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Targeting Amyotrophic Lateral Sclerosis with Gene Therapy: From Silencing Genes to Enhancing Neuroprotection

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Gene therapy is emerging as a transformative approach for treating amyotrophic lateral sclerosis (ALS), a progressive and fatal neurodegenerative disease. While gene replacement has shown a groundbreaking success in spinal muscular atrophy, the complexity of ALS—due to frequent gain-of-function mutations and a heterogeneous etiology—presents significant challenges. Importantly, approximately 90% of ALS cases are sporadic, with unknown genetic mutation, further complicating patient stratification and therapeutic targeting. As a result, gene therapy strategies must often address multiple pathological mechanisms simultaneously. So far, current gene therapy strategies aim to either suppress toxic gene expression or promote neuroprotection, predominantly via viral-mediated delivery systems. This review will provide an overview of emerging preclinical and clinical gene therapy approaches for ALS, focusing on two main strategies: gene silencing and neuroprotection. Gene silencing techniques, including antisense oligonucleotides (ASOs), viral-mediated RNA interference, and gene editing, have demonstrated efficacy in reducing mutant gene expression, particularly in SOD1 and C9orf72 models, although clinical translation has so far yielded limited success. The recent Food and Drug Administration's approval of the ASO therapy Qalsody for SOD1-ALS underscores the clinical potential of these approaches. Neuroprotective strategies aim to enhance motor neuron survival through delivery of trophic factors, often targeting both central and peripheral tissues to harness retrograde transport mechanisms. We will discuss the advantages and limitations of various delivery vectors, targeting specificity, timing of intervention, and translational challenges, alongside current clinical trial data. This review aims to synthesize how these approaches may converge to address the multifaceted nature of ALS and guide the development of next-generation therapeutics.

Keywords: ALS, delivery routes, gene augmentation, gene silencing, motor neuron, neuromuscular

INTRODUCTION

Therapeutic landscape of amyotrophic lateral sclerosis

Gene therapies offer significant potential in addressing rare, devastating, and life-threatening diseases such as amyotrophic lateral sclerosis (ALS), as they usually focus on correcting the underlying genetic cause of the condition rather than just alleviating the symptoms. While gene therapy—including vector-based strategies, antisense oligonucleotides (ASOs), and RNA interference—has recently gained significant attention, a range of nongenetic strategies have also been explored preclinically in ALS. These

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include small molecules, peptide- or protein-based systems, and stem cell-derived therapies. Currently, only three nongenetic treatments are approved for ALS: riluzole, edaravone, and Relyvrio. Riluzole, the first approved drug, likely acts via antiglutamatergic mechanisms but offers only a modest survival benefit of 3–4 months.[1,2] Edaravone, which is thought to be a free radical scavenger, showed efficacy only in a narrow patient subgroup and was later withdrawn in Europe due to insufficient benefit.[3] Finally, clinical trials of Relyvrio suggest a modest delay in disease progression by reducing endoplasmic reticulum (ER) and mitochondrial stress.[4,5]

The notable success in treating infants with spinal muscular atrophy (SMA) underscores the promise of gene therapy for other neuromuscular disorders and motoneuron diseases (MNDs). However, unlike SMA, ALS is uncommonly a monogenic disorder, complicating the development of therapeutic strategies. Additionally, in cases with a genetic basis, the disease is most often associated with gain-of-function mutations, rendering gene replacement therapies unfeasible. Nevertheless, numerous gene therapy strategies have been investigated in preclinical settings, primarily focusing on gene supplementation with factors capable of mitigating ALS pathological alterations (outlined in Table 1). These approaches commonly involve the direct delivery of viral vectors—such as adeno-associated (AAV), adenoviral (Ad), or lentiviral (LV) systems—or the transplantation of genetically engineered cells. In parallel, the targeted suppression of pathogenic genes has been extensively explored using ASOs and viral-mediated knockdown via short hairpin RNAs (shRNAs) or microRNAs (miRNAs) (summarized in Table 2). Notably, clinical trials employing these approaches have shown promise, particularly targeting *SOD1* mutations (Qalsody), with ongoing evaluations directed to *FUS* (NCT04768972) and *C9ORF72* (NCT04931862 and NCT03626012) mutations.

Gene therapy approaches have focused on targeting either motoneurons (MNs) or the skeletal muscles and their synaptic connections. Local MN pools can be directly reached through intraspinal (i.s.) injections for lower MNs and intracerebral (i.c.) injections for upper MNs. Alternatively, MNs can be broadly accessed through cerebrospinal fluid (CSF) infusion via intrathecal (i.t.), intracisternal magna (i.c.m.), or intracerebroventricular (i.c.v.) routes. To deliver therapeutics to the muscles, strategies have involved intramuscular (i.m.) or intravenous (i.v.) administrations, complemented with the use of retrograde viral vectors or transgenic proteins that, upon expression, will be taken up at the synapse (Fig. 1).

In this review, we explore the current landscape of gene therapy strategies for ALS, examining preclinical and clinical approaches, delivery methods, and the challenges that arise from the disease complexity.

