Gene therapy for ALS: A review

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Amyotrophic lateral sclerosis (ALS) has historically posed unique challenges for gene-therapy-based approaches, due to a paucity of therapeutic targets as well as the difficulty of accessing both the brain and spinal cord. Recent advances in our understanding of disease mechanism and ALS genetics, however, have combined with tremendous strides in CNS targeting, gene delivery, and gene editing and knockdown techniques to open new horizons of therapeutic possibility. Gene therapy clinical trials are currently underway for ALS patients with SOD1 mutations, C9orf72 hexanucleotide repeat expansions, ATXN2 trinucleotide expansions, and FUS mutations, as well as sporadic disease without known genetic cause. In this review, we provide an in-depth exploration of the state of ALS-directed gene therapy, including antisense oligonucleotides, RNA interference, CRISPR, adeno-associated virus (AAV)-mediated trophic support, and antibody-based methods. We discuss how each of these approaches has been implemented across known genetic causes as well as sporadic ALS, reviewing preclinical studies as well as completed and ongoing human clinical trials. We highlight the transformative potential of these evolving technologies as the gene therapy field advances toward a true disease-modifying treatment for this devastating illness.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a uniformly fatal disease characterized by degeneration of upper and lower motor neurons, leading to progressive paralysis, respiratory failure, and death in 2–5 years. It is also the most common adult motor neuron disease, with a prevalence of 5 per 100,0001 and a lifetime risk of 1:400–1:800.2 The search for disease-modifying therapies has long been hampered by a poor understanding of disease mechanism. In fact, out of >80 human clinical trials, only riluzole4,5 and edaravone6–8 have slowed progression, both of them modestly and by targeting non-specific factors such as excitotoxicity or oxidative stress. Recent advances in our understanding of ALS pathophysiology, including the discovery of many of its genetic underpinnings, have enabled the development of increasingly targeted therapies, with today’s clinical trials holding growing promise and hope for efficacious treatments.

A pathologic hallmark of ALS, affecting over 97% of patients, is ubiquitinated cytoplasmic inclusions consisting largely of Tar-DNA binding protein of 43 kDA (TDP-43).9,10 TDP-43 pathology is also found in 50% of frontotemporal dementia (FTD) patients and is seen in nearly all ALS-FTD spectrum cases.9–13 Normally found in the nucleus, TDP-43 broadly mediates transcription, translation, and splicing.14–17 Acting with ~30% of the transcriptome,14 its cytoplasmic mislocalization is thought to be detrimental on two fronts: (1) toxicity of cytoplasmic aggregates,18,19 and (2) loss of normal nuclear function.20–24 In motor neurons from ALS patients, TDP-43 associates with cytoplasmic stress granules (SGs),25,26 conglomerates of proteins and RNA that become maladaptive in disease. SGs additionally bind a host of nuclear import, export, and transcription and translation factors, resulting in loss of nucleocytoplasmic shuttling and eventual cell death.27–29 This intracellular cascade provides several therapeutic targets aimed at pathologic elements common to most ALS cases.14

Another approach, and one particularly amenable to gene therapy techniques, is targeting genetic mutations known to cause ALS. Ten percent of ALS can be classified as familial, with ~70% of familial cases explained by known gene mutations.28 The first ALS-associated gene, superoxide dismutase-1 (SOD1),30 was described in 1993 and accounts for 12%–20% of familial and 1%–2% of sporadic ALS.28,29 For the decade and a half following its discovery, this remained the only major known heritable cause and therefore was the basis of all preclinical ALS models.30,31 However, unlike the 97% of cases described above, SOD1-ALS patients do not demonstrate TDP-43 pathology at autopsy, raising concern that SOD1 animal models similarly do not represent most ALS pathology, and studies conducted in these models may not broadly translate.32–34 Indeed, most early studies failed in human clinical trials.34–36 It was not until 2008 that a second contributor to heritable ALS, TARDBP—which encodes TDP-43—was discovered.34,35 TARDBP mutations account for 4% of familial and 1% of sporadic cases, and the discovery of both this direct genetic cause and the identification of TDP-43 in motor neuron inclusions yielded additional mouse models.30,31,36,37 A year later, mutations in the fused in sarcoma (FUS) gene were discovered, accounting for another 4% of familial ALS,36,39 although, like SOD1, FUS patients lack TDP-43 pathology. In 2010, intermediate-length CAG trinucleotide repeat expansions in the ataxin-2 (ATXN2) gene were found to be associated with up to 4.7% of all ALS.40 The most significant genetic discovery to date has been that of C9orf72 in 2011;41,42 G4C2 hexanucleotide repeat expansions in this gene have

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been found in 40% of familial and 8% of sporadic cases, or 11% of all ALS,\textsuperscript{28,43} and some mouse models recapitulate ALS pathophysiology.\textsuperscript{44,45} More than 20 additional ALS-associated genes together account for an additional 12%–15% of familial ALS,\textsuperscript{28,46} leaving 25%–30% of familial cases unexplained. Aside from SOD1 and FUS, TDP-43 pathology is seen across all mutations, with nearly all known ALS-associated genes also being identified in FTD or acting as FTD phenotype modifiers, SOD1 being a notable exception.\textsuperscript{47}

Gene therapy involves the delivery of genetic material to cells in order to introduce functional copies of dysfunctional genes, introduce trophic factors and other disease-modifying genes, or silence harmful gene expression using antisense oligonucleotides (ASOs), RNA interference (RNAi), or gene-editing technology such as CRISPR. In order to target the central nervous system (CNS), this material can be delivered naked, as is often the case for ASOs; by viral vectors including adenovirus-associated virus (AAV) and others; or by physical or chemical systems such as nanoparticles.\textsuperscript{24,25} Methods to access the CNS across the blood-brain barrier include intravenous (i.v.), intracerebroventricular (i.c.v.), intrathecal (i.t.), or intraparenchymal delivery. An ALS-specific challenge is the need to reach both the motor cortex and the spinal cord.

Viral vectors have the advantage of conferring sustained expression of genetic material in transduced cells after a single treatment. Among these, AAV has emerged as the lead vector for CNS delivery, based on its ability to transduce terminally differentiated cells and to establish nuclear episomes without incurring the risk of insertional mutagenesis.\textsuperscript{28,48} Equally advantageous is the selective tropism of its many different serotypes, both naturally occurring and modified.\textsuperscript{47,48} Thus far, the serotypes that have dominated ALS preclinical studies and clinical trials are the naturally occurring AAV9 and AAVrh10, due to their ability to target motor neurons.\textsuperscript{72,73} In particular, the discovery that self-complementary AAV9 (scAAV9) vectors cross the blood-brain barrier\textsuperscript{11} led to their use in treatment of spinal muscular atrophy (SMA), an inherited lower motor neuron disorder in which patients lack a functioning copy of the SMN1 gene. i.v. delivery of scAAV9 encoding SMN1 rescued SMA mouse models,\textsuperscript{53–55} and subsequent human clinical trials showed dramatic efficacy in SMA patients,\textsuperscript{56} leading to US Food and Drug Administration (FDA) approval of Zolgensma in 2019. This served as an important proof of principle for ALS therapeutics, which also must target lower motor neurons.\textsuperscript{49} Recently, various modifications have been made to increase ASO stability, protect against nuclease degradation, improve cellular uptake, recruit RNase H, and reduce immunogenicity.\textsuperscript{64} Although they do not cross the blood-brain barrier, ASOs achieve widespread CNS distribution after i.t. delivery,\textsuperscript{63,64} making them an important tool in the arsenal against ALS. Indeed, ASOs were successfully developed to treat SMA, providing an important proof-of-concept for ALS. For SMA therapy, delivery of a splice-altering ASO resulting in the conversion of SMN2 to SMN1 showed efficacy in mouse models of SMA as well as distribution to affected areas of the spinal cord in nonhuman primates (NHPs).\textsuperscript{65} This was followed by clinical trials showing marked improvements in strength and longevity in children with SMA,\textsuperscript{67–69} leading to FDA approval of nusinersen in 2016 and its current widespread use in the clinic.

