The clinical trial landscape in amyotrophic lateral sclerosis—Past, present, and future

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Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease marked by progressive loss of muscle function. It is the most common adult-onset form of motor neuron disease, affecting about 16,000 people in the United States alone. The average survival is about 3 years. Only two interventional drugs, the antiglutamatergic small-molecule riluzole and the more recent antioxidant edaravone, have been approved for the treatment of ALS to date. Therapeutic strategies under investigation in clinical trials cover a range of different modalities and targets, and more than 70 different drugs have been tested in the clinic to date. Here, we summarize and classify interventional therapeutic strategies based on their molecular targets and phenotypic effects. We also discuss possible reasons for the failure of clinical trials in ALS and highlight emerging preclinical strategies that could provide a breakthrough in the battle against this relentless disease.

Keywords
ALS preclinical strategies, amyotrophic lateral sclerosis, clinical trials, edaravone, riluzole
1 | INTRODUCTION

It was 1938 when the Yankees’ first baseman Lou Gehrig, arguably one of the greatest baseball players of all time, showed signs of a dip in form. Or rather, it was more than that. In the words of the sports journalist James Kahn: “I think there is something wrong with him. Physically wrong, I mean. I don’t know what it is, but I am satisfied that it goes far beyond his ball-playing. I have seen ballplayers ‘go’ overnight, as Gehrig seems to have done. But they were simply washed up as ballplayers. It’s something deeper than that in this case, though. I have watched him very closely and this is what I have seen: I have seen him time a ball perfectly, swing on it as hard as he can, meet it squarely—and drive a soft, looping fly over the infield. In other words, for some reason that I do not know, his old power isn’t there... He is meeting the ball, time after time, and it isn’t going anywhere.” Shortly after, he was diagnosed with amyotrophic lateral sclerosis (ALS).

ALS is a disorder characterized by the death of motor neurons in the motor cortex and spinal cord as well as spinal interneurons. The loss of motor neurons, which control voluntary movement, in turn, causes progressive muscle weakness. Over time, patients lose the ability to walk, speak, and swallow.

ALS is the most common adult-onset form of motor neuron disease, with a prevalence of around five to six in 100,000 people in the United States and Europe.\(^2\,^3\) Since the first disease-associated gene, superoxide dismutase 1 (SOD1), was identified in 1993, more than 30 genes have been genetically linked to familial (5%-10% of cases) and sporadic forms (ie, without ascertainable family history; 90%-95% of cases) of the disease.\(^4\,^5\) By far the most common mutation identified to date is a G4C2 hexanucleotide repeat expansion in the gene C9ORF72, which is linked to 30% to 50% of familial and 5% to 7% of sporadic cases.\(^5\) Histopathologically, ALS is characterized by inclusions of mislocalized, aggregated proteins. In around 97% of ALS cases—excluding those caused by mutations in the genes SOD1 and FUS—the principal constituent of these inclusions is a RNA-binding protein (RBP) with a prion-like domain (PrLD), TDP-43.\(^7\,^8\,^9\)

Most ALS patients die within 3 to 5 years after diagnosis,\(^4\) usually as a result of respiratory failure. Sadly, Lou Gehrig was no exception: in 1941, he succumbed to the disease aged 37. Today, almost 80 years later, ALS remains almost as intractable as it was back then. Scores of clinical trials have only yielded two interventional treatments: Riluzole, approved in 1995; and edaravone, approved by the FDA in 2017 (Table 1). In this review, we summarize the state of play of interventional drugs currently or formerly in the clinic for the treatment of ALS. We also highlight exciting novel therapeutic avenues built on our rapidly expanding understanding of the pathogenesis of this devastating disease.

2 | APPROVED INTERVENTIONAL DRUGS

2.1 | Riluzole and other antiexcitatory therapeutic strategies

In the late-1980s and early-1990s, studies first implicated impaired glutamate homeostasis in ALS.\(^48,102,103\) Today, excitotoxicity—excessive, deleterious glutamate signaling—is believed to underlie or contribute to motor neuron degeneration in ALS.\(^104\) Consistent with this hypothesis, transcranial magnetic stimulation experiments have revealed a cortical hyperexcitability phenotype in ALS patients.\(^105,106\) Consequently, many therapeutic efforts have focused on stemming excessive excitatory signaling (Figure 1). To date, however, riluzole remains the only antiexcitatory therapy that has proven effective. It was approved by the FDA in 1995 based on positive survival data in two clinical trials (chemical structures of riluzole and other antiexcitatory small molecules, see Figure 2).\(^34,35\) A more recent meta-analysis combining results of three trials concluded that riluzole, at a dose of 100 mg per day, improves the probability of surviving 1 year by about 10% and extends median survival by about 3 months.\(^36\)

Limited bioavailability in the central nervous system due to efflux via the P-glycoprotein transporter may limit the efficacy of riluzole.\(^107\) A recent study by Abrahao et al\(^108\) demonstrated that the blood-brain barrier (BBB) can be
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<tr>
<td>SM</td>
<td>EH301</td>
<td>Nicotinamide riboside, boosts levels of NAD+</td>
<td>Ph 2</td>
<td>ALSFRS-R</td>
<td>99</td>
</tr>
<tr>
<td>ASO</td>
<td>BIIB078</td>
<td>ASO targeting C90RF72</td>
<td>Ph 1</td>
<td>Safety, tolerability</td>
<td>100</td>
</tr>
<tr>
<td>SM</td>
<td>MD1003</td>
<td>High dose biotin, supports remyelination</td>
<td>Ph 2</td>
<td>Safety, tolerability, hematology panel</td>
<td>101</td>
</tr>
</tbody>
</table>

Note: The two drugs in bold indicate that they are the approved drugs.

Abbreviations: AALSRS, Appel amyotrophic lateral sclerosis rating scale; ACTH, adrenocorticotropic hormone; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASO, antisense oligonucleotide; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; CAFS, combined assessment of function and survival; CNTF, ciliary neurotropic factor; CSF, cerebrospinal fluid; CSF-1R, colony stimulating factor 1 receptor; DLK, dual leucine zipper kinase; EAAT2, excitatory aminoacid transporter 2; EPO, erythropoietin; FVC, forced vital capacity; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; IL-6R, interleukin-6 receptor; JNK, c-Jun N-terminal kinase; MAALS, mean Appel ALS score; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MMT, manual muscle testing; MAO-B, monoamine oxidase-B; mTOR, mammalian target of rapamycin; MVIC, maximum voluntary isometric contraction; NF-κB, nuclear factor κB; NMDA, N-methyl-D-aspartate; NMJ, neuromuscular junction; PDE, phosphodiesterase; PFT, pulmonary functional testing; PPAR-γ, peroxisome proliferator-activated receptor γ; RIPK1, receptor-interacting serine/threonine-protein kinase 1; S1PR1, sphingosine-1-phosphate receptor 1; SICI, short-interval intracortical inhibition; SVC, supine vital capacity; TGF-α, tumor necrosis factor α; VC, vital capacity; VEGF, vascular endothelial growth factor; ZFP, zinc finger protein.