Pathophysiological mechanisms and experimental animal models

The identification of ALS-associated genes has enabled the generation of transgenic models replicating key features of the disease, uncovering the pathogenic events underlying MN degeneration and serving as essential tools for preclinical assays. An ideal rodent model for ALS should recapitulate ALS phenotypes and pathologies, particularly involving motor deficits and MN degeneration. As the first gene discovered to be causative of ALS, transgenic mice overexpressing mutant forms of the human *SOD1* gene remain the most widely used and best-characterized models.[118] The first ALS animal model featured a high copy number of the human mutant *SOD1* gene (m*SOD1*) with a glycine-to-alanine transition at the 93rd codon.[119] These mice start motor signs around 12 weeks of age, developing trembling, hindlimb weakness, and muscle atrophy, culminating in paralysis and death by 16–20 weeks, which is preceded by axonal degeneration and neuromuscular junction (NMJ) denervation. Histopathological characteristics of the most common profile of patients with ALS, with MN loss accompanied by glial reactivity, except for the absence of mislocalization and accumulation of TDP-43 inclusions are also replicated in these mice.[120] Alterations in SOD1 protein have also been found in sporadic patients with ALS, thus increasing the interest of this murine model.[121] Other *SOD1* mutants (*e.g., G37R* and *G85R*) and low-copy-number *G93A* lines have been developed, generally displaying slower disease progression.

Beyond *SOD1*, additional genetic models have provided insight into ALS pathophysiology. The main pathological finding in most sporadic and familial ALS cases is the presence of ubiquitinated inclusions of TDP-43 in the cytoplasm of neurons and glia in the brain and spinal cord. Models for *TARDBP* mutations have been generated with wild-type or mutant *TARDBP* sequences showing ranging degrees of MN degeneration and phenotypes, although most of these models fail in reproducing the ALS motor involvement.[122] The initial Prp-TDP-43 mouse models show cortical and descending corticospinal tract pathology before developing lower MN degeneration. A recent knock-in *TDP-43*^{Q331K} model recapitulates both ALS and frontotemporal dementia (FTD)-like phenotypes, including reduced muscle mass and impaired motor function, being the most physiological TDP-43 model to date.[123]

The *C9ORF72* G_4C_2 hexanucleotide repeat expansion is the most common genetic alteration in patients with ALS and is believed to induce toxicity through gain-of-function mechanisms including RNA foci, sequestration of RNA-binding proteins, and repeat-associated non-ATG translation leading to toxic aggregates of dipeptide repeat proteins (DRPs). Mouse models expressing this repeat recapitulate distinct disease-related pathological, functional, and behavioral phenotypes,[124] although many fail to exhibit TDP-43 pathology, whereas others fail to induce MN loss and severe motor alterations.[125–127]

Mutations in the gene encoding profilin 1 (*PFN1*), a cytosolic protein that participates in the assembly of

