplam is not recommended for patients with hepatic impairment and may also cause male infertility. Common adverse reactions occurring in $\geq$10% of infants receiving risdiplam were upper respiratory tract infections (60%), fever (40%), pneumonia (26%), rash (26%), diarrhea (15%), and vomiting (15%), which mirrored adverse effects observed in the later-onset SMA cohort (42). Potential retinal toxicity was closely monitored during clinical trials of risdiplam, as an earlier trial of its predecessor small molecule RG7800 was halted due to retinal toxicity observed in preclinical models.

Molecular Target Engagement

Understanding the required timing of target engagement and expression levels required for efficacy while avoiding toxicity (i.e., defining the therapeutic window) is critical to the clinical success of gene-targeting therapeutics. In the case of SMA, the markedly increased benefit obtained with treatment soon after birth may relate in part to endogenous changes of SMN expression during the course of development. SMN protein levels are particularly high during fetal development and fall perinatally in human spinal cord, suggesting a particular requirement for SMN during early developmental periods (65). Importantly, in the case of SMA, therapeutic benefit appears to arise with various induction levels of the SMN protein, with little evidence to date that supraphysiological levels of SMN expression are toxic. As both nusinersen and risdiplam act via splice

modulation, the level of SMN induction is limited by the levels of existing *SMN2* pre-mRNA; however, in the case of onasemnogene abeparvovec, virally delivered *SMN* cDNA is driven by the chicken β-actin promoter and thus is not constrained in this way (9). In some cases, potential toxic effects can result from gene expression in nontarget tissues or due to overexpression of the target gene (31, 76). Similarly, excessive therapeutic knockdown may be deleterious, as might occur in the case of excessive knockdown of SOD1 or HTT (77, 78). The durability of gene-targeting strategies also requires further study, as cellular compensations such as host DNA epigenetic alterations or episomal silencing are possible. Preclinical data for risdiplam showed off-target effects, including alternative splicing of genes such as *FOXM1* and *MADD* (42), and similar effects could potentially be a drawback of small-molecule treatments in general.

Evolution of Disease Pathogenesis and Novel Biomarkers

The timing of irreversible neurodegenerative events relative to treatment initiation has been one of the most difficult challenges in the field of neurodegenerative disorders. In the case of SMA, preclinical studies suggested that disease pathology may start at fetal stages, and this prompted clinical trials to deliver therapeutics as soon after birth as possible. Recent characterizations of type I SMA human tissues integrated with detailed SMA mouse model studies reaffirm that abnormal development of motor neuron axons begins in utero and is followed by fulminant degeneration of immature motor units neonatally (79). These data provide a pathological understanding of the temporal window relevant to more efficacious treatment in patients and emphasizes the importance of very early treatment. To achieve this goal, newborn genetic screening is being implemented in various countries. In 2018, the Advisory Committee on Heritable Disorders in Newborns and Children recommended that SMA be added to the Recommended Uniform Screening Panel (RUSP). Minnesota was the first US state to begin screening (80), and as of April 2020, 22 states are screening for SMA in newborns.

As gene-targeting therapeutics continue to advance, the need for novel pharmacodynamic biomarkers to monitor disease progression and therapeutic efficacy has become increasingly urgent. Blood and/or CSF neurofilament protein levels may be useful biochemical indicators of disease severity and progression (81, 82). NF-H, NF-M, and NF-L (neurofilament-heavy, -medium, and -light) are cytoskeletal proteins expressed specifically in neurons and are released during degeneration (83). NF-L has emerged as a prognostic blood biomarker in predicting disease severity and progression in various neurological disorders, including Parkinson's disease, ALS, and multiple sclerosis (84–87). In the ENDEAR nusinersen trial, phosphorylated NF-H levels were ∼10 times higher in children with SMA than in age-matched controls (81) and decreased dramatically in the nusinersen-treated group (81). SMN protein levels in blood could potentially serve as a biomarker for SMN induction in neuronal tissues following risdiplam administration (88), but it is unclear if SMN levels in blood correlate with clinical outcomes. In addition to circulating biomarkers, novel imaging and electrophysiological biomarkers are being developed for various neurological diseases (89, 90).

CONCLUSIONS

Three new gene targeting treatments, each with distinct routes of administration, are now FDA approved for the treatment of SMA patients. Despite this remarkable progress, variability in clinical outcomes highlights the need for treatment optimization. Continued basic and translational research efforts are needed to define factors that limit cell and tissue drug biodistribution and target engagement, and to characterize long-term durability and potential toxicities. Lessons learned

from this work in SMA will be invaluable in advancing efficacious gene-targeting therapeutics for other neurogenetic disorders. The high price tags of SMA therapeutics have received extensive press coverage. Onasemnogene abeparvovec costs $2.1 million for a single dose; nusinersen costs $125,000 per dose, and over the course of multiple doses, its treatment cost surpasses $1 million; risdiplam is set to be price-capped at up to $340,000 per year (91, 92). The costs of such gene-targeting therapeutics are not sustainable, particularly as the US FDA predicts it will approve 10 to 20 cell or gene therapy products per year by the year 2025. Efforts must be undertaken to address these skyrocketing costs such that all patients can benefit from the promise of such precision healthcare.

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