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Emerging antisense oligonucleotide and viral therapies for amyotrophic lateral sclerosis

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Purpose of review
Amyotrophic lateral sclerosis (ALS) is a rapidly fatal disease for which there is currently no effective therapy. The present review describes the current progress of existing molecular therapies in the clinical trial pipeline and highlights promising future antisense oligonucleotide (ASO) and viral therapeutic strategies for treating ALS.

Recent findings
The immense progress in the design of clinical trials and generation of ASO therapies directed towards superoxide dismutase-1 (SOD1) and chromosome 9 open reading frame 72 (C9orf72) repeat expansion related disease have been propelled by fundamental work to identify the genetic underpinnings of familial ALS and develop relevant disease models. Preclinical studies have also identified promising targets for sporadic ALS (sALS). Moreover, encouraging results in adeno-associated virus (AAV)-based therapies for spinal muscular atrophy (SMA) provide a roadmap for continued improvement in delivery and design of molecular therapies for ALS.

Summary
Advances in preclinical and clinical studies of ASO and viral directed approaches to neuromuscular disease, particularly ALS, indicate that these approaches have high specificity and are relatively well tolerated.

Keywords
amyotrophic lateral sclerosis, antisense oligonucleotide, C9orf72, superoxide dismutase 1, viral therapy

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron loss and paralysis. Although the majority of cases are singleton ('sporadic'), genetic advancements in the 10% of patients with familial ALS have provided valuable insights into disease pathomechanisms and pinpointed therapeutic targets in patients with specific gene mutations. Hexanucleotide repeat expansions in chromosome 9 open reading frame 72 (C9orf72) and mutations in superoxide dismutase-1 (SOD1) are the most common forms of hereditary ALS, constituting nearly 35–50% [1] and nearly 20% [2] of cases, respectively. For SOD1-related and C9orf72-related ALS, the establishment of gain-of-function mechanisms as causes for disease and creation of reliable disease models have catalyzed generation of targeted antisense oligonucleotide (ASO) [3] therapies that are now or are soon to be in clinical trials. Despite our increasing understanding of ALS pathogenesis, many barriers remain for generating effective therapeutic strategies for sporadic disease including the heterogeneity of disease mechanisms, incomplete understanding of cell autonomous and non-cell autonomous contributions to disease, and limitations in gene delivery methods.

Gene therapy approaches, including ASO and viral-directed gene delivery, have shown enormous potential to enable therapeutic modulation of gene expression in diverse monogenic neurological conditions from Huntington's disease, myotonic dystrophy, spinal muscular atrophy (SMA), and ALS. ASOs are synthetic single-stranded DNA sequences, 8–50 base pairs in length, that complement selected sequences of target RNA, leading to mRNA

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KEY POINTS

- ASO therapies for SOD1-related familial ALS have shown promise in preclinical studies and progressed to early clinical trials.
- Advances in viral-based gene therapy for SMA suggest that this approach may enable durable and versatile gene manipulation in other motor neuron diseases such as ALS.
- Preclinical gene therapy studies targeting multiple processes commonly disrupted in sporadic ALS show benefit.

degradation through RNase H enzyme recruitment. ASOs can also be modified to promote alternative splicing rather than causing mRNA degradation. Moreover, ASOs distribute widely throughout the central nervous system (CNS) when delivered intrathecally, a crucial feature for treatment of neurological disorders [4▪].

However, viral-mediated gene delivery may offer additional flexibility for drug administration and provide longer-lasting genetic alterations. Viral vectors, such as adeno-associated virus (AAV), can provide greater ease in drug administration because they more readily cross the blood–brain barrier when given systemically and can be manipulated to favor tropism to specific cell types and structures [5,6]. Furthermore, AAVs express stable extrachromosomal nuclear episomes, ensuring long-lasting gene expression [3].