Table 1. *Gene Delivery Strategies Tested in ALS Models*

Delivery	*Target*	*Vector (dose)*	*Promoter*	*Model*	*T. Age*[a]	*Survival*	*Main Findings*	*Reference*
Trophic factors								
GDNF								
t.p.: medial and lateral	GM	RV MPC 50,000/site	PGK1	B6SJL.*SOD1*^{G93A} mice	P42 (Pre)	0%	Increased large spinal MNs and slowed muscle atrophy. Delayed onset by 15 days and improved motor function	6
i.m.: GM + TB	Skeletal muscles	AAV2 ($3 \times 10^{10} + 2 \times 10^{10}$ vg)	CMV	*SOD1*^{G93A} male mice	P63 (Pre)	16.6%	>120-fold GDNF in muscles. Rescued muscle mass loss and atrophied myofibers. Motor function deterioration was delayed	7
i.m.: TA + GM + QD + PSM	Skeletal muscles	Ad5 (5×10^{9} PFU/muscle)	CMV	B6SJL.*SOD1*^{G93A} mice	P5-7 (N)	12.2%	Delayed onset. Motor performance declined more slowly. MNs preserved until 4 months. No differences in CMAP	8
i.m.: right GM	GM	Ad5 (1×10^{8} PFU)	CMV	B6SJL(*SOD1*^{G93G})^{dl} mice	P245 (LS)	N/R	No differences in clinical grade, voluntary movement, or rotarod. Preserved p-Akt^{+} large MNs	9
i.m.: QD + lnC	Spinal MNs	AAV2 (1×10^{10} vp)	CMV	B6SJL.*SOD1*^{G93A} mice	P60, 90 (Pre, LS)	8.9%	Delayed onset (17.5%). Did not slow disease progression	10
i.s.; i.c.: FMN	Lumbar or facial MNs	LV (50/100 ng p24 antigen/site)	PGK1	B6SJL.*SOD1*^{G93A} mice	P40 (Pre)	N/R	Appearance of SFP in GM was not delayed and GM CMAP was not preserved. Local GDNF did not prevent lumbar MNs loss	11
t.p.: lumbar spinal cord	Spinal cord	LV NPC (1.2×10^{5} bilateral)	PGK1	SD *SOD1*^{G93A} rats	P90 (Pre)	0%	GDNF correlated with increased ChAT expression within axons. MNs not preserved. No differences in BBB scores	12
i.m.: TA + TB + LT	Spinal MNs	LV MSC; 3 times (120,000/site)	PGK1	Fast; Slow progressing SD *SOD1*^{G93A} rats	P80 (Pre)	15.8%; 17.5%	Less denervation of NMJs, maintained MNs, and delayed onset by 28 days. Slower rate of motor decline (BBB test)	13
i.v.: tail	Broad	AAV9 (4×10^{12} vp)	CAG	SD *SOD1*^{G93A} rats	P25 (Pre)	0%	Modestly improved grip strength and delayed forelimb paralysis. Slower weight gain, reduced activity, and working memory	14
i.c.: motor cortex	Upper MNs	LV iNPC; 20 sites (10,000/site)	PGK	SD *SOD1*^{G93A} rats	P80 (Pre)	8.2%	Delayed disease onset, improved motor performance in BBB test, and preserved large CTIP^{+} upper MNs and ChAT^{+} lower MNs	15
i.v. tail	Skeletal muscles	AAV8 (1.8×10^{14} vg/kg)	Desmin	B6.*SOD1*^{G93A} mice	P35 (Pre)	N/R	Reduced glial reactivity and preserved LMNs and NMJs. Delayed onset and slowed ALS progression	16
i.s.: lumbar	MNs	LV iNPC, 3 sites (10,000/site)	PGK	SD *SOD1*^{G93A} rats	P70-95 (Pre)	0%	Provides protection to MNs but no differences in BBB scores	17
IGF-1								
i.m.: QD + lnC	Spinal MNs	AAV2 (1×10^{10} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre); P90 (LS)	30%; 17.7%	Delayed onset (34%) and motor deficits, along with 34% more surviving MNs at end stage. Reduced apoptosis, astrogliosis, and aggregates	10
i.s.: L4 and L5 bilateral	Spinal MNs	AAV2 (1.2×10^{10} vg/site)	CAG	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	10.7% (males)	Rescued MNs loss. Delayed onset (12.4%) and slowed grip strength and weight declines, all selectively in males	18
i.c.b.: DCN bilateral	CNS	AAV1, AAV2 (2×10^{10} vp/site)	CMV	*SOD1*^{G93A} mice	P90 (LS)	11.6%	Preserved spinal MNs, in cervical regions only AAV1. Grip strength and rotarod were improved until end stage. Reduced gliosis	19
i.s.: cervical unilateral	Spinal MNs	AAV2 (4.12×10^{10} vg)	CAG	SD *SOD1*^{G93A} rats	P80 (Pre)	0%	Reduced MNs loss within treated C3–C5 spinal segments. Only males had full preservation of ipsilateral grip strength	20
i.m.: GM + TA + lnC + TB	MNs or muscles	LV-αCAR or LV-VSV-G (7.1×10^{10} vg)	CMV	B6.*SOD1*^{G93A} mice	P28 (Pre)	9% (αCAR)	Disease onset by weight was delayed 27% (αCAR) or 22.7% (VSVG). αCAR preserved MNs and improved rotarod test and gait	21
i.v.: tail	Broad	scAAV9 (1.5×10^{11} vg)	CMV	B6SJL.*SOD1*^{G93A} female mice	P90 (LS)	7.7%	Decreased reactive glia, weight, and MN loss. Mitigation of myelin pathology in ventral roots. Inhibition of apoptosis and less TNFα levels	22
i.m.: 8 muscles	MNs	scAAV9 (1×10^{10} vg/muscle)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre); P90 (LS)	21.8%; 11.7%	Protection of MNs, locomotion, and weight. Less atrophic muscles and reactive glia. Benefits explained by upregulated DAO	23
i.m.: multiple	MNs	scAAV9 ($2–3 \times 10^{10}$ vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	N/R	Protection of mitochondria from apoptosis and upregulated mitophagy: increased MMP and decreased CytC release	24
IGF-2								
i.m.: QD + lnC	Spinal MNs	AAV9 (11×10^{11} vg)	CBA	B6.*SOD1*^{G93A} mice	P80 (ES)	10%	Improved rotarod test and preserved MNs and axonal density in ventral roots. Induced MNs regeneration and sprouting in NMJ	25
HGF								
i.m.: TA + GM + VL	Spinal MNs	AAV6 (3.12×10^{8} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P90 (LS)	9.8%	No effect on body weight or limb strength. Improved muscle integrity and function could be explained by the FOXO signaling pathway	26
i.t.: lumbar	Spinal cord	AAV1 (5×10^{9} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	6.5%	No effect on weight. Rotarod, hanging wire, and grip strength tests were improved. Two-fold increase in spinal MNs and restored NMJs	27
i.t.: lumbar	CNS	AAV9 (1×10^{9} vg)	EF-1α	C57BL/6 prp*TDP-43*^{A315T} mice	P60 (Pre)	N/A	Reduces cortical gliosis, ubiquitin deposits, and UMNs loss.	28
VEGF								
i.m.: GM + FC + DIA + TG + lnC	MNs	Rabies-G LV (~9×10^{9} TU)	LV (EIAV)	B6SJL.*SOD1*^{G93A} mice	P21 (Pre); P90 (LS)	30%; 14.9%	P21 VEGF delayed onset (28%). Treatments preserved stride length until end stage. MNs survival increased 44–125% depending on MN pool	29
i.c.m; i.c.: ICap + MC (P50); i.v. (P2, P50)	Spinal and cortical MNs	AAV1, scAAV9 ($10^{12}–10^{13}$ vg/kg)	CMV	LIX1$^{-/-}$ cats	P2 (N), 50 (Pre)	N/R	I.c.m resulted in spinal MNs transduction and neurons in the cerebellum, thalamus and MC. I.v. bore low spinal vg and no VEGF protein was detected. No therapeutic effect	30
i.t.: lumbar	Spinal MNs	scAAV9 (1×10^{12} vg/kg)	CMV	B6SJL.*SOD1*^{G93A} mice	P90 (LS)	9.1%	Improved footprint and rotarod test. Preserved MNs and decreased apoptosis and gliosis, switching microglia from a M1 to a M2 state	31
i.t.: lumbar	Spinal MNs	scAAV9 (1×10^{12} vg/kg)	CMV	B6SJL.*SOD1*^{G93A} female mice	P60 (Pre)	13.3%	Delayed onset (11.2%), preserved weight, and rotarod, modulating microglia, inhibiting inflammation, and macrophage infiltration	22
i.t.: lumbar	Spinal MNs	scAAV9 (1×10^{12} vg/kg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	12.1%	Delayed onset (11.5%), preserved weight, and rotarod, upregulating aromatase and modulating oxidative stress and autophagy	32
NT-3 + CNTF								
i.m.: unilateral GM + TB + LT	Skeletal muscles	Ad (1×10^{9} PFU)	RSV LTR	*pmn* mice	P3-5 (N)	51.7%	Onset not affected but treated mice had less atrophy and better motor performance. Recovery of GM CMAP and protected axons. Effects were potentiated with AdCNTF	33,34