*Survival defined as time to death, long-term ventilation, or tracheostomy.
safely and reversibly opened using transcranial magnetic resonance-guided focused ultrasound (MRgFUS). Thus, in the future, MRgFUS may be coupled with therapeutics with limited BBB permeability such as riluzole to improve therapeutic efficacy.

The exact mechanism of action (MOA) of riluzole is not entirely understood, as a multitude of studies, many of them carried out in vitro and at nonphysiological concentrations, have identified various antiexcitatory features of this drug (for a detailed review of riluzole’s MOA, see Reference 109). At concentrations approximating those reached in the human brain, riluzole inhibits fast and persistent sodium currents, inhibits presynaptic glutamate release and may also increase glutamate reuptake.

Despite intense efforts to identify other therapeutic interventions targeting excitatory signaling, riluzole remains the only approved drug in this class. Other therapies that modulate concentrations of glutamate at synapses were not effective. For example, removing excess glutamate from the synaptic cleft by increasing expression of the glutamate transporter EAAT2/SLC1A2 with ceftriaxone did not improve functional decline or survival in a phase 3 trial. Another trial examining the effects of nonsteroidal anti-inflammatory celecoxib also disappointed. Celecoxib is an inhibitor of cyclooxygenase-2, an enzyme in the synthesis pathway of prostaglandin E2, which stimulates the release of glutamate from astrocytes.

Other potential therapeutic approaches that directly target glutamate receptors also fared poorly (Figure 1). Memantine, an N-methyl-D-aspartate receptor antagonist used to improve cognitive function in Alzheimer’s disease, failed to slow the rate of functional decline in the ALS functional rating scale revised (ALSFRS-R), a compound score comprising...
12 functional categories including speech, handwriting, swallowing, salivation, walking, stair climbing, and respiration.\textsuperscript{111} An $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, talampanel, showed a potential effect on muscle strength and function in a small phase 2 study, but failed a subsequent larger, unpublished trial.\textsuperscript{40} Perampanel, another AMPA receptor antagonist with a longer half-life than talampanel, demonstrated preservation of motor neurons and motor function in an ALS mouse model,\textsuperscript{41} and is under investigation in a phase 2 study.\textsuperscript{42} Compounds that modulate voltage-gated ion channels have also undergone clinical testing. For example, the potassium channel opener retigabine, an approved antiepileptic drug recently withdrawn from the market, is being tested in the clinic based on data showing improved neuronal excitability and survival of ALS patient-derived induced pluripotent stem cell (iPSC)-derived motor neurons harboring the SOD1 A4V mutation.\textsuperscript{37} The sodium channel blockers lamotrigine and mexiletine failed to slow down the rate of functional decline but may prove beneficial as a symptomatic treatment for muscle cramps.\textsuperscript{43,44} The $\gamma$-aminobutyric acid analogue gabapentin is thought to suppress excitotoxicity by inhibiting branched-chain amino-transferase, an enzyme in the glutamate synthesis pathway, as well as by inhibiting L-type calcium channels.\textsuperscript{112} However, gabapentin failed to slow the decline of arm muscle strength in a phase 3 trial.\textsuperscript{49} Another L-type calcium channel blocker, nimodipine, did not progress to a larger study after a trial of 87 patients suggested that it was ineffective in slowing down the decline in forced vital capacity (FVC), a measure of respiratory function.\textsuperscript{48}

\subsection*{2.2 Edaravone and other antioxidant therapeutic strategies}

The only other current ALS therapy, edaravone, was approved based on a significant effect on the rate of decline in the ALSFRS-R in a subset of Japanese ALS patients.\textsuperscript{50} Furthermore, a recently published retrospective study of a small number of patients suggested that edaravone prolongs tracheostomy-free survival in human patients.\textsuperscript{113}
However, the small number of subjects and imbalances in the number of patients with the more aggressive bulbar-onset form of the disease between the edaravone and control groups could have skewed the survival outcome numbers. A 5-year prospective observational study (SUNRISE) investigating the effects of edaravone on survival is ongoing.114

The precise MOA of edaravone is unknown. It is a radical oxygen scavenger shown to protect both neurons and glia from oxidative stress.115 Oxygen radicals are thought to contribute to the degeneration of motor neurons in ALS, and markers of oxidative stress are upregulated in patient tissue.116 Edaravone may reduce oxidative stress in ALS and other models of disease.117-119 In cells exposed to oxidative stress, edaravone increases the expression of peroxiredoxin 2, which reduces hydrogen peroxide.120 Furthermore, edaravone increases expression of nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor that regulates the expression of antioxidant proteins.121 Overexpression of NRF2 in astrocytes improved survival in the SOD1-G93A mouse model of ALS.122

However, with the exception of edaravone, antioxidant therapeutic strategies have thus far failed (chemical structures of edaravone and other antioxidants, see Figure 3). The radical oxygen scavengers N-acetylcysteine, vitamin E, and coenzyme Q10 did not show significant beneficial effects in their respective clinical studies.51-54 One reason for the failure of these compounds might be poor or no BBB permeability. Monoamine oxidase-B (MAO-B) inhibitors have also been studied as potential interventional treatments for ALS. MAO-B inhibitors such as selegiline and rasagiline are approved for the management of Parkinson’s disease and work by suppressing the breakdown of dopamine. A byproduct of this enzymatic reaction is hydrogen peroxide. Thus, inhibition of MAO-B in ALS could decrease the oxidative stress burden on motor neurons. Furthermore, in vivo studies demonstrated that selegiline displayed antioxidant properties in the striatum of aged rats at high concentrations by increasing the activity of SOD and catalase.123,124 Selegiline failed to decelerate functional decline measured using the Appel ALS rating scale,125 but was administered in doses far below those used to elicit its antioxidative effects.55 More recently, the second-generation MAO-B inhibitor rasagiline also showed no beneficial effects on survival and disease progression in two studies.56,57 In a different strategy to combat oxidative stress in ALS, Epi-589 purportedly increases levels of the antioxidant glutathione and is now being trialed in a phase 2 study. However, to date, there have been no publications detailing its MOA or its effects in animal models of ALS.126

3 | CLINICAL CANDIDATES

3.1 | Anti-inflammatory/immune-modulatory drugs

Motor neuron death and skeletal muscle denervation are the defining, though not the only, pathological features of ALS. The factors that drive this selective degeneration in ALS patients are still poorly understood and may vary
between cases. Many lines of evidence suggest that cell autonomous as well as noncell autonomous mechanisms can drive the degeneration of motor neurons. In recent years, neuroinflammation has emerged as a process closely linked to neurodegeneration. Aberrant activation of astrocytes and microglia, as well as infiltration of CD4+ and CD8+ T lymphocytes, have been demonstrated in ALS patient tissue. Furthermore, coculture systems using astrocytes, microglia, or T cells of mouse primary or human iPSC origin have shown that these cells exert direct cytotoxic effects on motor neurons. These cytotoxic effects might at least in part be mediated through proinflammatory cytokines or other factors released as a result of inflammatory signaling.