**SOD1**

Mutations in SOD1 are believed to cause ALS through toxic gain of function caused by aggregation of misfolded SOD1 protein.\textsuperscript{30,70} Although more than 150 ALS-associated SOD1 mutations have been described, the G93A mutation, rare in humans, is the most commonly studied in preclinical models, as there exists a readily available SOD1\textsuperscript{G93A} mouse\textsuperscript{172} and rat.\textsuperscript{71} In a key preclinical study, a 20-mer ASO targeting SOD1 was delivered via continuous infusion in the lateral ventricle of rats and the lumbar spine of NHPs and demonstrated significant and widespread ASO concentrations throughout the brain and spinal cord with deep tissue penetration.\textsuperscript{74} In SOD1\textsuperscript{G93A} rats treated pre-symptomatically, there was substantial SOD1 mRNA and protein knockdown in the brain and spinal cord that was associated with slowed disease progression and extended survival.\textsuperscript{74} This led to a first-in-human phase I clinical trial (ClinicalTrials.gov: NCT01041222), where ASO ISIS 333611 was delivered via a single, 11.5-h i.t. infusion to patients with SOD1-associated ALS.\textsuperscript{75} While the study demonstrated safety and tolerability as well as establishing the use of cerebrospinal fluid (CSF) SOD1 as a pharmacokinetic marker, there were no reductions in CSF SOD1 protein at the conservative concentrations used (maximum dose, 3 mg). A subsequent trial (ClinicalTrials.gov: NCT02623699) delivered the ASO, now named BIBO677/Tofersen (IONIS-SOD1Rx), via serial lumbar i.t. injections over 12 weeks, with patients cohorts into dosing groups ranging from 20–100 mg.\textsuperscript{76} The trial demonstrated safety at these higher doses, as well as reductions in CSF SOD1 protein of...
33% in the highest-dose group. Exploratory analyses showed promising reductions in rate of decline as measured by the revised ALS functional rating scale (ALSFRS-R) in the high-dose group, especially among fast progressors. Safety and efficacy are currently being evaluated in a phase 3, randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov: NCT02623699) and its extension study (ClinicalTrials.gov: NCT03070119). In addition to the traditional delivery of naked ASOs described in these studies, another group reported a novel approach using AAV to achieve targeted and sustained ASO expression. AAVrh10 expressing antisense sequences targeting \(SOD1\) embedded in a U7 small-nuclear RNA was delivered both i.v. and i.c.v. to \(SOD1^{G93A}\) mice, at birth or in pre-symptomatic adulthood, leading to nonsense-mediated \(SOD1\) decay and markedly increased survival, strength, and weight in both age groups.

**C9orf72**

Although the full mechanisms by which \(C9orf72\) hexanucleotide repeat expansions cause ALS are unknown, studies suggest a prominent role for a toxic gain of function, mediated in part by the \(G\_C\_G\_C\) repeat RNA and in part by dipeptide repeat proteins produced by repeat-associated non-ATG (RAN) translation. The first ASO-based strategies focused on reducing this toxicity in induced pluripotent stem cells (iPSCs) derived from humans with \(C9orf72\) expansions. Three such studies published in tandem in 2013 found that ASO-mediated reduction of the \(C9orf72\) transcript—whether through binding within the repeat expansion or within the surrounding regions—reduced nuclear RNA foci in \(C9orf72\)-associated ALS. Two of the groups found additionally that ASO treatment reversed aberrant gene expression and reduced susceptibility to excitotoxicity in iPSC-differentiated neurons, while the third group demonstrated persistence of aberrant RNA expression after sense-strand targeting and evidence suggesting the importance of simultaneously targeting the antisense strand. This group also performed the first in vivo studies, demonstrating sustained efficacy and tolerability of knocking down \(C9orf72\) RNA in wild-type mice for up to 18 weeks after a single injection of ASO into the lateral ventricle. In a subsequent study, the authors used a single i.c.v. injection to deliver ASOs targeting \(G\_C\_G\_C\) repeat-containing sense-strand RNAs to pre-symptomatic adult

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**Figure 1. Summary of gene therapy strategies**

(A) Non-viral strategies include using ASOs to induce alternate splicing or RNase H-mediated degradation. (B and C) Viral strategies include (B) AAV-mediated gene silencing, through RNA interference or CRISPR-Cas9 or (C) AAV-mediated gene delivery including neurotrophic factors. AAV, adeno-associated virus; ASO, antisense oligonucleotide; Cas, CRISPR-associated system; miRNA, microRNA; PAM, protospacer adjacent motif; RISC, RNA-induced silencing complex; RNAi, RNA interference; shRNA, small hairpin RNA.
mice expressing up to 450 repeats. They demonstrated sustained reduction in RNA foci and dipeptide repeat proteins in the cortex and spinal cord, as well as amelioration of cognitive deficits.92 This work has ultimately led to a phase I clinical trial of the ASO BIB078 (ClinicalTrials.gov: NCT03626012) for C9orf72-ALS patients.

ATXN2
Although long trinucleotide repeat expansions in ATXN2 have been known to cause spinocerebellar ataxia type 2,13–38 intermediate-length expansions were discovered in 2010 to be a relatively common cause of heritable ALS.40,87–89 Ataxin-2 serves diverse functions in the cell, including RNA processing and receptor endocytosis, but its critical functions in the formation of stress granules27,90 and induction of aberrant TDP-43 cleavage by caspase 391,92 are of particular relevance in ALS. In 2018 it was demonstrated that a host of nucleocytoplasmic transport factors are bound in ataxin-2-containing SGs upon induction of stress in HEK293T cells. Further, delivery of ASOs targeting ataxin-2 to neuronal-differentiated iPSCs from C9orf72-ALS patients reversed cytoplasmic mislocalization of nuclear proteins.27 Seminal work in vivo used a rapidly progressive TDP-43 ALS mouse model to demonstrate that a one-time delivery of Atxn2-ASO to the lateral ventricle at birth resulted in sustained, marked reduction of Atxn2 mRNA as well as prolonged survival and improved gait.92 While this strategy can be of benefit to patients with ATXN2-ALS, importantly, it also offers a therapeutic avenue for the broader ALS population, as TDP-43 localization to ATXN2-dependent stress granules is a shared pathologic endpoint.25 A phase I clinical trial of the ASO BIBI105 (ClinicalTrials.gov: NCT04494256) is currently underway, enrolling patients both with and without CAG repeat expansions in ATXN2.

FUS
FUS is the most commonly mutated gene found in juvenile and pediatric ALS, with the p.P525L mutation causing a particularly aggressive and early-onset form4,39 through a toxic gain-of-function mechanism. In 2019, Ionis Pharmaceutical, in conjunction with Columbia Medical Center, developed an ASO targeting this mutation via i.t. delivery and obtained FDA approval for experimental use in a young woman, for whom the therapy, jacifusen, is named.96 Jaci has since passed away, but jacifusen has been used to treat three additional FUS-ALS patients through the FDA’s compassionate use protocol, and 8 more patients are approved for investigational treatment with funding from the ALS association and Project ALS.97,98

AAV-MEDIATED GENE SILENCING
AAV-mediated silencing has the advantage over ASOs of eliminating the need for readministration, which is of use when targeting both the brain and spinal cord. AAVs are frequently used to deliver small non-coding RNAs to achieve RNAi, a naturally occurring process in which double-stranded RNA molecules regulate the expression of mRNA through homologous base pairing and subsequent cleavage via the RNAi-induced silencing complex (RISC; Figure 1B).399 Two types of small noncoding RNAs, small hairpin RNA (shRNA) and artificial microRNA (miRNA), are in increasingly widespread use as a therapeutic strategy against neurodegenerative disease, particularly miRNA due to its more favorable safety profile.100,101 Further refinement has been achieved using bioinformatic tools to minimize passenger strand loading102 and optimize promoter, serotype, and dosing choices,99 leading to greatly decreased off-target effects. The first AAV-RNAi human clinical trial for a neurodegenerative disease (ClinicalTrials.gov: NCT04120493) is currently in phase I/II and uses AAV5 to deliver a miRNA targeting Huntingtin to treat Huntington’s disease.2,103 This study will provide important safety and feasibility data for the ALS field.