(*continued*)

Table 1. (Continued)

Delivery	Target	Vector (dose)	Promoter	Model	T. Age[a]	Survival	Main Findings	Reference
CT-1								
i.m.: GM + TB + LT	Skeletal muscles	Ad5 ($2.5 \times 10^9 + 1.5 \times 10^9 + 2 \times 10^9$ PFU)	RSV LTR	B6SJL.*SOD1*^{G93A} mice	P5-6 (N)	7.7%	Postponed disease onset. Increased GM mass, reduced atrophy, and denervation. At end stage, CMAP decreased at a lower rate	35
G-CSF								
i.m.: GM + LT; i.s.: lumbar	Spinal MNs	AAV1 (i.m.: 9×10^9 vg; i.s.: 3.78×10^9 vg)	CBA	B6.*SOD1*^{G93A} female mice	P70 (ES)	10%	Contrarily to i.s., i.m. fails to transduce and preserve MNs. I.s. improved strength and rotarod at end stage. Delayed weight loss	36
NDNF								
i.t.	CNS	AAVPHP.eB (5×10^{11} vg)	CBh	B6.*SOD1*^{G93A} mice	P61 (Pre),85 (ES), 100 (LS)	N/R	Positive effects on locomotion, weight loss, MNs, aggregation, and NMJ. Survival mildly extended at P100	37
Modulators of NMJs								
NRG1								
i.m.: GM	GM	AAV1 (1×10^{11} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P56 (Pre); P84 (ES)	N/R	Nrg1-1 preserved GM CMAP and NMJ by collateral sprouting. Rotarod performance and disease onset did not improve	38
i.s.: bilateral	Spinal MNs	AAV1 (6 sites; 3.6×10^8 vg)	CAG	B6SJL.*SOD1*^{G93A} mice	P65 (Pre)	6.5%	Only Nrg1-3, but not Nrg1-1, restored the number of C-buttons and large MNs	39
i.v.: tail	Skeletal muscles	AAV8 (7.6×10^{13} vg)	Desmin	B6SJL.*SOD1*^{G93A} mice	P42 (Pre)	N/R	Nrg1-1 preserved CMAP of PL, TA, and GM with higher MEPs. Preserved NMJ and MNs and decreased gliosis	40
i.t.: lumbar	MNs	AAVrh10 (1×10^{11} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P56 (Pre)	N/R	Nrg1-3 preserved NMJ and CMAP of GC, TA, and PL. Improved locomotion, surviving MNs, and reduced gliosis. Not in males	41
DOK7								
i.v.: tail	NMJ	AAV9 (1.2×10^{12} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P90 (LS)	7.5%	Enhanced MuSK activation and clustered AChR in NMJ. Protected from nerve and muscle atrophy but not from MNs loss	42
NRIP								
i.m.: GM + TA + TB + BB	NMJ	AAVDJ/8 (8×10^{10} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	0%	Improved locomotion, increased myofiber size, reduced NMJ and axonal degeneration, and preserved MNs and CMAPs	43
Apoptosis								
Bcl-2								
i.s.: bilateral lumbar	Spinal MNs	AAV2 (9.5×10^7 vg/site)	CMV	B6SJL.*SOD1*^{G93A} mice	P35 (Pre)	0%	Onset defined by SFP was delayed (9.8%). At end stage, CMAP amplitude was higher. Rescued ~50% of MNs that degenerate	44
SYT13								
i.m.: QD + ^{TM}	MNs	AAV9 (11×10^{11} vp)	N/A	B6.*SOD1*^{G93A} mice	P80 (LS)	14%	Improved inverted grip test and reduced MNs loss, ER stress, and apoptosis. Improved axonal density in ventral roots. Preserved NMJ	45
Aggregation								
scFv								
i.t.	CNS	scAAV1 (3×10^9 vp)	CMV	B6.*SOD1*^{G37R} mice	P45 (Pre)	28%	Lifespan correlated with scFv titer. Delayed onset. scFv D3H5 reduced neuronal stress, MNs loss, and gliosis by reducing mSOD1	46
MIF								
i.s.: lumbar	Spinal cord	AAV9 (5.35×10^8 vg)	CMV	B6.*SOD1*^{G93A} mice B6.*SOD1*^{G37R} mice	P1 (N)	20.3%	Delayed onset (11.6%), maintained weight and strength. Reduced mSOD1 but not gliosis. Outcomes enhanced in *SOD1*^{G37R} mice	47
i.v.: tail	CNS	AAV-PHP.eB (1.2×10^{14} vg/kg)	CAG	C57BL/6.Lox*SOD1*^{G37R}	P210 (ES)	6.78%	Improves motor function, delays progression, and extends survival. MIF reduces neuroinflammation, mSOD1 accumulation, rescues MNs, and corrects dysregulated pathways	48
SRCP1								
i.c.: bilateral	Motor cortex	AAV9 (8×10^{11} vg)	N/R	B6SJL.*SOD1*^{G93A} mice	P70-80 (ES)	0%	Small but significant reduction in insoluble and soluble SOD1	49
Glutamatergic								
ADAR2								
i.v.: tail	Upper and lower MNs	AAV9 (2.14×10^{12} vg)	SYN	ADAR2$^{flox/flox}$/VAChT-Cre.Fast; AR2 mice	P63-91 (Pre); P105 (Sym)	N/R	Model is unable to edit the Q/R site of GluA2 RNA. Treatment ameliorated the decline in rotarod, but not grip strength or weight loss. 10–20% of MNs and TDP-43 mislocalization were rescued	50
GLT1a								
i.s.: bilateral cervical	Astrocytes	AAV8 (1×10^{10} vg)	Gfa2	B6.*SOD1*^{G37R} mice	P110 (Pre)	0%	High expression in astrocytes. PhMNs, NMJ innervation, and CMAP of diaphragm were not preserved. Disease progression unaltered	51
DAO								
i.t.: lumbar	Spinal MNs	AAV9 (1×10^{12} vg/kg)	CMV	B6SJL.*SOD1*^{G93A} mice	P90 (LS)	4.6%	Improved hindlimb stretching but not motor function. Decreased MNs and muscle atrophy, and NF-κB activation, alleviating gliosis	52