In recent years, the application of these strategies in a form of motor neuron disease, SMA, has led to FDA approval of nusinersen and recent initiation of phase III trials of an AAV9-based AVXS-101 therapy (AveXis; NCT03461289, NCT03306277, NCT03505099). These successes in tandem with early advances in the development of ASOs in ALS serve as valuable guides for future therapeutic endeavors (Fig. 1).

ASO THERAPEUTICS IN SUPEROXIDE DISMUTASE-1–RELATED AMYOTROPHIC LATERAL SCLEROSIS

The discovery of SOD1 mutations as a cause of familial ALS in 1993 precipitated a molecular revolution in our understanding of the disease. Similar to human SOD1 postmortem tissue, multiple SOD1 rodent models exhibit SOD1 aggregates, TDP-43 inclusions, and motor neuron degeneration, suggesting toxicity through a gain-of-function mechanism, whereas SOD1 knockout mice fail to develop motor neuron loss [7]. These cumulative findings indicated that SOD1 lowering could be a viable therapeutic strategy. Pioneering studies of SOD1 ASO in rats and nonhuman primates demonstrated widespread distribution of ASOs in CNS, effective knockdown of SOD1, and extension of survival [8].

A first-in-man phase I double-blind, placebo-controlled clinical trial of intrathecally-delivered ASO 333611 (ISIS-SOD1$_{RX}$) in SOD1-related ALS was completed in January 2012 (NCT01041222). The study employed conservatively low single doses of ASO that did not modify SOD1 expression but established that the treatment approach was well tolerated. Moreover, ISIS-SOD1RX concentrations measured in plasma, cerebrospinal fluid (CSF), and even spinal cord autopsy tissue of one participant, provided valuable pharmacokinetic data [9].

A more potent second generation SOD1 targeting ASO, BIIB067 (IONIS-SOD1$_{RX}$) [10▪▪], is currently being tested in an ongoing phase I A/B randomized, placebo-controlled escalating dose trial that began in late January 2016, and is planned to complete in February 2019 (NCT02623699). The study includes participants with SOD1-related ALS and functional vital capacity (FVC) more than 50% with an enrollment target of 84. In part A, a single infusion of BIIB067 or placebo will be administered, whereas in part B, SOD1-ALS participants will receive multiple administrations of BIIB067. A phase I long-term extension study of the effects of BIIB067 in participants who have completed part A/B is also ongoing and estimated to complete in January 2020 (NCT03070119). The primary goal of these studies is to establish the safety profile and pharmacokinetics of BIIB067. Unlike the initial phase I study, doses of BIIB067 are anticipated to be sufficiently high to lower SOD1 levels. Thus, an important secondary measure involves detection of SOD1 protein levels in CSF as a marker for target engagement [11].

Human SOD1 has a long half-life in CSF [12], a characteristic that limits how early traditional methods can measure ASO-mediated changes in patients. A promising approach for measuring early changes in SOD1 expression involves monitoring protein kinetics via stable isotope labeling combined with mass spectrometry, a method that provides high specificity and sensitivity [12]. Using this technique, measurements of newly synthesized SOD1 protein may provide earlier assessments of target engagement, a parameter that will serve as a valuable efficacy measure and help determine optimal frequency of dosing for future clinical trials.

As SOD1-targeting ASO clinical trials advance, a critical consideration will be the challenge of recruiting participants with a rare genetic disorder. A recent natural history study of patients with