An alternative approach to treat gain-of-function disorders is gene editing, which can be achieved using CRISPR-associated (Cas) systems, the most widely used of which is the RNA-guided Cas9 endonuclease104 (Figure 1B). CRISPR-Cas9 enables specific targeting of genomic sequences and induction of double-stranded DNA breaks, causing frameshift-inducing base insertions or deletions (indels) with diverse applications,105 although its use in the ALS field is relatively nascent.106 Additional CRISPR-based systems have been developed that have transformative potential.107–110 These include CRISPR-interference (CRISPRi), which uses a catalytically dead Cas9 to repress DNA without the potential mutagenic effects associated with cleavage;111 the Cas13 family, which targets RNA for degradation12,113 and variants of which are small enough for AAV packaging;114 and prime editing, which is a method of introducing specific genomic insertions or deletions without inducing double-stranded breaks.115 The application of these technologies to the ALS field is highly anticipated as they are further developed.

SOD1
As the first-identified form of ALS, SOD1 has been the target of the majority of AAV-RNAi-based approaches, with increasingly promising results. The first AAV9 studies delivering shRNA against SOD1 compared i.v. administration in SOD1pG93A mice at differing ages and found a 39% increase in survival after P1-injection that decreased substantially with age and disease progression;116 they noted increasingly preferential glial targeting over neurons with later injections.116,117 They also found markedly increased survival in the slower-progressing SOD1G37R mouse model even when treated after disease onset, and they demonstrated robust SOD1 mRNA knockdown throughout the spinal cord in NHPS after lumbar i.t. injection.118 Another group used bilateral direct cortical injection of AAV9-shRNA targeting SOD1 in presymptomatic adult SOD1pG93A rats and found that upper motor SOD1 suppression alone was able to preserve motor function and increase survival by 20 days.118 A 2016 study used AAV9 to deliver an artificial miRNA against SOD1 (mi-SOD1) to the ventricles in neonatal SOD1pG93A mice. The authors found a 50% extension in survival with significant delay in hindlimb paralysis, as well as improvements across multiple histopathologic parameters including number of spinal motor neurons.119 Notably, neonatal treatment has limited translatability, as most patients present in adulthood after symptom onset, and the authors therefore conducted a separate study using RAAVRh10 to deliver mi-SOD1 systemically to adult SOD1pG93A mice. They found substantially delayed
disease onset and 21% extended survival, with preserved strength and respiratory function. \(^{120}\) They then tested the vector in marmosets via i.t. injection and found significant lowering of SOD1 in lower motor neurons throughout the spinal cord without short-term toxicity. \(^{120}\) Subsequent studies of rAAVRh10.mi-SOD1 in macaques, delivered i.t. with the head down at 30 degrees, demonstrated robust silencing at the mRNA level in laser-captured motor neurons throughout the spinal cord that was proportionate to the strength of the promoter used. \(^{121}\) The authors demonstrated a low off-targeting profile and found no adverse events up to 92 days post-infusion, paving the way for a human study published in July 2020. In that study, two patients with SOD1-ALS were treated with i.t. infusion of rAAVRh10.mi-SOD1. The first patient had transient improvement in strength in his right leg and slightly lowered CSF SOD1 levels throughout the 12-month period reported. \(^{122}\)

The DRG toxicity seen in the above clinical trial has also been seen with AAV9 when administered systemically to neonatal pigs and juvenile primates, \(^{123}\) although the NHPs did not show clinical signs of toxicity. Further meta-analyses of CSF and systemic administration across 33 NHP studies (a total of 256 NHPs) showed DRG toxicity in 83% of CSF-treated and 32% of systemic-treated NHPs that was independent of serotype, sex, promoter, or transgene; importantly, however, pathology was dose dependent and overall mild, and no clinical sequelae were seen. \(^{123}\) Interesting alternative approaches to avoid DRG toxicity have been tried with varying degrees of success. For instance, the AAV-Rh10-mediated miSOD1 treatment described above also resulted in 50-day survival prolongation after direct injection of the tongue and intrapleural space of adult presymptomatic SOD1 \(^{G93A}\) mice, \(^{123}\) areas in which weakness is the lead cause of death in ALS patients. Recent studies successfully used DRG-specific miRNAs to downregulate transgene expression in these cells while preserving intended CNS transduction, an efficient detargeting strategy that can be employed across any AAV-based therapeutic. \(^{126}\)

Finally, one notable study used a novel subpial delivery method, administering AAV9-shRNA to the narrow space between the spinal cord parenchyma and the innermost layer of the meninges. The authors achieved long-term suppression of motor neuron disease in SOD1 \(^{G37R}\) mice treated pre-symptomatically as well as potent blocking of disease progression in mice treated after disease onset. They then used this delivery method in adult pigs and NHPs and found homogeneous and robust transgene expression throughout the spinal cord, including motor neurons. \(^{127}\) Although technically challenging, the demonstration of feasibility in these larger animal models speaks well to the promise of subpial AAV delivery in humans, who share comparable spinal anatomy and size.

The first study using CRISPR in ALS was published in 2017. The authors used a modified AAV9 to deliver *Staphylococcus aureus*-derived Cas9 (SaCas9) and a single-guide RNA (sgRNA) targeting the hSOD1 gene via the facial vein to neonatal SOD1 \(^{G93A}\) mice. \(^{128}\) They found concentrated SaCas9 expression throughout the ventral horn cells of the spinal cord, with >2.5-fold decrease in SOD1 protein levels. Treated mice showed preserved motor neurons with improved motor function, a 37% delay in disease onset, and a 25% increase in survival, with a low off-target indel rate across spinal cord transgenes. \(^{128}\) Another group used AAV9-SaCas9-sgRNA to treat neonatal SOD1 \(^{G93A}\) mice via i.c.v. injection and also found reduced SOD1 in anterior horn cells. These studies also reported an increase in motor neurons and greatly improved motor function, as well as a notable 54.6% increase in survival, with a low off-target indel rate in predicted off-target sequences. \(^{129}\) In a subsequent study by the first group, an intein-mediated trans-splicing system was devised for *in vivo* single-base editing of SOD1, in order to minimize the potential mutagenic outcomes of double-stranded DNA breaks. The authors used dual AAV9 vectors to deliver this system i.t. to pre-symptomatic adult SOD1 \(^{G93A}\) mice and found a 40% reduction in SOD1 inclusions accompanied by improved neuromuscular function and muscle bulk, as well as 11% increase in survival, \(^{130}\) despite astrocyte-predominant targeting. These CRISPR-based strategies hold great promise as refinement continues.