(*continued*)

Table 1. (Continued)

Delivery	*Target*	*Vector (dose)*	*Promoter*	*Model*	*T. Age*[a]	*Survival*	*Main Findings*	*Reference*
Multitarget								
IGF-1 + VEGF								
i.c.v.: lateral and 4th ventricle	Ependymal cells	AAV4 (8×10^{10} vp)	CMV	B6SJL.*SOD1*^{G93A} mice	P80-90 (LS)	12.2%, 6%	VEGF increased survival to a greater extent than IGF-1. Both preserved strength but not rotarod performance. No additive effect.	53
Combinations of GDNF, VEGF, IGF-1, and/ or BDNF								
t.p.: GM	GM	LV MPC (2.5×10^5/each NTF)	CMV	B6.*SOD1*^{G93A} mice	P90 (LS)	9.3%	Combined MPC preserved NMJ (88%), rescued MNs axons (6-fold), and delayed onset (52%). Reduced benefits from single MPC-NTF	54
t.p.: TA + TB + LT	Skeletal muscles	LV MPC; 3 times (1.5×10^5/site.)	PGK1	SD *SOD1*^{G93A} rats	P90 (LS)	10.4%, 7.5%, 16.2%	GDNF and VEGF prolonged survival (10.4 and 7.5%), and VEGF delayed onset (5.8%). Both slowed BBB scores, preserved NMJ and MNs, to a larger extent when combined. GDNF+VEGF also delayed onset and extended survival (16.2%).	55
EAAT2, GDH2, and NRF2								
i.c.m. + i.m.: GM	Upper and lower MNs	LV (5×10^8 vp)	CMV	B6.*SOD1*^{G93A} mice	P65 (Pre)	7.9–13.7%	Only the combination improved NS, weight loss, hindlimb reflex, motor function, and delayed onset. Two-fold preservation in MN numbers	56
Galectin 1 + SOD1 shRNA								
i.c.v.: lateral	CNS	scAAV9 (7.5×10^{10} vg)	CAG; H1; H1/CAG	B6SJL.*SOD1*^{G93A} mice	P1-2 (N)	0%; 37%; 64%	Gal1 alone had no effect, but combined with shRNA improved survival and further reduced microglial inflammation	57
Other Neuroprotective Strategies								
NRF2, PRDX3								
i.m.: FC + TG + InC + DIA + HL	Skeletal muscles	AAV6 (1.2×10^{11} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P30 (Pre)	0%	Neither showed differences in NS, disease onset, rotarod performance, survival, or MN death	58
IL-10								
i.s.: lumbar	Spinal cord	AAV1 (N/A)	N/R	B6SJL.*SOD1*^{G93A} mice	P0 (N)	15%	No changes in body weight or in GFAP$^+$ or Iba1$^+$ cells, but the upregulation of a subset of innate immunity genes was attenuated	59
MCP1								
iM: TA + GM + GLU + TB	Skeletal muscles	scAAV9 (2.18×10^{11} vg/site)	CMV	B6.*SOD1*^{G93A} mice; 129Sv*SOD1*^{G93A} mice	P42; P56 (Pre)	N/R	Promoted axonal regeneration and prevented inflammation and MN loss. Disease progression and onset were ameliorated	60
Caveolin-1								
s.p.: lumbar	MNs	scAAV9 (2×10^{11} vg)	SYN	B6.*SOD1*^{G93A} mice	P56 (Pre)	10%	Delayed disease onset, greater running-wheel performance, preserved MNs, and NMJ integrity.	61
s.p.: cervical	MNs	scAAV9 (4×10^{11} vg)	SYN	SD *SOD1*^{G93A} rats	2 months (Pre)	N/R	Preserved forelimb grip strength and MEPs in the brachial and GM muscles	61
VDAC1								
i.s.: lumbar	Spinal cord	AAV5 (10^{10} vg)	CAG	B6.*SOD1*^{G93A} mice	P2 (N)	N/R	Rescues respiratory chain complex I and sirtuins function	62
OPTN								
i.t.: lumbar	CNS	AAV9 (3.61×10^{10} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P30-40 (Pre)	10.5%	Improved stride length, rotarod performance, and weight	63
i.c.v.: right lateral	CNS	LV (5×10^6 vp)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre); P90 (LS)	7.2%; 0%	Only presymptomatic treatment delayed disease onset (9.7%) and improved motor performance. Ameliorated MNs loss by 46.8%	64

[a]Treatment ages: asymptomatic (Asym), neonatal (N), presymptomatic (Pre), symptomatic (Sym), early symptomatic (ES), and late symptomatic (LS).