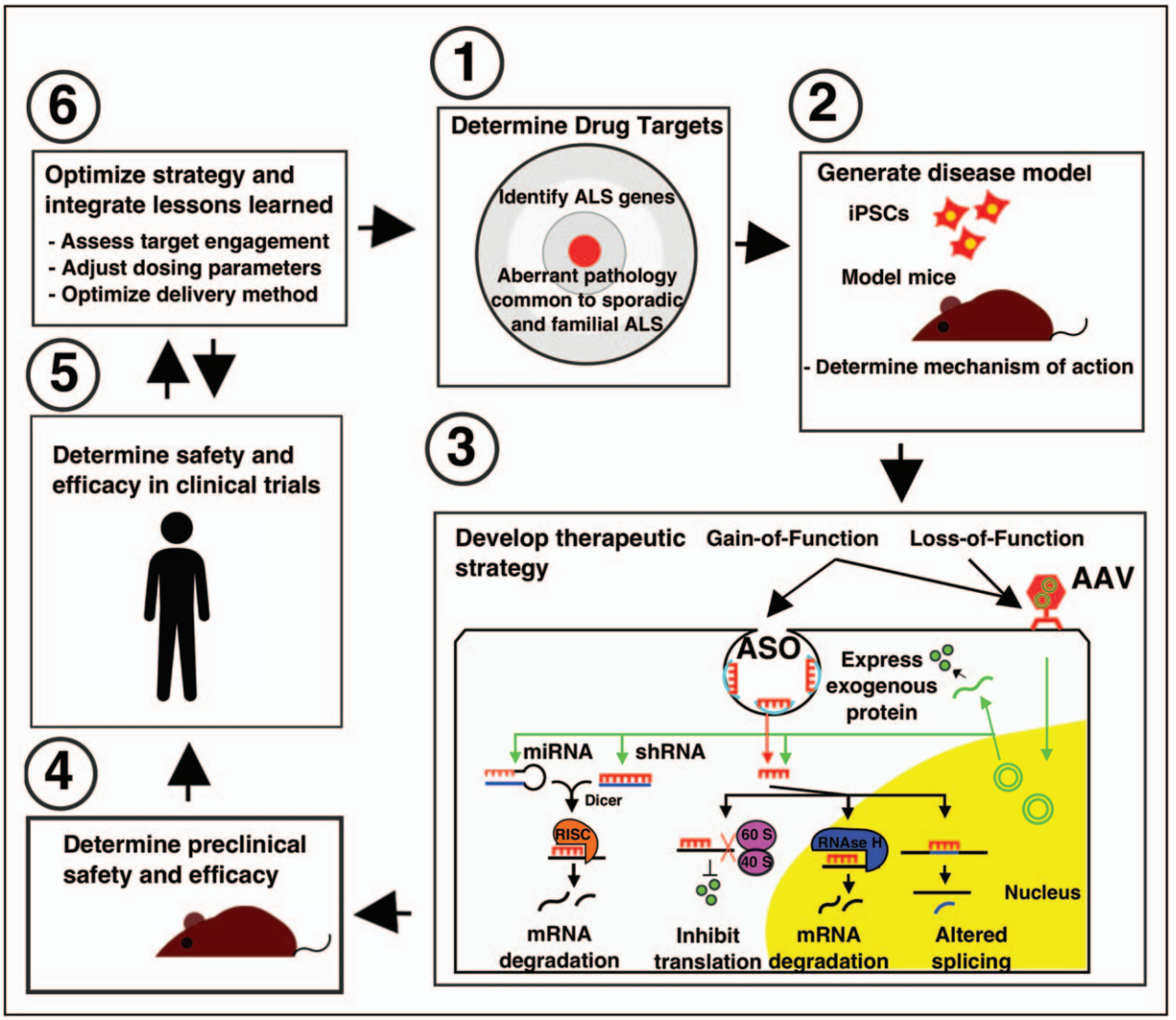

FIGURE 1. Cycle of gene therapy development. The development of gene therapies for ALS begins with 1) pinpointing promising disease targets which have historically derived from identification of genes in familial ALS and the discovery of aberrant cellular processes in affected patients or animal models. 2) Generation of cellular or animal disease models helps to clarify how gene mutations or pathologic features impact disease. 3) Mechanistic knowledge of disease pathogenesis derived from disease models informs development of a therapeutic strategy. Disease mechanisms governed by gain-of-function (i.e. SOD1-ALS, C9-ALS) may benefit from approaches that enable gene knockdown (i.e. ASOs, AAV-mediated delivery of ASOs, shRNA, or miRNA) while disease caused by loss-of-function could benefit from restoration of the defective gene using AAV. If gene therapies show 4) preclinical efficacy, they may progress to 5) human clinical trials. 6) The cumulative lessons derived from iterations of this cycle will hopefully streamline the process of drug development in ALS.