Various classes of anti-inflammatory drugs are aimed at suppressing the production of proinflammatory cyto-
kines and chemokines as well as the proliferation and polarization of glial cells, through mechanisms that are not always completely understood (Figures 4 and 5). Several of these drugs have failed to prove efficacious in ALS clinical trials. Two approved drugs with anti-inflammatory properties, the peroxisome proliferator-activated receptor γ agonist pioglitazone, used for the treatment of type 2 diabetes, and the antibiotic minocycline, protected neurons from degeneration and improved survival in SOD1 mouse models of ALS. However, both failed in clinical trials, with minocycline proving harmful to patients as evidenced by a faster rate of decline in the ALSFRS-R compared with placebo-treated patients. Other drugs that target glial responses through inhibition of rho kinase (ROCK; fasudil), NF-xB, and mitogen-activated protein kinase signaling (CC100) or phosphodiesterases (PDEs; ibudilast) are being trialed in ALS patients. Ibudilast recently garnered positive feedback from the FDA to commence a phase 3 study. Previously, treatment with pentoxifylline, another nonselective PDE inhibitor, led to a worse outcome in 18-month survival in a phase 3 trial. Thalidomide, which reduces messenger RNA (mRNA) levels of the inflammatory cytokine tumor necrosis factor α, was not effective in a small, open-label study compared with historical data, and may have had adverse effects on disease outcome. Ono-2506 was developed as a modulator of astrocyte activity and was shown to suppress the expression of S110b and GFAP. A phase 2 study failed to show a therapeutic benefit in pulmonary function. The most unconventional anti-inflammatory therapeutic agent undergoing clinical testing in a phase 2 trial for ALS is perhaps RNS60. It consists of charge-stabilized, oxygen-filled nanobubbles in a saline solution. RNS60 has been shown to exert its anti-inflammatory effects by suppressing activation of the NF-xB pathway.

The receptor-interacting serine/threonine-protein kinase 1 (RIPK1) kinase inhibitor DNL747 was recently shown to be safe in a phase 1 study. It is thought to work by suppressing RIPK1-dependent production of proinflammatory cytokines in microglia. It has also been shown to prevent RIPK1-dependent death of oligodendrocytes in two ALS mouse models. Masitinib, a CSF1R/c-kit inhibitor that reduces proliferation of inflammatory microglia in the SOD1-G93A rat model of ALS, met its primary endpoint, a change in the ALSFRS-R score in a phase 2/3 study. However, the European Medicine Agency’s Committee for Medicinal Products for Human Use subsequently recommended refusal of marketing authorization due to concerns about the reliability of the results and its safety. Other anti-inflammatory treatment strategies in ALS focus on T lymphocytes (Figure 4). Fingolimod, an approved drug for the treatment of relapsing-remitting multiple sclerosis (MS), binds to and causes the internalization of the sphingosine-1-phosphate receptor 1. As a result, T cells become trapped in lymph nodes, reducing the number of circulating cells. Another approved MS drug, glatiramer acetate, which is thought to exert its effects by modulating the proliferation, migration, and balance of T helper cells failed to slow the rate of decline in the ALSFRS-R score in a phase 2 trial. More recently, the MIROCALS study was launched to investigate the efficaciousness of interleukin 2 (IL-2), which promotes the differentiation of regulatory T cells.

The aforementioned drugs seek to modulate proinflammatory activation and cytokine release by glia, T cells, or macrophages. By contrast, other interventional therapeutic strategies are aimed at suppressing the downstream effects of proinflammatory cytokines. Tocilizumab is an antibody targeting the interleukin-6 receptor (IL-6R) and is approved for the treatment of several forms of arthritis. A phase 2 trial was recently completed, but no results have been published as of the date of this publication. Another approved rheumatoid arthritis drug, the IL-1 receptor antagonist anakinra, was deemed safe in a single-arm pilot study in ALS patients.
Anti-inflammatory clinical treatment strategies for ALS. Astrocytes, microglia, and T cells promote inflammation in ALS, causing immune cell infiltration and the production of inflammatory cytokines. Anti-inflammatory strategies suppress proinflammatory signaling. Ono-2506 suppresses astrogliosis. Various small molecules are under investigation that suppress microglial activation including masitinib (inhibitor of CSF-1R), fasudil (ROCK1 inhibitor), DNL747 (RIPK1 inhibitor), and the PDE inhibitors pentoxyfilline and ibudilast. Several therapeutics are aimed at suppressing NF-κB signaling through various and sometimes unknown mechanisms including CC100, NP001, pioglitazone (an approved drug for the treatment of diabetes), and RNS60. Minocycline suppresses microglial cytokine production through unknown mechanisms. Thalidomide decreases levels of TNF-α. Other therapies are aimed at suppressing T cell response including antibodies that target IL-1R (anakinra) and IL-6R (tocilizumab) and thus suppress signaling through these receptors. Glatiramer acetate, approved for the treatment of multiple sclerosis, is a mixture of peptides resembling myelin basic protein and modulates the reactivity of T cells. IL-2 peptide activates regulatory T cells that modulate T cell response. ALS, amyotrophic lateral sclerosis; IL, interleukin; IL-1R, interleukin receptor 1; MHC II, major histocompatibility complex class II; NF-κB, nuclear factor κB; RIPK1, receptor-interacting serine/threonine-protein kinase 1; PDE, phosphodiesterase; TNF-α, tumor necrosis factor α [Color figure can be viewed at wileyonlinelibrary.com]
3.2 | Troponin binders