AAV, adeno-associated virus; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BB, biceps brachii; BBB score, Basso–Beattie–Bresnahan locomotor rating score; CB, cytomegalovirus enhancer/β-actin promoter; Cb, combined; CBA, chicken beta-actin; CBE, cytosine base editor; CMAP, compound muscle action potential; CMV, cytomegalovirus promoter; CNS, central nervous system; CRISPR, clustered regularly interspaced short palindromic repeats; CT-1, cardiotrophin 1; CTNF, ciliary neurotrophic factor; DAO, D-amino acid oxidase; DCN, deep cerebellar nuclei; DIA, diaphragm; FC, facial muscles; FL, forelimb; FMN, facial motor nucleus; G-CSF, granulocyte-colony stimulating factor; GDNF, glial cell-derived neurotrophic factor; GLT1, glial glutamate transporter 1; GLU, gluteus maximus; GluA2, subunit of the AMPA receptor; GM, gastrocnemius; HGF, hepatic growth factor/hepatocyte growth factor; HL, hindlimb; HP, hippocampus; i.c., intracerebral; ICap, internal capsule; i.c.b., intracerebellar; i.c.m., intracisterna magna; i.c.v., intracerebroventricular; IGF-1, insulin-like growth factor; i.l., intralingual; InC, intercostal muscles; i.p., intrapleural; iPSCs, induced pluripotent stem cells; i.s., intraspinal; i.s.t., intrastriatal; LV, lentivirus; MC, motor cortex; MIF, macrophage migration inhibitory factor; MMP, measurement of mitochondrial function; MNs, motoneurons; MPC, muscle progenitor cells; MSC, mesenchymal stem cells; MuSK, muscle-specific receptor tyrosine kinase; N/A, not applicable; NAb, neutralizing antibodies; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NHP, nonhuman primate; NMJ, neuromuscular junction; NPC, neural progenitor cells; N/R, not reported, NRG1-I, neuregulin type I; NRG1-III, neuregulin 1 type III; NS, neurological score; NT-3, neurotrophin-3; PD, pharmacodynamics; PGK1, phosphoglycerate kinase-1; PhMNs, phrenic motoneurons; PK, pharmacokinetics; pmn, progressive motor neuronopathy; p-NF, phospho-neurofilament; PSM, paraspinal muscles; QD, quadriceps; RSV LTR, Rous sarcoma virus long terminal repeat promoter; SD, Sprague–Dawley rats; SFP, spontaneous fibrillation potentials; SYN, synapsin I; SYT13, synaptotagmin 13; T Age, Treatment Age; TA, tibialis anterior; TB, triceps brachii muscles; TC, thoracic muscles; TG, tongue; t.p., transplant; TS, triceps surae muscle; TU, transducing units; vg, viral genomes.