SOD1-related ALS has provided guidance for design of future clinical trials and estimated enrollment goals needed to adequately assess therapeutic efficacy [13▪]. Notably, median survival of SOD1 A4V mutation carriers was only 1.2 years compared to 6.8 years for non-A4V carriers, and 2.7 years for all patients with SOD1. Because A4V constitutes the most common SOD1 mutation in North America, and A4V patients are more clinically homogeneous, future therapeutic efficacy studies may consider stratifying results from these patients.

GENE TARGETING STRATEGIES IN AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED WITH C9ORF72 REPEAT EXPANSIONS

Hexanucleotide repeat expansions in C9orf72 were discovered to be the most common genetic cause of ALS and frontotemporal dementia (FTD) early this decade, yet tremendous progress has been made to understand how this mutation confers neuronal toxicity. Several mechanisms are thought to play

a role. First, the DNA encoding the expansion is transcribed bidirectionally and forms nuclear RNA inclusions that cause gain-of-function toxicity. Second, through noncanonical repeat-associated non-ATG-mediated (RAN) translation, up to five distinct aggregate-prone dipeptides can be generated from sense and antisense strands. Third, C9orf72 haploinsufficiency has been implicated particularly given its newly appreciated roles in lysosomal maintenance and inflammatory regulation [14,15].

Initial development of ASO-based therapeutics for C9orf72 was ushered by availability of induced pluripotent stem cell (iPSC) and fibroblast models from patients with C9orf72-related ALS. These early studies established design considerations for ASO development and focused on reducing gain-of-function toxicity associated with the repeat expansion. ASOs designed to bind within or immediately upstream of the intronic expansion did not significantly alter C9orf72 mRNA levels while those binding downstream of the expansion led to significant reduction of C9orf72. Regardless of the targeted region, ASOs reduced RNA foci, increased survival from glutamate excitotoxicity, and abrogated aberrant gene expression patterns [16–18].

Several transgenic mouse models expressing human C9orf72 repeat expansions recapitulate pathological disease features [14,19,20] and cause neurological deficits [19,21]. In the first *in vivo* test of C9orf72 ASO, investigators demonstrated that single dose, intraventricular administration of ASO could attenuate RNA foci and dipeptide aggregates and improve the behavioral and cognitive deficits associated with the C9orf72 repeat expansion [19]. Furthermore, poly (GP) dipeptide can be detected in the CSF of C9orf72 expansion carriers and its levels are reduced with ASO treatment in experimental models indicating that it could serve as a marker of target engagement [22▪▪,23]. These encouraging results provided a springboard for the initiation of a phase I clinical trial of C9-ASO anticipated to start soon. These disease models will continue to aid in further refinement of ASO design, safety testing, biomarker development, and practical planning for future therapeutic trials.

Existing ASO strategies preferentially target sense strand transcripts. However, antisense RNA foci have also been identified in C9orf72 patient neuronal tissue and may also contribute to disease [17]. Thus, future ASO strategies may need to target toxic RNA transcribed from both directions in order to adequately treat the condition. One approach is to employ sense-directed and antisense-directed ASOs in tandem. Alternatively, recent studies suggest that knockdown of Spt4, a transcription elongation factor selectively involved in expression of expanded repeats, inhibits development of sense and antisense RNA foci and dipeptide inclusions in C9orf72 patient fibroblasts [24]. However, knockout of the mouse homolog, Supt4 h, causes embryonic lethality [25]. Thus, clarifying the endogenous role of Spt4 will be necessary to determine the clinical viability of this strategy.