Dysfunction and degeneration of spinal cord motor neurons underlie the progressive muscle atrophy in ALS, as muscle fibers do not receive sufficient electrical input. Thus, increasing muscle contraction in response to a given input signal could slow down the deterioration of muscle strength in ALS. With this hypothesis in mind, the first-generation fast skeletal muscle activator tirasemtiv (CK-2017357) was developed. Tirasemtiv binds to fast skeletal muscle troponin and increases its affinity for calcium (Figures 6 and 7A). While a phase 2 study failed to show an effect of tirasemtiv on the rate of functional decline in the ALSFRS-R, muscle strength and slow vital capacity (SVC) declined more slowly. However, these results could not be confirmed in a subsequent phase 3 study, possibly due to a high rate of dropouts in the high dose cohort as a result of poor tolerability. A second-generation fast skeletal muscle troponin activator, CK-2127107 (Figure 7A), is thought to be more potent and better tolerated and is under investigation in a phase 2 trial. Oral levosimendan, a troponin binder and calcium sensitizer in fast and slow skeletal muscle fibers, recently completed a phase 2 trial. The primary outcome—SVC when sitting—did not show significant differentiation compared with the placebo. The secondary efficacy variable, supine SVC, improved in the levosimendan groups while it deteriorated in the placebo group. Despite missing its endpoints, a phase 3 trial was launched in 2018 to investigate positive trends in supine SVC observed in the phase 2 study.

3.3 | SOD1-targeting therapeutic approaches

SOD1 is a cytoplasmic copper/zinc-binding enzyme that converts superoxide radicals (O$_2^-$) into oxygen (O$_2$) and hydrogen peroxide (H$_2$O$_2$). Mutations in SOD1 were the first identified genetic cause of ALS, accounting for 5% to 10% of familial cases. Therapeutic strategies aimed at mutant SOD1 present a targeted therapeutic approach tailored to a well-defined patient population (chemical structures of SOD1-targeting small molecules, see Figure 7B). Several ALS-associated mutations confer poor binding affinity for copper and zinc ions to SOD1, which may affect SOD1 stability, aggregation behavior, and binding to other proteins. Thus, delivery of zinc or copper to mutant SOD1 may be beneficial in SOD1-ALS. Indeed, treatment of mutant SOD1 mouse models with Cu(II)-ATSM has been shown to improve locomotor function and survival and preserve motor neurons, despite increased levels of total SOD1 in the spinal cord. A phase 1 dose escalation and pharmacokinetic study began in 2016, and is open to both familial ALS patients with SOD1 mutations as well as sporadic patients.
Several lines of evidence suggest that mutant SOD1 exerts its detrimental effects through a gain-of-function rather than a loss-of-function mechanism. While mutant SOD1-expressing mice develop ALS phenotypes, SOD1 knockout mice do not. Furthermore, while some ALS-associated SOD1 mutations cause a decrease in dismutase activity, others have little effect or even increase activity. Perhaps most tellingly, knocking out murine SOD1 in an ALS mouse model expressing the enzymatically inactive G85R mutant has no effect on survival. Thus, rather than altering the metal-binding status of SOD1, other interventional strategies are aimed at reducing total SOD1 protein levels. Pyrimethamine, a drug used to treat toxoplasmosis and human immunodeficiency virus-associated pneumonia, was shown to reduce SOD1 levels in cerebrospinal fluid (CSF) of ALS patients with SOD1 mutations by ~13.5%. Antisense oligonucleotides (ASOs) targeting SOD1 mRNA were beneficial in the SOD1-G93A rat model of ALS. A first study in man demonstrated that intrathecal delivery of a SOD1 ASO was safe but did not decrease SOD1 protein levels in the CSF at the low doses used. A phase 1 trial of the second-generation ASO BIIB067 (IONIS-SOD1Rx) commenced in 2016. While detailed results of the study were not publicly available at the time of the submission of this article, an interim analysis showed that treatment with BIIB067 over a 3-month period led to lower SOD1 levels in the CSF and a trend toward a slower rate of decline in the ALSFRS-R. In addition to ASOs, SOD1 mRNA can also be targeted by RNA interference approaches. A single administration of an adeno-associated virus gene therapy vector (VY-SOD101) has been shown to reduce levels of SOD1 mRNA in spinal cord motor neurons by greater than 70% in non-human primates. However, this therapeutic strategy has not yet entered the clinic.

### 3.4 Autophagy enhancers

Autophagy is the process by which long-lived, misfolded and aggregated proteins, as well as organelles, and pathogens are cleared from cells under homeostatic and pathological conditions. Several lines of evidence indicate that impaired autophagy is fundamentally implicated in the pathogenesis of ALS. As in other age-related neurodegenerative diseases, a
major histopathological hallmark of ALS is the accumulation of misfolded proteins in insoluble aggregates, suggesting that autophagic clearance of these misfolded proteins is insufficient. In 97% of ALS cases, the major protein found in these inclusions of aggregated protein is a RBP with a PrLD, TDP-43. Second, mutations in a remarkable number of autophagy genes cause ALS, including C9ORF72, UBQLN2, TBK1, VCP, and OPTN. Last, post mortem tissues of human patients and ALS mouse models display markers of impaired autophagy. Thus, therapeutic strategies aimed at enhancing autophagy could be a viable therapeutic avenue for the treatment of ALS (chemical structures of small-molecule autophagy enhancers, see Figure 7C). Lithium is an inhibitor of inositol monophosphatase, leading to decreased levels of inositol 1,4,5-trisphosphate, a negative regulator of mammalian target of rapamycin (mTOR)-independent autophagy. However, lithium did not improve survival in phase 2 and 3 studies. A phase 2 trial investigating the effects of rapamycin is ongoing. Rapamycin is an inhibitor of the mammalian target of rapamycin complex 1, a complex containing the kinase mTOR that serves as a master regulator of cell growth, survival, metabolism, autophagy, and protein synthesis. mTOR inhibits autophagy by phosphorylating Unc-51 like autophagy activating kinase 1, which regulates the initiation of autophagy. However, mTOR inhibitors do not robustly stimulate autophagy in neurons, and thus in the brain are only likely to stimulate autophagy in neighboring glia. It is unclear whether stimulating glial autophagy alone will be neuroprotective in ALS. Novel compounds that stimulate neuronal autophagy of TDP-43 are beginning to emerge. A clinical study examining tamoxifen, which increases levels of beclin-1, a central protein in autophagosome formation and maturation, was completed with no results yet published.