**C9orf72**

Due to the challenges of targeting intronic, GC-rich repeat expansions \(^{131}\) and achieving knockdown in the nucleus, where C9orf72-mediated RNA foci are predominantly found, RNAi-based approaches for C9orf72 have been challenging compared to SOD1. In 2015, a group was able to engineer duplex RNAs to overcome these hurdles and effectively target both sense and antisense G\(_4\)C\(_2\) expansions in ALS-patient-derived fibroblast cells. \(^{132}\) By introducing mismatches in key regions, they destabilized the parent duplex to allow entry into the RISC complex, with resultant 40%–60% reduction in nuclear RNA foci from both strands. The authors subsequently engineered single-stranded silencing RNAs (ss-siRNAs) that function like ASOs but mediate degradation through RNAi, and found that these achieved even more potent inhibition of sense and antisense G\(_4\)C\(_2\) repeat expansions in human mutant C9orf72 fibroblast cells. \(^{133}\) The biotech company uniQure developed a different bidirectional miRNA-based approach, using concatenated miRNA hairpins targeting C9orf72 (miC), and achieved 50% reduction of sense and antisense nuclear RNA foci in G\(_4\)C\(_2\) expressing cells. They further incorporated these miC constructs into AAV5 and effectively silenced C9orf72 in iPSC-derived neurons from an FTD patient. \(^{134}\) In a subsequent study, they used miC constructs to reduce both nuclear and cytoplasmic C9orf72 mRNA in FTD patient-derived iPSCs. \(^{135}\) They then delivered their AAV5-miC constructs to the striatum of adult transgenic C9orf72 \(_{3}\) line 112 mice, a strain harboring several tandem copies of human C9orf72 that does not develop neurodegeneration but exhibits RNA foci and poly(GP) protein. They found 20%–40% reduction of C9orf72 mRNA and sense intronic transcripts in transduced regions, although the mice were not followed long enough to determine poly(GP) reduction. \(^{135}\)
There have been few CRISPR-mediated studies for C9orf72, but a recent study of note used CRISPR-Cas9 to generate deletions in the promoter region of iPSCs derived from patients harboring C9orf72 expansions. They found that these deletions reduced the expression of the C9orf72 variant containing the repeat expansion, which in turn nearly eliminated production of all dipeptide repeats and markedly reduced axonal degeneration. Another recent study identified Ku80 as a DNA repair protein that is overactivated in in Drosophila harboring G4C2 expansions and in C9orf72-ALS patient-derived iPSCs, leading to premature apoptosis. The authors used CRISPR-Cas9 to reduce Ku80 and found reductions in pro-apoptotic pathways; they also found decreased nuclear RNA foci after direct CRISPR-mediated deletion of the G4C2 repeat expansion. These are promising approaches for future in vivo studies.

**ATXN2**

To date there are no published RNAi or CRISPR-based approaches targeting ATXN2. However, the potential for this approach can be inferred from related studies in the spinocerebellar ataxia field and from the ASO-mediated reduction of Atxn2 in mouse models of SCA2. Viral delivery of RNAi-based approaches has demonstrated success in mouse models of SCA1, SCA3, and SCA7, while a CRISPR-based approach in patient-derived iPSCs has shown early promise for SCA3. Additional ASO, RNAi, and CRISPR-based approaches for spinocerebellar ataxia are reviewed extensively elsewhere and demonstrate the feasibility and promise of this approach if adapted to motor neuron-targeting AAV delivery methods.

**AAV-MEDIATED GENE DELIVERY**

The most long-established application of gene therapy is delivery of a therapeutic transgene. Unlike in the case of Zolgensma for SMA, however, no ALS-causing mutations have been found to have an exclusive loss-of-function mechanism, and so therapeutic transgene delivery has focused on neurotrophic factors to provide support for degenerating neurons (Figure 1C). Neurotrophins are typically secreted proteins involved in neuronal growth or survival, and several neurotrophins have been found to decline over time in ALS patients and animal models, suggesting a potential role in prolonging neuronal health. While nonspecific and unlikely to be curative, these methods are attractive for their potential to prolong survival across all forms of ALS. Non-gene-therapy-mediated growth factor studies have unfortunately not been successful in clinical trials, which is felt to be in large part due to inadequate dosing to affected areas of the nervous system. For this reason, gene therapy approaches have been pursued by numerous groups in an effort to improve CNS delivery and dosing. Early gene therapy studies for neurotrophic support purposes have been extensively reviewed elsewhere. Here, we focus on recent AAV-mediated work, most of which was conducted in SOD1G93A mice. We also discuss AAV-mediated delivery of non-trophic factors, including neuromuscular junction modulators and aggregation-directed therapies.

Intramuscular delivery of insulin-like growth factor 1 (IGF1) using AAV9 was shown in two separate studies to preserve spinal motor neurons and modestly extend lifespan in SOD1G93A mice treated pre-symptomatically or in early post-symptomatic adulthood. Similar results were obtained using systemic AAV9-IGF1 delivery. In a different approach, i.c.v. delivery of AAV4 was used to target ependymal cells for IGF1 secretion into the CSF in early symptomatic SOD1G93A mice. The authors found improved motor function and modestly improved survival, effects that were slightly greater with delivery of AAV4-mediated vascular endothelial growth factor (VEGF), which has been hypothesized to act in the same pathway as IGF1. Delivery of both AAV4-IGF1 and AAV4-VEGF did not provide further benefit. VEGF has been adapted for ALS therapy at both the gene therapy and protein levels. Using i.t. delivery of AAV9, one group demonstrated that VEGF expression in adult symptomatic SOD1G93A mice improved motor function and extended survival. When AAV1 and AAV9 were used to deliver VEGF to a feline model of lower motor neuron disease via i.c.v., i.v., or intracisterna magna (i.c.m.) injection, only i.c.m. delivery resulted in sustained, high levels of VEGF expression in the spinal cord; however, there was no therapeutic benefit.

Although early studies of AAV-mediated glial-derived neurotrophic factor (GDNF) delivery showed promise, effects were modest and seen only after intramuscular delivery. A 2017 study of a more translatable, systemic AAV9-GDNF delivery in pre-symptomatic SOD1G93A rats showed modest improvement in strength and delay of forelimb paralysis. However, there was no extension in survival, and adverse side effects were seen, including decreases in working memory, activity levels, and weight. Another neurotrophic factor, granulocyte-colony stimulating factor (G-CSF), was found to preserve motor units and extend survival by 10% when delivered to adult pre-symptomatic SOD1G93A mice via direct intraspinal injection of AAV1. Hepatocyte growth factor (HGF) has also been evaluated, using i.t. AAV1 or intramuscular AAV6 to treat adult pre-symptomatic SOD1G93A mice, with modest improvements noted in strength and survival using both delivery methods. Factors that act at the neuromuscular junction have also been assessed, including intramuscular delivery of AAV1-neuregulin to promote collateral sprouting and electrophysiologic function in adult symptomatic SOD1G93A mice. However, there were no effects on strength or survival. Systemic administration of AAV9 delivering the gene encoding the neuromuscular junction protein DOK7 preserved the neuromuscular junction, improved locomotion, and modestly increased lifespan in early symptomatic SOD1G93A mice, without affecting motor neuron counts. D-amino acid oxidase (DAO) is thought to modulate excitotoxicity, and i.t. delivery of AAV9-DAO to early symptomatic SOD1G93A mice resulted in motor neuron preservation and a modest survival extension.