Recent studies indicate that both gain-of-function and loss-of-function mechanisms may act in concert to cause pathogenesis in C9orf72-related disease. Arguing against loss-of-function, C9orf72-deficient mice do not display neurodegeneration [14,15] and lowering of C9orf72 using ASOs in mice is well tolerated [17]. However, C9orf72 knockout mice have a pronounced immune phenotype characterized by splenomegaly and leukocyte, particularly macrophage, abnormalities [15]. Given the prominent neuroinflammation in ALS, these findings suggest an underappreciated link between immune regulation and ALS. A recent study in iMNs suggested more directly that C9orf72 deficiency is related to neurodegeneration. Under conditions of excitotoxic or proteostatic stress, C9orf72-deficient iMNs undergo degeneration stemming from pleiotrophic consequences of perturbed vesicle trafficking including glutamate receptor accumulation and impaired lysosomal function [26▪▪]. Thus, an integrated therapeutic approach to knockdown expansion-related mRNA and protein products and restore native C9orf72 expression may be necessary to fully address the cellular deficits in C9orf72-related disease.

ASOs TO TARGET TDP-43 PATHOLOGY

Although ASOs demonstrate therapeutic promise for monogenic gain-of-function conditions such as SOD1-related and C9orf72-related ALS, a challenge in developing treatments for the broader ALS population is the heterogeneity of cellular pathways disrupted. Notably, aggregates of the ribosomal protein, transactive response DNA binding protein 43 kDa (TDP-43), are a pathological hallmark of nearly 97% of ALS cases [27,28] and mutations in TDP-43 that promote protein aggregation are also sufficient to cause disease [29]. In addition, the abundance of TDP-43 inclusions in mouse models that overexpress wild-type or mutant protein correspond to symptomatic burden [30,31].

Recent studies suggest that knockdown of ataxin-2, a protein that regulates stress granule formation and promotes aggregation of TDP-43, may hold therapeutic promise. ASOs targeting ataxin-2 in mice expressing human TDP-43 were shown to have reduced formation of TDP-43 aggregates, slowed disease progression, and markedly

prolonged survival [32▪▪]. Ataxin-2 knockout adult mice exhibit weight gain but are viable and fertile [33] whereas removal of TARDBP, the gene encoding TDP-43, causes embryonic lethality [34,35], suggesting that direct manipulation of TDP-43 may not be feasible. A recent study further showed that inhibiting stress granule formation using ataxin-2 ASOs or small molecules suppressed nucleocytoplasmic transport defects and neurodegeneration in C9-ALS iMNs and C9-ALS fly models [36▪▪]. These preclinical data indicate that destabilizing stress granules by targeting ataxin-2 or other factors required for their assembly has potential to be beneficial in a broader ALS population.

EMERGING VIRAL-BASED THERAPEUTICS IN AMYOTROPHIC LATERAL SCLEROSIS

Recent data using viral delivery in SMA are impressive and may provide lessons for implementation of viral-mediated strategies in ALS. A phase I trial of a single-dose adenoviral intravenous gene replacement therapy of human survival motor neuron (hSMN; AVXS-101) in SMA1 (AveXis; NCT02122952) demonstrated striking gains in motor milestones and longer survival compared to a historical cohort [37▪▪]. Side effects included elevated transaminases in a subset of participants though safety and tolerability will continue to be examined in future studies. Enrollment has already been initiated for phase III open-label, single-dose studies of AVXS-101 in a larger SMA I cohort (AveXis; NCT03461289; NCT03306277) and in a broader presymptomatic cohort with SMA types 1–3 (AveXis; NCT03505099) to further assess drug safety and efficacy. The AAV9-mediated therapy has been shown to cross the blood–brain barrier, target neurons diffusely within the spinal cord, and lead to sustained expression of hSMN [37▪▪,38]. Thus, viral-mediated therapies may provide additional ease and versatility for gene delivery as it can be given systemically and may be efficacious with single or infrequent dosing.