3.5 | Neurotrophic factors

Growth factors have long been studied as a therapeutic avenue for the treatment of neurodegenerative diseases including ALS. Neurotrophic factors promote prosurvival signaling, axon outgrowth, synapse formation, and metabolic function, and confer protection against toxic or oxidative insults and injury. However, clinical studies thus far
have not yielded any viable therapies. Subcutaneously administered brain-derived neurotrophic factor, ciliary neurotrophic factor (CNTF), and insulin-like growth factor 1 were not shown to be beneficial in large-scale studies.\textsuperscript{65–67,70,72,73} This could be due to low brain exposure resulting from insufficient dosing, short protein half-life, or lack of BBB penetration.\textsuperscript{165} Furthermore, intravenous injection of the hematopoietic growth factor erythropoietin did not improve survival in a phase 3 study.\textsuperscript{69} Intravenously administered GM604, a peptide sequence identical to an active site of motoneurotrophic factor, was recently found safe,\textsuperscript{71} as was intrathecal injection of human recombinant hepatocyte growth factor.\textsuperscript{77} Xaliproden is a 5-HT\textsubscript{1A} agonist and the first discovered nonpeptide neurotrophic factor enhancer (Figure 8A). It prolongs neuronal survival in vitro and in vivo and enhances the secretion of neurotrophic factors. In two phase 3 trials to assess xaliproden as a monotherapy and in combination with riluzole, primary outcome measures (survival and vital capacity <50%) did not justify clinical approval.\textsuperscript{68}

Given issues of short half-lives or poor BBB penetration, producing neurotrophic factors at the site of motor neuron degeneration may result in better outcomes. This could be achieved by intramuscular or intracerebroventricular administration of plasmids encoding neurotrophic factors or transcription factors that regulate the expression of neurotrophic factors, and trials investigating this approach have been initiated.\textsuperscript{74–76}

3.6 Modulators of mitochondrial function

Mitochondrial dysfunction is a central feature of ALS and manifests in a variety of ways. Disturbances in energy metabolism, impairment of mitochondrial fission and transport and abnormal mitochondrial morphology have been described in various cell and animal models (for a thorough review describing the role of mitochondria in ALS, see Reference \textsuperscript{167}). Thus, therapeutic treatment strategies have been aimed at rescuing mitochondrial function. Dexpramipexole and olesoxime are inhibitors of the mitochondrial permeability transition pore, a nonselective mitochondrial inner membrane channel that
opens in response to insults such as excitotoxicity, excess oxidative stress, or decreased adenosine triphosphate (ATP) levels (Figure 8B). Opening of the transition pore causes the breakdown of the electrochemical proton gradient, increases the production of reactive oxygen species and disrupts ATP production. Both drugs failed to improve survival in their respective trials. Creatine supplementation to boost the intracellular pool of phosphocreatine, which acts as an energy buffer for regeneration of ATP, was also not found to be efficacious in several clinical studies (Figure 8B). A small study investigating the effects of acetyl-L-carnitine, which improves transportation of fatty acids across mitochondrial membranes via the carnitine shuttle, found a significant effect on the proportion of patients becoming non-self-sufficient—an unusual primary endpoint, but thus far no larger follow-up study has been reported.

4 | STEM CELL APPROACHES

Another way of improving trophic support locally in affected tissues is stem cell transplantation. Stem cells can differentiate into support cells such as astrocytes, oligodendrocytes, or microglia, which may benefit degenerating motor neurons by producing growth factors and anti-inflammatory cytokines, providing nutrients and buffering excessive glutamate. A variety of stem cell therapies have entered early clinical phases (for a comprehensive overview of stem cell trials in ALS see Reference 169). Mesenchymal stem cells (MSCs) can be harvested from bone marrow or adipose tissue. As they are an autologous source of stem cells, transplant recipients do not require treatment with immunosuppressants. The most common delivery routes are intrathecal or intramuscular. Most current trials are small and primarily aimed at evaluating safety. However, a phase 3 study of bone-marrow derived MSCs induced to secrete neurotrophic growth factor was initiated and will assess efficacy using the ALSFRS-R. Neural stem cells (NSCs) are pluripotent stem cells capable of differentiating into neural and glial cell types. Early theories posited that NSC transplantation could replace degenerating motor neurons in ALS. However, these neurons would need to integrate into and form functional circuits while projecting axons over great distances. It is thus more likely that NSC transplantation therapies could confer therapeutic benefit by differentiating into glial cells and interneurons that provide trophic and structural support and suppress excitotoxicity. Results of phase 1/2 studies have demonstrated that intraspinal transplantation of human spinal cord-derived NSCs is safe and well-tolerated, and that grafts can survive up to 2.5 years. Comparison of a phase 1/2 study to historical datasets suggested a significant clinical benefit in functional decline measured using the ALSFRS-R. Due to the invasive nature of stem cell implantation, early phase trials should be carried out without a sham surgery control group. Rather, outcome measures are compared with historical controls or to data gathered from trial participants before the procedure. Where the data suggest a significant benefit of the stem cell procedure, a controlled follow-up trial with a sham surgery control group is then warranted to substantiate the findings.

4.1 | Why have drugs failed in ALS clinical trials?

In over 20 years of clinical trials only two drugs—riluzole and edaravone—have been approved for the treatment of ALS. Many more failed to efficaciously stall the disease, or worse had deleterious effects. There are many possible reasons for these failures. First, the drug target itself may not be appropriate to treat the disease. It is difficult to either prove or disprove this argument, and the intention of this review is not to critique the choice of target of any one clinical trial. However, the question of appropriate target selection leads to another problem: the choice of animal models for in vivo experiments. The most frequently used mammalian models of ALS are mutant SOD1 transgenic animals. However, given that only ~10% of familial and 1% of sporadic patients carry SOD1 mutations, efficacy in mutant SOD1 transgenic mice may be inadequate to predict efficacy in a heterogeneous human patient population. Furthermore, inadequate study designs may have confounded results from animal studies. In 2008, Scott et al demonstrated that when various drugs that had failed in the clinic after showing efficacy in
SOD1-G93A mouse studies, such as minocycline, creatine, and ceftriaxone, were retested in an optimized testing paradigm controlled for sex, transgene copy number, litter, and exclusion criteria, none provided a significant survival benefit. In addition, treating animals before onset of symptoms is a common strategy for mouse efficacy studies, but does not reflect the human situation.