Factors with direct effects on misfolded ALS-associated proteins are also being studied. For example, the cytosolic chaperone, macrophage migration inhibitory factor (MIF), inhibits mutant SOD1 misfolding. AAV9-mediated expression of MIF after direct intraspinal delivery to neonatal SOD1G93A mice or the slower-progressing
| Gene therapy | Date | Model | Route | Outcome | Citation |
|--------------|------|-------|-------|---------|----------|
| Antisense oligonucleotides | | | | | |
| SOD1 | 2006 | rat; NHP SOD1<sup>G93A</sup> | i.c.v. (rat), i.t. (NHP) | increased survival; good biodistribution in NHP | Smith et al.74 |
| | 2013 | human SOD1-ALS phase I | i.t. | safe; no benefit | Miller et al.75 |
| | 2017 | mouse SOD1<sup>G93A</sup> | i.c.v. + i.v. | increased survival, strength, and weight | Biferi et al.77 |
| | 2020 | human SOD1-ALS phase I/II | i.t. | safe; reduced CSF SOD1 | Miller et al.76 |
| | current | human SOD1-ALS phase III | i.t. | TBD | ClinicalTrials.gov: NCT02623699 |
| C9orf72 | 2013 | human iPSC C9-ALS | | reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression | Donnelly et al.78 |
| | 2013 | human iPSC C9-ALS | | reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression | Sareen et al.81 |
| | 2013 | human iPSC C9-ALS; mouse (WT) | | reduced nuclear RNA foci; persistent aberrant expression; safe in mice | Lagier-Tourenne et al.80 |
| | 2016 | mouse C9<sup>(450)</sup> | i.c.v. | sustained reduction in nuclear RNA foci and DPRs in motor neurons; cognitive benefit | Jiang et al.82 |
| | current | human C9-ALS phase I | i.t. | TBD | ClinicalTrials.gov: NCT03626012 |
| ATXN2 | 2017 | mouse hTDP-43 | i.c.v. | reduced Atxn2 mRNA; increased survival; improved gait | Becker et al.93 |
| FUS | 2018 | human iPSC C9-ALS | | reduced nuclear RNA foci and excitotoxicity; reversed aberrant gene expression | Zhang et al.94 |
| | Current | human ATXN2-ALS & sporadic ALS phase I | i.t. | TBD | ClinicalTrials.gov: NCT04494256 |
| FUS | 2019 | human FUS-ALS | i.t. | patient passed; further clinical data not reported | Arnold95 |
| | Current | human FUS-ALS | i.t. | TBD | Figueiredo97 |
| AAV-mediated gene silencing | | | | | |
| AAV-RNAs | | | | | |
| SOD1 | 2013 | mouse SOD1<sup>G93A</sup> & SOD1<sup>G37R</sup>; NHP | i.v. (mice), i.t. (NHP) | increased survival, decreasing with age of administration; reduced SOD1 mRNA in NHP spinal cord | Foust et al.116 |
| | 2014 | rat SOD1<sup>G93A</sup> | cortex | delayed disease onset and increased survival; preserved motor function | Thomsen et al.118 |
| | 2016 | mouse SOD1<sup>G93A</sup> | i.c.v. | increased survival; delayed paralysis; increase in motor neurons | Stoica et al.119 |
| | 2016 | mouse SOD1<sup>G93A</sup>; NHP | i.v. (mice), i.t. (NHP) | delayed onset; increased survival; preserved motor and respiratory function in mice; reduced SOD1 mRNA in NHP spinal cord | Borel et al.120 |
| | 2018 | NHP | i.t. | reduced SOD1 mRNA in spinal cord | Borel et al.121 |
| | 2019 | mouse SOD1<sup>G93A</sup> | tongue, subplural | increased survival; lowered SOD1 mRNA in muscle, tongue, and diaphragm | Keeler et al.122 |

(Continued on next page)
| Gene therapy               | Date     | Model                                      | Route   | Outcome                                                                 | Citation                                      |
|---------------------------|----------|--------------------------------------------|---------|-------------------------------------------------------------------------|-----------------------------------------------|
| SOD 1                     | 2017     | mouse SOD1<sup>G93A</sup>                  | i.v.    | increased survival and strength with decreased SOD1 protein in spinal cord | Gaj et al. 128                                |
|                           | 2020     | mouse SOD1<sup>G93A</sup>                  | i.c.v.  | increased survival and strength with decreased SOD1 protein in spinal cord | Duani et al. 129                             |
|                           | 2020     | mouse SOD1<sup>G93A</sup>                  | i.t.    | increased survival and strength with decreased SOD1 inclusions in spinal cord | Lim et al. 128                               |
| C9orf72                   | 2019     | Drosophila C9; human iPSC C9-ALS           |         | reduced apoptotic pathway activation; reduced nuclear foci              | Lopez-Gonzalez et al. 137                     |
|                           | 2020     | human iPSC C9-ALS                          |         | reduced C9orf72 expression; near elimination of dipeptide repeats; reduced axonal degeneration | Krishnan et al. 136                          |

**Neurotrophic support**

| Neurotrophic support     | Year     | Model                                      | Route   | Outcome                                                                 | Citation                                      |
|--------------------------|----------|--------------------------------------------|---------|-------------------------------------------------------------------------|-----------------------------------------------|
| IGF                      | 2010     | mouse SOD1<sup>G93A</sup>                  | i.c.v.  | improved motor function and increased survival                         | Dodge et al. 151                             |
|                           | 2016     | mouse SOD1<sup>G93A</sup>                  | i.m.    | preserved motor neurons; increased survival                            | Allodi et al. 128                            |
|                           | 2018     | mouse SOD1<sup>G93A</sup>                  | i.m.    | preserved motor neurons; increased survival                            | Lin et al. 129                               |
|                           | 2018     | mouse SOD1<sup>G93A</sup>                  | i.v.    | preserved motor neurons; increased survival                            | Wang et al. 130                              |
|                           | 2010     | mouse SOD1<sup>G93A</sup>                  | i.c.v.  | improved motor function and increased survival                         | Dodge et al. 121                             |
| VEGF                     | 2013     | feline Lxi<sup>Lm</sup> (LMN disease)      | i.c.v., i.v., or i.c.m. | only i.c.m. delivery resulted in sustained VEGF in spinal cord, without therapeutic benefit | Bucher et al. 135                           |
|                           | 2016     | mouse SOD1<sup>G93A</sup>                  | i.t.    | improved motor function and increased survival                         | Wang et al. 136                              |
| GDNF                     | 2017     | rat SOD1<sup>G93A</sup>                   | i.v.    | improved strength, but no effect on survival; worsened cognitive function, decreased activity | Thomsen et al. 159                           |
| G-CSF                     | 2011     | mouse SOD1<sup>G93A</sup>                  | intra-spinal | preserved motor units; increased survival                          | Henriques et al. 170                          |
| HGF                       | 2019     | mouse SOD1<sup>G93A</sup>                  | i.t.    | improved motor function and increased survival                         | Lee et al. 170                               |
|                           | 2019     | mouse SOD1<sup>G93A</sup>                  | i.t.    | improved motor function and increased survival                         | Lee et al. 170                               |

**Neuromuscular junction modulators**

| Neuromuscular junction modulators | Year | Model | Route | Outcome                                                                 | Citation                                      |
|----------------------------------|------|-------|-------|-------------------------------------------------------------------------|-----------------------------------------------|
| Neuregulin                       | 2016 | mouse SOD1<sup>G93A</sup>                  | i.m.  | no effect on strength or survival                                       | Mancuso et al. 162                           |

(Continued on next page)
loxSOD1G37R mouse model significantly reduced misfolded SOD1 levels in the spinal cord of the SOD1G93A mice and improved strength and survival in both models.\textsuperscript{166} i.t. delivery of AAV1 expressing a monoclonal antibody targeting misfolded SOD1 to pre-symptomatic adult SOD1G93A mice resulted in a notable 28% extension of lifespan accompanied by reduced misfolded spinal SOD1 and attenuated neuronal stress signals and gliosis.\textsuperscript{167} A similar strategy was tested in symptomatic TDP-43G348C mice, which manifest cytoplasmic accumulation of TDP-43 as well as memory impairment. AAV9-mediated cortical delivery of an antibody targeting an aggregation-prone region of TDP-43 as well as memory impairment. AAV9-mediated cortical delivery of an antibody targeting an aggregation-prone region of TDP-43 reduced TDP-43 proteinopathy, cognitive impairment, motor defects, and neuroinflammation.\textsuperscript{168}

CONCLUSIONS AND PERSPECTIVES

The ability to provide targeted, sustained treatment gives gene therapy the potential to permanently alter the therapeutic landscape for diseases of the CNS, as it has already done in the case of SMA. ALS in particular is in dire need of impactful, disease-modifying approaches capable of reaching some of the body’s most protected regions, the cerebral cortex and the anterior horn cells of the spinal cord, which can increasingly be achieved through minimally invasive means by harnessing the tremendous potential of gene therapy. Once delivered to their target, these therapies can act through knockdown-mediated amelioration of gain-of-function mechanisms or through delivery of protective agents, enabling approaches ranging from targeting a specific ALS-causing mutation to modifying a common pathologic endpoint across sporadic cases. As we continue to hone our delivery methods, vector specificity, and cargo efficacy, there is great hope that truly impactful treatments for ALS will emerge in the near future.