Viral-based treatment strategies in ALS have predominantly been explored in preclinical studies. Similar to the ability of ASO-based strategies to reduce toxic gene products, viral vectors have also been modified to deliver RNA-based therapeutics to suppress gene expression. Adenoviral or lentiviral vectors carrying SOD1 short hairpin RNA (shRNA) [39–42], SOD1 micro-RNA (miRNA) [43,44], or SOD1 ASO [45▪] have been shown to suppress toxic mutant SOD1, slow disease progression, and/or improve survival in SOD1 G93A mice when given intravenously and/or intrathecally. Notably, AAV-based delivery of SOD1-directed miRNA in nonhuman primates has been shown to effectively silence SOD1 in the spinal cord, setting the stage for development of future clinical trials [44].

A particularly valuable application of viral vectors is that they enable restoration or overexpression of genes. Neurotrophic factors are secreted proteins valuable to the growth and survival of multiple neuronal types and levels of various neurotrophic factors have been shown to decline in patients with ALS and animal models [46]. Multiple approaches have been explored to deliver neurotrophic factors in ALS to favorably alter the cellular milieu surrounding motor neurons to impact their survival. This sort of intervention has the potential to modify both sporadic and familial forms of the disease. Over the years, various factors including glial-derived neurotrophic factor (GDNF), insulin-like growth factor 1/2, and vascular endothelial growth factor have been shown to provide benefit when virally delivered using various administration routes in preclinical models of ALS [47▪]. Moreover, the delivery method and cell types targeted have been shown to have a significant impact on the therapeutic benefit gained from this strategy. For instance, although systemic delivery of AAV9-GDNF in G93A rats failed to extend survival [48▪], specific induction of GDNF expression in muscle was shown to prolong disease progression and increase survival [49,50].

Adenoviral gene therapy has also provided a tool for clarifying the therapeutic potential of targeting molecular and pathological derangements observed in sporadic ALS. For instance, the 'dying back' of motor nerve terminals is a common pathological feature observed in ALS model mice and patients. In a recent study, SOD1 G93A mice were given systemic gene therapy to express Dok7, a regulator of synaptogenesis. Systemic viral delivery of the gene was found to reduce motor nerve denervation and prolonged lifespan, suggesting that stabilizing the neuromuscular junction in ALS may provide additional therapeutic benefit, particularly if combined with other treatment strategies [51▪]. Glutamatergic excititoxicity is another pathway widely implicated in sporadic and familial ALS. Accumulation of D-serine, an NMDA receptor co-agonist that can facilitate receptor overactivation, has been seen in sporadic ALS and SOD1 model mice. D-serine is degraded by D-amino acid oxidase (DAO), an enzyme that has lowered expression in ALS model mice. Increasing DAO expression in G93A mice using a single intrathecal injection of ssAAV9-DAO reduced D-serine in the spinal cord, lessened motor neuron loss, and extended survival [52▪]. The durability and versatility of viral-based gene therapies motivates further exploration of these strategies in ALS preclinical and clinical trials.

CONCLUSION

Considerable strides in developing gene therapy for ALS and SMA have been made in recent years. ASO-based therapeutics for familial ALS caused by SOD1 and C9orf72 mutations have shown promise in preclinical studies and continue to advance in the clinical pipeline. Success of viral-based gene delivery in SMA highlights its potential to improve ease of drug administration and expand possibilities for gene modification. The complexity and heterogeneity of cellular processes disrupted in sporadic ALS is a significant challenge for therapeutic development. However, targeting pathological features broadly observed in ALS, such as TDP-43 inclusions, provide an intriguing approach. In addition, combining gene therapies to target independent processes could provide synergistic benefit [53▪].

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Conflicts of interest

C.V.L. has no conflicts to disclose. Washington University has equity ownership interest in C2N Diagnostics and may receive royalty income based on technology licensed by Washington University to C2N Diagnostics. C2N has licensed IP regarding protein kinetics measurements. Washington University has submitted a nonprovisional patent application 'SOD1 kinetics measurements' (T.M.M.). T.M.M. has served on a medical advisory board for Biogen. Ionis pharmaceuticals provided support for research. T.M.M. serves as a consultant for Cytokinetics.

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