Table 2. *Gene Silencing and Editing Strategies Tested in ALS Models*

Delivery	*Target*	*Vector (Dose)*	*Promoter*	*Model*	*T. Age*[a]	*Survival*	*Main Findings*	*Reference*
shRNA/siRNA								
SOD1								
i.m.: HL + FC + TG + DIA + InC	Skeletal muscles	RGP-LV (total of 120 µL, 0.7–1 × 10^9 TU/mL)	H1	B6SJL.*SOD1*^{G93A} mice	P7 (N)	77%	Strong protection of MNs and improved motor performance, resulting in a delay in the onset by more than 100%	65
i.s.: bilateral	Lumbar spinal cord	LV (90 ng p24 antigen/ site)	H1	B6SJL.*SOD1*^{G93A} mice	P40 (Pre)	0%	Retarded onset and the progression rate of the disease. Preserved MNs and GM CMAP amplitude	66
i.m.: hindlimbs	Skeletal muscles	AAV1(1 × 10^{10} vg); LV (1 × 10^7 vp)	H1	B6SJL.*SOD1*^{G93A} mice	P40 (Pre)	N/R	AAV1 preserved muscle mass and force whereas LV pseudotyped to lack retrograde transport failed to do so	67
i.t. pump	CNS	Modified siRNA	—	B6SJL.*SOD1*^{G93A} mice	P85 (ES)	6%	Widespread distribution of siRNA knocked down m*SOD1* expression and slowed the disease progression	68
i.c.: 16 sites motor cortex	UMNs	AAV9 (N/R)	H1	SD *SOD1*^{G93A} rat	P70 (Pre)	12%	Significant delay of disease onset and enhanced survival of LMNs and maintenance of NMJs	69
s.p.: lumbar + cervical	CNS	scAAV9 (5.6 × 10^{10} vg)	H1	B6SJL.*SOD1*^{G37R} mice	P120 (Pre)	Corrected	Treatment before onset prevents MND. Preservation of MEP, α-MNs, and interneurons. Suppression of peripheral denervation. No spinal atrophy and misfolded SOD1, and no changes in inflammation or gene expression	70
s.p.: lumbar + cervical	CNS	scAAV9 (5.6 × 10^{10} vg)	H1	B6SJL.*SOD1*^{G37R} mice	P348 (LS)	Corrected	Treatment after onset stops progression, partly preserving strength, escaping paralytic state, and preserving MNs	70
i.c.m	Spinal cord	scAAV9 (8 × 10^{12} vg/kg)	H1	B6SJL.*SOD1*^{G93A} mice	P1 (N)	42%	First report to show no significant off-targets. 88% decrease in MN death. Improved motor and neurological deficits. Decreased gliosis and CSF hSOD1 levels	71
i.c.m	Spinal cord	scAAV9 (5 × 10^{13} vg/kg)	H1	B6SJL.*SOD1*^{G93A} mice	P40 (Pre)	13.8%	Longer latency to fall from rotarod. Decreased body weight loss and enhanced neuroscore	71
i.v.: temporal vein	MNs	AAV9 (3.6 × 10^{14} vg/kg)	H1	B6SJL.*SOD1*^{G93A} mice	P1 (N)	39%	Treatment at all ages improved motor function and retained muscle mass. Only P1 delayed disease onset (38%), due to more efficient MNs *SOD1* silencing	72
i.v.: tail vein	MNs	AAV9 (1.7 × 10^{14} vg/kg)	H1	B6SJL.*SOD1*^{G93A} mice	P21 (Pre)	29.5%	P21 injection slowed disease progression compared with P1, which had less transduced non-neuronal cells	72
i.v.: tail vein	MNs	AAV9 (1.6 × 10^{14} vg/kg)	H1	B6SJL.*SOD1*^{G93A} mice	P85 (ES)	22.7%	Treatment improved motor function and retained muscle mass	72
i.v.: tail vein	MNs	AAV9 (1.1 × 10^{14} vg/kg)	H1	B6SJL.*SOD1*^{G37R} mice	P215 (ES)	22%	Treatment after onset slowed disease progression, preserving weight and force	72
i.v.: tail vain	Broad	AAV6 (2 × 10^{11} vg)	H1	B6SJL.*SOD1*^{G93A} mice	P42 (Pre)	0%	No benefit was conferred	73
i.m.: HL + FL + InC + FC + TC + TG	MNs	AAV6 (2 × 10^7 TU/site, TC: 8 × 10^7 TU)	H1	B6SJL.*SOD1*^{G93A} mice	P1-5 (N)	0%	Protected transduced MNs and myofiber from atrophy, decreased NMJ denervation. No changes in glial reactivity. Delayed loss of TS CMAP and increased weight, but swimming latency was unaltered	74
i.n.: SCN	Spinal MNs	rAd, AAV2 (1.25 × 10^9 vp)	U6	B6SJL.*SOD1*^{G93A} mice	P94 (LS)	7.5%	rAd increased survival and attenuated weight loss, but not AAV2, which targeted fewer MNs	75
FUS								
i.c.: right MC	Cortical MNs	AAV9 (1 × 10^{11}vg)	H1	NHP	57, 73 months.	N/A	∼80% KO across cell types. MC Iba1+ and GFAP+ signal was higher in shFUS-treated cortex than in control-injected left cortex	76
ATXN2								
i.c.v.: bilateral	CNS	PM-AAV9 (2 × 10^{10}vg)	mU6	B6SJL *TAR4/4*	P1-2 (N)	45.5%	Increased strength and vertical activity. Reduced inflammation and pTDP-43	77
FTO								
i.t.: lumbar	CNS	scAAV9 (1 × 10^{11}vg)	H1	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	10%	Reduction of N^6-methyladenosine of RNAs delayed disease onset, preserved MNs, improved grip strength, and decreased gliosis	78
miRNA								
SOD1								
i.l. + i.p.	Respiratory MN	AAVrh10 (1 × 10^{11} vg)	H1	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	36%	Preserved diaphragm NMJ and axons in phrenic and hypoglossal nerves, but not MNs. Enhanced breathing but not lung phenotype	79
i.t.: lumbar infusion	MNs	AAVrh10 (3.5 × 10^{13} vg)	CBA, H1, U6	NHP	N/R	N/A	In MNs silencing correlated with promoter strength (CBA<H1-U6), greater in lumbar regions. Preexisting NAb did not affect distribution or silencing. No off-targets and NAb responses.	80
i.t. lumbar 2 speeds	Spinal cord	AAVrh10 (2.4 × 10^{10} vg; 8 min vs. 30 s)	CBA	B6SJL.*SOD1*^{G93A} mice	P30 (Pre)	8.8%; 5.3%	Slow and fast injections tended to delay onset (11.7 vs. 8.1%) and extend survival, better for slow injections, which displayed longer footprint and slowed weight loss	81
i.c.v.: lateral ventricles	CNS	AAV9 (5 × 10^{10} vg/ventricle)	CBA	B6SJL.*SOD1*^{G93A} mice	P0-1 (N)	50%	Reduction of m*SOD1* mRNA in UMNs and LMNs. Preserved numbers of LMNs, diameter of axons, and attenuated inflammation. Delayed hindlimb paralysis	82

(*continued*)

Table 2. (Continued)