Flawed clinical trial designs may have also contributed to candidate failures. ALS is a heterogeneous disease, both phenotypically and genetically. Since the discovery of SOD1 as the first gene linked to familial forms of ALS in 1993, mutations in over 30 genes have been found to be causative of both familial and sporadic forms of the disease. Hexanucleotide repeat expansions in C9ORF72, which account for up to 30% to 50% and 5% to 7% of sporadic and familial ALS, were only described in 2011. Different genetic contributions may indicate that mechanisms leading to the disease state are distinct, despite apparent similarities in presentation of symptoms. While older trials may not have distinguished different patient populations based on genetic background, it will likely become a key inclusion/exclusion criterion in the future. In trials to evaluate the efficacy of ASOs targeting disease-associated genes such as SOD1 and C9ORF72, genetic determination is already a prerequisite for trial enrolment. Indeed, SOD1 ASOs are likely to be ineffective in non-SOD1 ALS cases. Additionally, eligibility criteria based on phenotype may also become more stringent, such as disease stage, initial site of onset or individual progression rate, defined during a pretrial observational period. For example, following an unsuccessful phase 3 trial for the now approved edaravone, enrollment eligibility criteria were adjusted based on a posthoc subgroup analysis. In addition to the previous criteria, which among others included a decrease in the ALSFRS-R score of 1 to 4 points during a 12-week observation period, a second phase 3 trial also specified a disease duration of less than or equal to 2 years, scores of greater than or equal to 2 on all 12 items of the ALSFRS-R, FVC of at least 80% and definite or probable ALS according to the El Escorial and revised Airlie House criteria.

Last, unsuccessful clinical trials could be due to insufficient exposure to the therapeutic agent at the site of action. For example, insufficient brain exposure may have hampered trials investigating antioxidants such as coenzyme Q10. The troponin binder tirasemtiv was poorly tolerated at higher doses, causing patients to drop out of the study. The growth factor CNTF has a very poor half-life of under 3 minutes when administered intravenously. Finding appropriate biomarkers to confirm target engagement and rule out lack of exposure should be a vital aspect of early clinical development.

5 | EMERGING PRECLINICAL STRATEGIES

In addition to the wide array of treatment strategies currently under clinical investigation for the treatment of ALS, exciting preclinical research has uncovered a variety of possible disease mechanisms that could be targeted with novel therapeutic interventions. While a thorough discussion of these novel preclinical strategies for ALS is outside the scope of this review, we want to highlight a few approaches aimed at transforming the therapeutic landscape of this intractable disease.

5.1 | Emerging ASO approaches

ASOs are a powerful technology enabling long-lasting (several months) modulation of gene expression by binding to RNA targets with complementary sequence. ASOs exert their therapeutic effects either by targeting RNAs for degradation, correcting splicing defects or sequestering miRNA. They are particularly attractive for the treatment of diseases known or thought to arise from the dysfunction of a single gene product. Since their inception, a number of ASOs targeting neurological disorders including ALS, Huntington’s disease and familial amyloid polyneuropathy have entered the clinic. One of the biggest success stories thus far is nusinersen (Spinraza), an ASO approved for the treatment of spinal muscular atrophy (SMA), a disease characterized by lower motor neuron degeneration resulting in progressive
muscle atrophy. The most common form of SMA is caused by mutations in the gene survival of motor neuron 1 (SMN1), leading to loss of SMN1 function. Humans possess a SMN1 paralog, SMN2. SMN2 differs from SMN1 by only a few nucleotides including a cysteine-to-threonine mutation that causes skipping of exon 7, resulting in the expression of an unstable protein. Nusinersen targets the splice site containing this mutation, thus enhancing the inclusion of exon 7, which leads to the expression of more stable, full-length SMN protein. In the interim analysis of its phase 3 study, nusinersen demonstrated significant effects on motor function and survival, prompting early termination of the trial and subsequent approval of the drug.

To date, no ASO therapy has been approved for the treatment of ALS, but phase 1 trials investigating ASOs that reduce levels of mutant SOD1 and C9ORF72 with the hexanucleotide repeat expansion mutation were recently initiated. Furthermore, in May 2019 the US House of Representatives passed Jaci’s Bill. The bill’s passage and subsequent approval by the FDA allows treatment of Jaci Hermstad, a patient suffering from early-onset ALS, with a personalized ASO targeting the FUS mutation P525L she carries. This effort, spearheaded by Neil Shneider, marks only the second instance of a personalized ASO approach made public in the United States.

In addition to ASOs aimed at lowering the expression of ALS-causative genes or even specific mutations in genes such as described above, preclinical efforts are also focused on ASOs targeting mRNAs that encode disease-modifying proteins downstream in the disease cascade. One ASO has been developed to reduce expression of ataxin-2, a protein involved in the assembly of stress granules (SGs). Expansions in the polyglutamine (polyQ) stretch of ataxin-2 beyond 34 to 36 repeats cause the neurodegenerative disease spinocerebellar ataxia type 2 while intermediate-length expansions (22-33 repeats) are associated with an increased risk of developing ALS. The role of ataxin-2 in mediating toxicity of TDP-43 was first discovered in yeast, when it was shown that upregulation of the yeast ataxin-2 homolog Pbp1 enhanced, while knocking out Pbp1 suppressed, TDP-43 toxicity. Furthermore, heterozygous deletion of the ataxin-2 homolog Atx2 was shown to rescue eye degeneration and extend lifespan in Drosophila expressing TDP-43. In a subsequent study, reduction in ataxin-2 by genetic knockout or a single administration of an ASO resulted in improved survival and motor function in a TDP-43 transgenic mouse. As the vast majority of ALS patients present with TDP-43 proteinopathy, an ataxin-2 ASO approach could thus target the broadest possible patient base.

Another potential target for a therapeutic ASO approach is stathmin-2 (STMN2). RNAseq studies to identify transcripts regulated by TDP-43 found that levels of STMN2, which is required for normal outgrowth of motor neurons, are greatly downregulated in TDP-43-depleted cells. Furthermore, STMN2 was found decreased in human motor neurons and spinal cord sections of ALS patients. Reduced binding of TDP-43 to a site within intron 1 uncovers a cryptic exon encoding a polyadenylation (polyA) site in STMN2, and inclusion of this polyA sequence leads to the translation of a truncated, nonfunctional protein (Figure 9). Rescue of STMN2 levels by post-transcriptional stabilization or expression of a construct without the cryptic polyA site rescued the axonal outgrowth defect caused by loss of TDP-43. Thus, an ASO designed to prevent the inclusion of the cryptic exon to restore expression of STMN2 is an interesting therapeutic strategy. Crucially, as the cryptic polyA site within intron 1 is absent in mouse STMN2 mouse neuronal cells or in vivo models would not reveal a decrease in STMN2 expression upon TDP-43 depletion. This key difference between mouse and human demonstrates the importance of incorporating human cell systems such as iPSC-derived motor neurons or motor neurons generated by direct conversion.