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AUTHOR CONTRIBUTIONS

D.A.A. and B.L.D. planned the manuscript. D.A.A. wrote and coordinated the draft and designed the figure and Table 1. D.A.A. and B.L.D. reviewed and revised the manuscript.

DECLARATION OF INTERESTS

B.L.D. is a founder of Spark Therapeutics and Spiovant Sciences and is on the SAB of Patch Bio, Resilience, Saligen Therapeutics, Panorama Medicines, Homology Medicines, and Spiovant Sciences.

REFERENCES

1. Oskarsson, B., Gendron, T.F., and Staff, N.P. (2018). Amyotrophic Lateral Sclerosis: An Update for 2018. Mayo Clin. Proc. 93, 1617–1628.
2. Martier, R., and Konstantinova, P. (2020). Gene Therapy for Neurodegenerative Diseases: Slowing Down the Ticking Clock. Front. Neurosci. 14, 580179.
3. Chen, J.J. (2020). Overview of current and emerging therapies for amyotrophic lateral sclerosis. Am. J. Manag. Care 26 (9, Suppl), S191–S197.

4. Bensimon, G., Lacomblez, L., and Meininger, V.; ALS/Riluzole Study Group (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. N. Engl. J. Med. 330, 585–591.

5. Bellingham, M.C. (2011). A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? CNS Neurosci. Ther. 17, 4–31.

6. Abe, K., Itoyama, Y., Sobue, G., Tsuji, S., Aoki, M., Doyu, M., Hamada, C., Kondo, K., Yoneoka, T., Akinot, M., et al. (2014). Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients Amyotroph. Lateral Scler. Frontotemporal Degener 15, 610–617.

7. The Writing Group on Behalf of the Edaravone (MCI-186) ALS 17 Study Group (2017). Exploratory double-blind, parallel-group, placebo-controlled extension study of edaravone (MCI-186) in amyotrophic lateral sclerosis Amyotroph. Lateral Scler. Frontotemporal Degener 18 (Suppl 1), 20–31.

8. Yoshino, H., and Kimura, A. (2006). Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). Amyotroph. Lateral Scler. 7, 241–245.

9. Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., et al. (2006). Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. Science 314, 130–133.

10. Baradaran-Heravi, Y., Van Broeckhoven, C., and van der Zee, J. (2020). Stress granule mediated protein aggregation and underlying gene defects in the FTD-ALS spectrum. Neurobiol. Dis. 134, 104638.

11. Mackenzie, I.R.A., and Rademakers, R. (2007). The molecular genetics and neuropathology of frontotemporal lobar degeneration: recent developments. Neurogenetics 8, 237–248.

12. Johnson, J.K., Diehl, J., Mender, M.F., Neubau, J., Shapira, J.S., Forman, M., Chute, D.I., Roberson, E.D., Pace-Savitsky, C., Neumann, M., et al. (2005). Frontotemporal lobar degeneration: demographic characteristics of 353 patients. Arch. Neurol. 62, 925–930.

13. Josephs, K.A., Holton, J.L., Rossor, M.N., Geddes, A.K., Ozawa, T., Strand, K., Khan, N., Al-Sarraj, S., and Revesz, T. (2004). Frontotemporal lobar degeneration and ubiquitin immunohistochemistry. Neuropathol. Appl. Neurobiol. 30, 369–373.

14. Guo, L., and Shorter, J. (2017). Biology and Pathobiology of TDP-43 and Emerging Therapeutic Strategies. Cold Spring Harb. Perspect. Med. 7, a024554.

15. Freibaum, B.D., Chitta, R.K., High, A.A., and Taylor, J.P. (2010). Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. J. Proteome Res. 9, 1104–1120.

16. Ling, S.C., Polynemidou, M., and Cleveland, D.W. (2013). Converging mechanisms in TAR DNA-binding Protein 43 (TDP-43) Proteinopathies. J. Biol. Chem. 291, 19437–19448.

17. Boynaiems, S., Bogaert, E., Van Damme, P., and Van Den Bosch, L. (2016). Inside out: the role of nucleocytoplasmic transport in ALS and FTLD. Acta Neuropathol. 132, 159–173.

18. Zhang, Y.-I., Xu, Y.F., Cook, C., Gendron, T.F., Roettges, P., Link, C.D., Lin, W.L., Tong, J., Castaneda-Casey, M., Ash, P., et al. (2009). Aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity. Proc. Natl. Acad. Sci. USA 106, 7607–7612.

19. Liu, Y.-C., Ling, S.C., Polymenidou, M., and Cleveland, D.W. (2013). Stress granules as crucibles of ALS pathogenesis. J. Cell Biol. 201, 361–372.

20. Zhang, K., Daigle, J.G., Cunningham, K.M., Coyle, F., Amin, K., Grima, J.C., Brown, K.E., Wadhwa, H., Yang, P., Rigo, F., et al. (2018). Stress Granule Assembly Disrupts Nucleocytoplasmic Transport. Cell 173, 958–971.e17.

21. Chia, R., Chio, A., and Traynor, B.J. (2018). Novel genes associated with amyotrophic lateral sclerosis diagnostic and clinical implications. Lancet Neurol. 17, 94–102.

22. Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O’Regan, J.P., Deng, H.X., et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362, 59–62.

23. Phillips, T., and Rothstein, J.D. (2015). Rodent Models of Amyotrophic Lateral Sclerosis. Curr. Protoc. Pharmacol 69, 5.67.1–5.67.21.

24. Joyce, P.L., Fratta, P., Fisher, E.M.C., and Acevedo-Arozena, A. (2011). SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm. Genome 22, 420–448.

25. Mitsumoto, H., Brooks, B.R., and Silani, V. (2014). Clinical trials in amyotrophic lateral sclerosis: why so many negative trials and how can trials be improved? Lancet Neurol. 13, 1127–1138.

26. Rothstein, J.D. (2003). Of mice and men: reconciling preclinical ALS mouse studies and human clinical trials. Ann. Neurol. 53, 423–426.

27. Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E., et al. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.

28. Rutherford, N.L., Zhang, Y.J., Baker, M., Gass, J.M., Finch, N.A., Xu, Y.F., Stewart, H., Kelley, B.J., Kuntz, K., Crook, R.J., et al. (2008). Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. PLoS Genet. 4, e1000193.

29. Janssens, J., Wils, H., Kleinberger, G., Russ, M., Munsat, T.L., and Thijssen, E. (2013). Overexpression of ALS-associated pM337V human TDP-43 human TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc. Natl. Acad. Sci. USA 107, 3858–3863.

30. Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, I., Kasarskis, E.J., and Munsat, T.L., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.

31. Vance, C., Rogelj, B., Hortobagyi, T., De Vos, L., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., et al. (2009). Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323, 1208–1211.

32. Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., et al. (2010). Ataxin-2...
intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1069–1075.

41. Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., et al. 2011. ALS-FTD Genes Consortium (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.

42. Defeser-Hernandez, M., Mackenzie, I.R., Boone, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., et al. 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

43. Majounie, E., Renton, A.E., Mok, K., Dopper, E.G., Waite, A., Rollinson, S., Chiò, A., Renton, A.E., Majounie, E., Mok, K., Dopper, E.G., Waite, A., Rollinson, S., Chiò, A., Batra, R., and Lee, C.W. (2017). Mouse Models of ALS. Ann. Rev. Pharmacol. Toxicol. 57, 59–86.

44. Liu, Y., Pattamatta, A., Zu, T., Reid, T., Bardhi, O., Borchelt, D.R., Yachnis, A.T., and Wu, W.L., Yang, B., Huber, N., Pasca, S.P., and Gradinaru, V. (2016). Cre-dependent selection yields AAV variants for widespread and targeted gene delivery in rodents. Nat. Protoc. 11, 379–414.

45. Azzouz, M. (2006). Gene Therapy for ALS: progress and prospects. Biochim. Biophys. Acta. 1762, 1122–1127.

46. Suzuki, M., and Svendsen, C.N. (2016). Ex Vivo Gene Therapy Using Human Mesenchymal Stem Cells to Deliver Growth Factors in the Skeletal Muscle of a Familial ALS Rat Model. Methods Mol. Biol. 1382, 325–336.

47. Ly, C.V., and Miller, T.M. (2018). Emerging antisense oligonucleotide and viral therapies for amyotrophic lateral sclerosis. Curr. Opin. Neurol. 31, 648–654.

48. Schoch, K.M., and Miller, T.M. (2017). Antisense Oligonucleotides: Translation from Mouse Models to Human Neurodegenerative Diseases. Neuron 94, 1056–1070.

49. Bennett, C.F., and Swayne, E.E. (2010). RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu. Rev. Pharmacol. Toxicol. 50, 259–293.

50. Passini, M.A., Bu, J., Richards, A.M., Kinneconn, C., Sardi, S.P., Stanek, L.M., Hua, Y., Rigo, F., Matson, J., Hung, G., et al. (2011). Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. Sci. Transl. Med. 3, 72ra18.

51. Ekhtiari Bidhendi, E., Bergh, J., Zetterström, P., Forsberg, K., Pakkenberg, B., Andersen, P.M., Marklund, S.L., and Brännström, T. (2018). Mutant superoxide dismutase 1 gene therapy for amyotrophic lateral sclerosis. Curr. Opin. Neurol. 31, 654–665.

52. Ripps, M.E., Huntley, G.W., Hof, P.R., Lobsiger, C.S., Ward, C.M., McAlonis-Downes, M., Wei, H., et al. (2019). Systemic AAV vectors for widespread and targeted gene delivery in the nervous system. Nat. Neurosci. 22, 1772–1775.

53. Riggins, M.E., and Huntley, G.W., Hof, P.R., Morrison, J.H., and Gordon, J.W. (1995). Transgenic mice expressing an altered murine superoxide dismutase gene provide a model for early-onset familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. USA 92, 689–693.

54. Nagai, M., Aoki, M., Miyoshi, I., Kato, M., Pasinelli, P., Kasai, N., Brown, R.H., Jr., and Itoyama, Y. (2001). Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. J. Neurosci. 21, 9246–9254.

55. Smith, R.A., Miller, T.M., Yamanaka, K., Polchow, C.Y., Alexander, D.D., Caliendo, J., Hentati, A., Kwon, Y.W., Deng, H.X., et al. (1994). Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 264, 1772–1775.

56. Bennett, C.F., and Swayne, E.E. (2010). RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu. Rev. Pharmacol. Toxicol. 50, 259–293.

57. Chiriboga, C.A., Swoboda, K.J., Darras, B.T., Iannaccone, S.T., Montes, J., De Vivo, D.C., Norris, D.A., Bennett, C.F., and Bishop, K.M. (2016). Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. Neurology 86, 890–897.

58. Finkel, R.S., Chiriboga, C.A., Vassar, J., Day, J.W., Montes, J., De Vivo, D.C., Yamasita, M., Rigo, F., Hung, G., Schneider, E., et al. (2016). Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet 388, 3017–3026.

59. Norton, L.J., Nielsen, L., Alfano, L., Berry, K., Church, K., et al. (2017). Results from a phase 1b clinical trial of nusinersen in children with spinal muscular atrophy. Neurology 89, 2286–2293.

60. Azzouz, M. (2006). Gene Therapy for ALS: progress and prospects. Biochim. Biophys. Acta. 1762, 1122–1127.

61. Choudhury, S.R., Hudry, E., Maguire, C.A., Sena-Esteves, M., Breakefield, X.O., and Grandi, P. (2017). Viral vectors for therapy of neurologic diseases. Neuropharmacology 120, 63–80.

62. Suzuki, M., and Svendsen, C.N. (2016). Ex Vivo Gene Therapy Using Human Mesenchymal Stem Cells to Deliver Growth Factors in the Skeletal Muscle of a Familial ALS Rat Model. Methods Mol. Biol. 1382, 325–336.

63. Ly, C.V., and Miller, T.M. (2018). Emerging antisense oligonucleotide and viral therapies for amyotrophic lateral sclerosis. Curr. Opin. Neurol. 31, 648–654.

64. Schoch, K.M., and Miller, T.M. (2017). Antisense Oligonucleotides: Translation from Mouse Models to Human Neurodegenerative Diseases. Neuron 94, 1056–1070.
83. Imbert, G., Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J.M., Weber, C., Pulst, S.M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.N., Lopes-Cendes, Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Lee, T., Li, Y.R., Ingre, C., Weber, M., Grehl, T., Gredal, O., de Carvalho, M., Meyer, Yu, Z., Zhu, Y., Chen-Plotkin, A.S., Clay-Falcone, D., McCluskey, L., Elman, L., Van Damme, P., Veldink, J.H., van Blitterswijk, M., Corveleyn, A., van Vught, P.W., Nonhoff, U., Ralser, M., Welzel, F., Piccini, I., Balzereit, D., Yaspo, M.L., Lehrach, H., Hart, M.P., and Gitler, A.D. (2012). ALS-associated ataxin 2 polyQ expansions enhance toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron 76. Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Genge, A., Glass, J., Ladja, S., Ludolph, A.L., et al. (2020). Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N. Engl. J. Med. 383, 109–119.

84. Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J.M., Weber, C., Pulst, S.M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.N., Lopes-Cendes, Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Lee, T., Li, Y.R., Ingre, C., Weber, M., Grehl, T., Gredal, O., de Carvalho, M., Meyer, Yu, Z., Zhu, Y., Chen-Plotkin, A.S., Clay-Falcone, D., McCluskey, L., Elman, L., Van Damme, P., Veldink, J.H., van Blitterswijk, M., Corveleyn, A., van Vught, P.W., Nonhoff, U., Ralser, M., Welzel, F., Piccini, I., Balzereit, D., Yaspo, M.L., Lehrach, H., Hart, M.P., and Gitler, A.D. (2012). ALS-associated ataxin 2 polyQ expansions enhance toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron 76. Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Genge, A., Glass, J., Ladja, S., Ludolph, A.L., et al. (2020). Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N. Engl. J. Med. 383, 109–119.

85. Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J.M., Weber, C., Pulst, S.M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.N., Lopes-Cendes, Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Lee, T., Li, Y.R., Ingre, C., Weber, M., Grehl, T., Gredal, O., de Carvalho, M., Meyer, Yu, Z., Zhu, Y., Chen-Plotkin, A.S., Clay-Falcone, D., McCluskey, L., Elman, L., Van Damme, P., Veldink, J.H., van Blitterswijk, M., Corveleyn, A., van Vught, P.W., Nonhoff, U., Ralser, M., Welzel, F., Piccini, I., Balzereit, D., Yaspo, M.L., Lehrach, H., Hart, M.P., and Gitler, A.D. (2012). ALS-associated ataxin 2 polyQ expansions enhance toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. Neuron 76. Miller, T., Cudkowicz, M., Shaw, P.J., Andersen, P.M., Atassi, N., Bucelli, R.C., Genge, A., Glass, J., Ladja, S., Ludolph, A.L., et al. (2020). Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N. Engl. J. Med. 383, 109–119.
Keeler, A.M., Zieger, M., Semple, C., Pucci, L., Veinbuchs, A., Brown, R.H., Jr., Hinderer, C., Katz, N., Buza, E.L., Dyer, C., Goode, T., Bell, P., Richman, L.K., and Mueller, C., Berry, J.D., McKenna-Yasek, D.M., Gernoux, G., Owegi, M.A., Pothier, Gaj, T., Ojala, D.S., Ekman, F.K., Byrne, L.C., Limsirichai, P., and Schaffer, D.V. (2020). Adeno-Associated Virus-Expressing Human SMN. Hum. Gene Ther. 115, Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., and Liu, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149–157.

Foust, K.D., Salazar, D.L., Likhite, S., Ferriauolo, L., Ditsworth, D., Ilieva, H., Meyer, K., Schmelzer, L., Braun, L., Cleveland, D.W., and Kaspar, B.K. (2013). Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS. Mol. Ther. 21, 2148–2159.

Foust, K.D., Nurre, E., Montgomery, C.L., Hernandez, A., Chan, C.M., and Kaspar, B.K. (2009). Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat. Biotechnol. 27, 59–65.

Thomsen, G.M., Gowig, G., Latter, J., Chen, M., Vit, I.P., Staggenbore, K., Avalos, P., Alkalsasli, M., Ferriauolo, L., Likhite, S., et al. (2014). Delayed disease onset and extended survival in the SOD1G93A rat model of amyotrophic lateral sclerosis after suppression of mutant SOD1 in the motor cortex. J. Neurosci. 34, 15587–15600.

Stoica, L., Teodea, S.H., Cabrera, G.T., Salameh, I.S., ElMallah, M.K., Mueller, C., Brown, R.H., Jr., and Sana, E.H. (2016). Adeno-associated virus–delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model. Ann. Neurol. 79, 687–700.

Borel, F., Gernoux, G., Cardozo, B., Metterville, J.P., Tero Cabrera, G.C., Song, L., Su, Q., Gao, G.P., ElMallah, M.K., Brown, R.H., Jr., and Mueller, C. (2016). Therapeutic rAAVrH10 Mediated Silencing in Adult SOD1(G93A) Mice and Nonhuman Primates. Hum. Gene Ther. 27, 19–31.

Borel, F., Gernoux, G., Sun, H., Stock, R., Blackwood, M., Brown, R.H., Jr., and Mueller, C. (2018). Safe and effective superoxide dismutase 1 silencing using artificial micro-RNA in macaques. Sci. Transl. Med. 10, eaau6414.

Mueller, C., Berry, J.D., McKenna-Yasek, D.M., Gernoux, G., Owegi, M.A., Potheir, L.M., Douthwright, C.L., Gelevski, D., Luppino, S.D., Blackwood, M., et al. (2020). SOD1 Suppression with Adeno-Associated Virus and MicroRNA in Familial ALS. N. Engl. J. Med. 383, 151–158.

Hinderer, C., Katz, N., Buza, E.L., Dyer, C., Goode, T., Bell, P., Richman, L.K., and Wilson, J.M. (2018). Severe Toxicity in Nonhuman Primates and Pigs Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN. Hum. Gene Ther. 29, 287–298.

Hordeaux, F., Buza, E.L., Dyer, C., Goode, T., Mitchell, T.W., Richman, L., Denton, N., Hinderer, C., Katz, N., Schmid, R., et al. (2020). Adeno-Associated Virus-Induced Dorsal Root Ganglion Pathology. Hum. Gene Ther. 31, 808–818.

Keeler, A.M., Zieger, M., Semple, C., Pucci, L., Veinbuchs, A., Brown, R.H., Jr., Mueller, C., and ElMallah, M.K. (2019). Intraluminal and Intrapleural AAV Gene Therapy Prolongs Survival in a SOD1 G93A Mouse Model. Mol. Ther. Methods & Diagnostics 5, 15587–15600.

Keiser, M.S., Boudreau, R.L., and Davidson, B.L. (2014). Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: implications for spinocerebellar ataxia type 1 therapy. Mol. Ther. 22, 588–595.

Keiser, M.S., Montey, A.M., Corbau, R., Gonzalez-Alegre, P., and Davidson, B.L. (2016). RNA interference prevents and reverses phenotypes induced by mutant human ataxin-1. Ann. Neurol. 80, 754–765.

Nóbrega, C., Nascimento-Ferreira, I., Onofre, I., Albuquerque, D., Hirai, H., Déglon, N., and de Almeida, L.P. (2013). Silencing mutant ataxin-3 rescues motor deficits and neuropathology in Machado-Joseph disease transgenic mice. PLoS ONE 8, e52396.

Nóbrega, C., Nascimento-Ferreira, I., Onofre, I., Albuquerque, D., Déglon, N., and de Almeida, L.P. (2014). RNA interference mitigates motor and neuropathological deficits in a cerebellar mouse model of Machado-Joseph disease. PLoS ONE 9, e100066.

Ramachandran, P.S., Boudreau, R.L., Schafer, K.A., La Spada, A.R., and Davidson, B.L. (2014). Nonallelic specific silencing of ataxin-7 improves disease phenotypes in a mouse model of SCA7. Mol. Ther. 22, 1635–1642.

Ouyang, S., Xie, Y., Xiong, Z., Yang, Y., Xian, Y., Ou, Z., Song, B., Chen, Y., Xie, Y., Li, H., and Sun, X. (2018). CRISPR/Cas9-Targeted Deletion of Polyglutamine in Spinocerebellar Ataxia Type 3 Derived Induced Pluripotent Stem Cells. Stem Cells Dev 27, 756–770.

Buysen, R.A.M., Toonen, I.J.A., Gardiner, S.L., and van Room-Mom, W.M.C. (2019). Genetics, Mechanisms, and Therapeutic Progress in Polyglutamine Spinocerebellar Ataxias. Nature Neurotherapeutics 16, 263–286.

Petrov, D., Mansfield, C., Monosy, A., and Hermine, O. (2017). ALS Clinical Trials Review: 20 Years of Failure. Are We Any Closer to Registering a New Treatment? Front. Aging Neurosci. 9, 68.

Battus, R.T., and Johnson, E.M., Jr. (2017). Clinical tests of neurotoxic factors for human neurodegenerative diseases, part 1: Where have we been and what have we learned? Neurobiol. Dis. 97, 156–168.

Alldred, D., Comley, L., Niederwirtz, S., Nizzardo, M., Simone, C., Benitez, J.A., Tao, M., Corti, S., and Hedlund, E. (2016). Differential neuronal vulnerability identifies IGF-2 as a protective factor in ALS. Sci. Rep. 6, 25960.

Lin, H., Hu, J., Duan, W., Liu, Y., Tan, G., Li, Z., Liu, Y., Deng, B., Song, X., Wang, W., et al. (2018). Intramuscular Delivery of scAAV9-hsFGF1 Prolongs Survival in the hSOD1 G93A ALS Mouse Model via Upregulation of D-Amino Acid Oxidase. Mol. Neurobiol 55, 682–695.