5.2 | Targeting deleterious phase separation

Liquid-liquid phase separation (LLPS) is the process by which macromolecules condense into structures with dynamic fluid-like properties within a surrounding liquid phase. LLPS underlies the formation of membrane-less organelles, self-organizing complexes without delineating lipid bilayer that form functional compartments within cells. Membrane-less organelles exist both in the nucleus (eg, nucleoli, Cajal bodies) and cytoplasm (eg, SGs, P bodies).
SGs are transient structures that form in response to activation of stress pathways and sequester RBPs and untranslated RNA. RBPs implicated in ALS such as TDP-43, FUS, hnRNPA1 are recruited to SGs and can undergo LLPS in vitro. While these LLPS modalities are beneficial, LLPS can also have deleterious consequences in disease.

Critical research over the past several years has suggested that liquid assemblies of RBPs can solidify over time and transition to an insoluble state in vitro and in cells. Furthermore, SGs, as well as SG-independent cytoplasmic droplets, can have detrimental effects linked to disease. For example, G3BP1 and TIAR-2 form liquid-like granules in neurons that suppress axon regeneration. Cytoplasmic TDP-43 droplets that form independently of SGs deplete nuclear TDP-43, sequester components of the nucleocytoplasmic transport machinery and cause cell death while repetitive, persistent assembly of SGs causes a cell death phenotype. Thus, therapeutic strategies that drive disassembly or disaggregation or interfere with the genesis of cytoplasmic liquid-like droplets may be a viable option for the treatment of ALS.

In a recently published study, Fang et al screened for small molecules that modulate the number, size, and composition of SGs or have the ability to disassemble preformed SGs. The screen identified a number of planar compounds that reduced the accumulation of TDP-43 in SGs as well as the puromycin-induced formation of cytoplasmic TDP-43 puncta that persist after SGs are resolved. In another study, using a light-inducible model of TDP-43 LLPS, Mann et al demonstrated that binding of RNA suppresses TDP-43 LLPS and inclusion formation. Excitingly, treatment of neuronal cells with a bait RNA oligonucleotide with high affinity for TDP-43 inhibited aberrant phase transition of TDP-43 and rescued neuronal survival, suggesting that RNA oligonucleotides that antagonize aberrant TDP-43 phase separation may be a possible therapeutic strategy. These RNA oligonucleotides are intriguing therapeutic candidates as they might be readily delivered to patients like an ASO. A challenge for therapeutics targeting phase separation, however, will be to ensure that beneficial phases are preserved and deleterious phases are eliminated. Overcoming this challenge will require a detailed structural and mechanistic understanding of what differentiates beneficial phases from deleterious phases.

5.3 Poly(ADP-ribose) polymerase inhibition

A number of studies have provided evidence that poly(ADP-ribose) polymerases (PARPs) may be a viable therapeutic target for ALS and other neurodegenerative diseases. PARPs catalyze a type of posttranslational modification called ribosylation, the addition of monomers or polymers of ADP-ribose to proteins. The role of poly(ADP-ribosylation) (PARylation) has been extensively studied in the context of DNA damage response
signaling, where auto-PARylation of PARP-1 triggers recruitment of DNA damage repair factors. More recently, it was shown that TDP-43 is a PAR-binding protein and that PAR promotes TDP-43 LLPS. Furthermore, inhibition of PARP-1/2 reduced SG formation, TDP-43 recruitment to SGs and formation of cytoplasmic TDP-43 foci, and rescued TDP-43-induced cell death of NSC-34 and spinal cord motor neurons. In TDP-43 Drosophila models of ALS, knockout of PARP-1 or tankyrase, another member of the PARP family, rescued retinal degeneration and extended survival. Thus, inhibiting members of the PARP family could reduce aberrant TDP-43 phase transition and prevent TDP-43-associated cytotoxicity. Since PARP inhibitors are already safely in the clinic for various cancers it would seem that a clinical trial with PARP inhibitors for ALS is warranted.

5.4 Stimulating protein disaggregation

The accumulation of misfolded proteins is the defining hallmark of neurodegenerative proteinopathies including ALS. Aggregation of misfolded proteins can cause toxicity either by loss-of-function or gain-of-function mechanisms. Thus, reversing protein aggregation could be a viable therapeutic strategy. Hsp104 is a AAA+ (ATPases associated with diverse cellular activities) ATPase with disaggregase activity found in Saccharomyces cerevisiae where it modulates the physiological aggregation of yeast prion proteins. However, wild-type Hsp104 only displays moderate disaggregase activity against human proteins that undergo aberrant misfolding associated with neurodegenerative diseases. By introducing specific single missense mutations, potentiated Hsp104 variants have been engineered that efficiently suppress the toxicity and aggregation of TDP-43 and FUS as well as α-synuclein, the major protein component of Lewy body inclusions associated with Parkinson’s disease, in yeast (Figure 10). Curiously, while Hsp104 is conserved between bacteria and many eukaryotes, no homolog exists in the animal kingdom, so Hsp104 may be a possible exogenous therapeutic candidate. In support of this possibility, expression of enhanced Hsp104 variants reverses ALS-linked FUS aggregation in mammalian cells. Further studies will be required to

FIGURE 10 Hsp104 is a protein disaggregase. Hsp104 extracts misfolded proteins from soluble oligomers, amyloid fibrils, and amorphous aggregates and returns them to a natively folded state in an ATP-dependent manner. Humans (or any other members of the animal kingdom) have no Hsp104 homolog. Thus, a therapeutic disaggregase strategy would depend on delivery of Hsp104 or DNA encoding Hsp104 exogenously. Potentiated Hsp104 variants have been engineered with enhanced activity against different misfolded proteins. ATP, adenosine triphosphate [Color figure can be viewed at wileyonlinelibrary.com]
investigate (a) whether potentiated Hsp104 variants can effectively disassemble aggregates of TDP-43 in degenerating neurons, (b) whether expression of potentiated Hsp104 variants is effective in mammalian ALS models in vivo, and (c) whether expression of potentiated Hsp104 variants interferes with normal cellular folding processes in mammalian systems.

In addition to potentiated Hsp104 variants, nuclear import receptors with chaperone activity have emerged as another class of proteins capable of driving protein disaggregation. Indeed, nuclear-import receptors with chaperone function can prevent and even reverse aberrant phase transition and fibrillization of a number of ALS-associated RBPs harboring a nuclear-localization signal. Transportin 1 (TNPO), also known as karyopherin-β2, prevents fibrillization as well as disassembles preformed fibrils, liquid droplets, and hydrogels of FUS, and reduces the accumulation of FUS in SGs. Furthermore, a combination of importin-α and karyopherin-β1 prevents fibrillization and promotes disassembly of TDP-43. Thus, strategies that boost the disaggregase function of mammalian nuclear-import receptors could provide a viable therapeutic avenue.

5.5 Inhibition of repeat-associated non-ATG translation

G₄C₂ hexanucleotide repeat expansions in C9ORF72 are the most common genetic mutation associated with ALS, accounting for 30% to 50% of familial and 5% to 7% of sporadic cases. Three potential mechanisms could explain how C9ORF72 repeat expansions cause ALS: loss-of-function of C9ORF72 protein, gain-of-function toxicity caused by C9ORF72 repeat RNA or gain-of-function toxicity by expression of dipeptide repeat proteins (DPRs). DPRs are translated from RNA with nucleotide repeat expansions, a process termed repeat-associated non-ATG (RAN) translation. Bidirectional RAN translation of the repeats yields five DPRs (poly-GA, poly-GP, poly-PR, poly-GR, and poly-PA; Figure 11), which are detected in pathological inclusions in patients’ brains. Poly-GA, poly-PR, and poly-GR are toxic in vitro and in animal models. Poly-PR interacts with ribosomal proteins and inhibits translation, disrupts nucleolar function by affecting LLPS of nucleolar proteins, causes heterochromatin abnormalities and disrupts nuclear architecture. Thus, therapeutic strategies aimed at lowering the production of DPRs may be beneficial to stop or slow down the progression of ALS caused by mutations in C9ORF72.

**FIGURE 11** RAN translation of dipeptide repeats in C9ORF72-ALS. G₄C₂ hexanucleotide repeat expansions in intron 1 of the C9ORF72 gene are translated in the absence of a start codon (RAN translation). Translation from the sense and antisense strand yields five dipeptide repeats (poly-GR, poly-GP, poly-GA, poly-PR, poly-PA). Dipeptide repeats have detrimental effects in cells, causing heterochromatin abnormalities, translational stalling, disturbed LLPS and cytotoxicity. Preclinical therapeutic strategies are aimed at suppressing RAN translation. LLPS, liquid-liquid phase separation; RAN, repeat-associated non-ATG [Color figure can be viewed at wileyonlinelibrary.com]
In one recent study, Yamada et al.\textsuperscript{260} showed that RPS25, a eukaryotic-specific, nonessential component of the 40S ribosomal subunit, is required for RAN translation. Knockout of RPS25 in immortalized cells led to a reduction in poly-GP, poly-GR, and poly-GA levels without affecting RNA foci formation or total C9ORF72 mRNA levels. Furthermore, an ASO targeting RPS25 reduced the number of nuclear poly-GR and poly-PR foci in iPSC-derived motor neurons from C9ORF72 patients.\textsuperscript{260} An ASO approach targeting RPS25 or other modulators of RAN translation may thus represent a therapeutic strategy.

Another recent study suggested that DDX3X, an RNA helicase, remodels G4C2 repeats to inhibit RAN translation.\textsuperscript{261} DDX3X directly binds to (G4C2)\textsubscript{n} RNAs but not the antisense (C4G2)\textsubscript{n} RNAs.\textsuperscript{261} Indeed, elevating DDX3X expression decreased DPR levels, rescued nucleocytoplasmic transport defects, and enhanced survival of patient iPSC-differentiated neurons.\textsuperscript{261} Thus, agents that increase DDX3X expression or activity could be another therapeutic strategy.\textsuperscript{261} Finally, small-molecule screens to identify RAN translation inhibitors may also provide novel compounds and targets.\textsuperscript{262}

### 5.6 Selective inhibitors of nuclear export

A major pathological hallmark in the majority of ALS cases is the mislocalization of TDP-43 from the nucleus to the cytoplasm. Deletion of the TDP-43 nuclear localization signal causes a loss of nuclear function, cytoplasmic accumulation, and aggregation of TDP-43 and is deleterious both in vitro and in vivo.\textsuperscript{263,264} Furthermore, nucleocytoplasmic transport deficits have been described in a variety of cellular ALS models.\textsuperscript{265–267} Preventing the egress of TDP-43 from the nucleus might thus be a potential therapeutic strategy. TDP-43 harbors a leucine-rich nuclear export signal (NES) predicted to be recognized by the nuclear export factor exportin-1 (XPO1), which can be inhibited by selective inhibitors of nuclear export (SINE) compounds. In 2019, the FDA approved the first-in-class XPO1 inhibitor KPT-330 (selinexor) as a fifth line treatment for multiple myeloma.\textsuperscript{268} The inhibition of XPO1 causes the accumulation of tumor suppressor proteins in cancer cells, which eventually kills the cells. Inhibition of XPO1 was shown to reduce TDP-43-induced cell death in primary neuron cultures, ameliorate larval locomotor deficits in a TDP-43 Drosophila model, and improve grip strength in a rat TDP-43 model.\textsuperscript{267,269} However, recent studies have demonstrated that the NES of TDP-43 is not functional and that TDP-43 egress is the result of passive diffusion rather than active transport.\textsuperscript{270,271} Furthermore, at concentrations shown to effectively rescue TDP-43-induced cell death in neuronal cultures, no increase in nuclear TDP-43 signal was observed.\textsuperscript{269} Thus, the mechanisms of action of XPO1 SINEs that protect against TDP-43-induced cell death and locomotor deficits may be independent of the localization of TDP-43. In addition to its effects in TDP-43 cell and in vivo models of ALS, inhibition of XPO1 also rescued an eye degeneration phenotype in a Drosophila model expressing the C9ORF72 G\textsubscript{4}C\textsubscript{2} hexanucleotide repeat expansion and improved survival in mutant SOD1-expressing flies.\textsuperscript{272,273} While the mechanism through which XPO SINEs protect cells against ALS gene-induced cell death remains to be fully elucidated, a recent study suggested that XPO1 inhibition stimulates autophagy.\textsuperscript{272}

### 6 CONCLUSIONS

Despite extensive research, dedication, and a multitude of clinical trials, ALS remains a devastating, debilitating, and ultimately fatal disease. Approved treatments only provide limited benefits. In this review, we sought to give an overview of the major types of interventional treatments that have been or are being investigated in the clinic. There are many possible explanations for the high failure rates of ALS clinical trials, such as choice of the wrong target, flawed clinical trial design, and patient selection or insufficient exposure to the drug. It will be crucial to address these reasons for failure for new drugs entering the clinic, such as by selecting patients based on genetic status, which is already a key criterion in trials investigating ASOs targeting SOD1 and C9ORF72. In addition, emerging preclinical strategies with novel mechanisms of intervention provide hope for more effective treatment options in the future.
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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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