Delivery	*Target*	*Vector (Dose)*	*Promoter*	*Model*	*T. Age*[a]	*Survival*	*Main Findings*	*Reference*
i.v.: tail; i.t.: lumbar (NHP)	Spinal cord	AAVrh10 (i.v.:2×10^{11} vg; i.t.:6×10^{12} vg/kg)	U6, CB	B6SJL.*SOD1*^{G93A} mice; NHP	P50-68 (Pre);<4 years.	20%	In mice, treatment delayed disease onset and extended survival by ~ 20% for both promoters. It preserved limb strength, motor skills, and inspiratory muscle strength. In NHP, 21% (CB) or 93% (U6) silencing were achieved in lumbar MNs	[79]
i.c.v.: lateral left ventricle	MNs	AAV6 (1.6×10^{11} vg);	CMV	(*SOD1*^{G93G})^{dl} mice	P2 (N)	26%	AAV6-CMV corrected TS CMAP and swimming. Postponed disease onset (25%). Spinal MNs, muscle integrity, and NMJ were preserved	[83]
i.c.v.: lateral left ventricle	Astrocytes	AAV9 (6.8×10^{11}vg);	gfaABC$_1$D	(*SOD1*^{G93G})^{dl} mice	P2 (N)	14%	AAV9-gfaABC$_1$D corrected TS CMAP and swimming performance. Spinal MNs, muscle integrity, and NMJ were preserved	[83]
i.c.v.: left lateral	MNs and astrocytes	AAV6 (8×10^{11} vg) + AAV9 (3.41×10^{11}vg);	CMV; gfaABC$_1$D	(*SOD1*^{G93G})^{dl} mice	P2 (N)	10%	The combination corrected TS CMAP, swimming performance, and median survival. Delayed disease onset (12.5%). Spinal MNs, muscle integrity, and NMJ were preserved	[83]
i.t.: lumbar	MNs	AAV9 (2.4×10^{12}vg),	CMV	(*SOD1*^{G93G})^{dl} mice	P35 (Pre)	0%	Preserved MNs and NMJ improved TS CMAP and motor function. Disease onset, progression, and survival were not affected	[83]
i.t.: lumbar	Astrocytes	AAV9 (2.4×10^{12}vg),	gfaABC$_1$D	(*SOD1*^{G93G})^{dl} mice	P35 (Pre)	0%	Improved TS CMAP and motor function, preserved lumbar MNs and NMJ. Disease onset, progression, and survival were not affected. Targeting MNs showed better outcomes	[83]
i.t.: lumbar	MNs	AAVrh10 (2.4×10^{10}vg)	CBA	B6SJL.*SOD1*^{G93A} mice	P65 (Pre)	13.9%	Did not alter disease onset but slowed progression. Extended survival, correlating with transduction	[84]
i.c.v.: left lateral	Astrocytes	AAV9 (4.2×10^{11}vg)	gfaABC$_1$D	B6SJL.*SOD1*^{G93A} mice	P2 (N)	N/R	Rescues NMJ by collateral sprouting, preserves fast-fatigable MU, GM CMAP amplitude, and motor function	[85]
i.c.v.: bilateral	CNS	AAV9-miR30a (5×10^{10}; 1.5×10^{10}; 4×10^{10}; 8×10^{10}; 1.6×10^{11} vg)	CAGG	B6.*SOD1*^{G93A} mice	P0 (N)	13.1; 21.3; 48.8; 58.8; 103.1%	Dose-dependent preservation of TA CMAP amplitude and reduction of NFL serum levels	[86]
C9ORF72								
i.s.t.	CNS	AAV5 (5×10^{10} or 1×10^{10} vg)	CAG	C57BL/6J BAC 112 mice	P90 (Asym)	N/A	20–40% lowering of *C9ORF72* mRNA and 20% less cells containing RNA foci	[87]
i.s.t.; i.c.v.; i.v.: temporal	CNS	AAV9 (1×10^{11}; 2×10^{10}; 2×10^{11} vg)	H1	C57BL/6J BAC 112 mice	4mo (Asym); P1; P1 (N)	N/A	Reduction of all *C9ORF72* mRNAs, protein, and polyGP DPRs	[88]
miR-17~92								
i.t.: lumbar	Spinal MNs	scAAV9 (1×10^{11} vg)	CMV	B6SJL.*SOD1*^{G93A} mice	P60 (Pre)	14%	miR-17~92/PTEN axis is related to MN degeneration and innervation, being dysregulated in *SOD1*^{G93A} mice. Overexpression lightly ameliorated GM CMAP	[89]
ATXN2								
i.t.: lumbar	CNS	AAV-PHP.eB (8×10^{11}vg)	CAG	FVB/NJ *PFN1*^{C71G}	P42-63 (Pre)	N/R	Reduces aberrant TDP-43 and NFL, delaying neurodegeneration and improving weight, strength, clasping, rotarod, and muscle fiber caliber	[90]
ASO								
SOD1								
i.c.v. pump	CNS	ASO (100 μg/d)	—	SD *SOD1*^{G93A} rat	P65-75 (Pre)	8%	Reduced SOD1 protein and mRNA in brain and spinal cord. Significantly slowed disease progression	[91]
i.t. pump	CNS	ASO (0.15 mg, 0.5 mg, 1.5 mg, 3 mg /12 h)	—	Human	Sym	0%	First-in-human clinical phase I trial. Safe, no dose-limiting toxicities. Re-dosing was well tolerated. Dose-dependent CSF ASO concentrations	NCT01041222[92]
i.t. pump	CNS	ASO (20–100 mg/over 12w)	—	Human	Sym	0%	Phase I/II clinical trial. Dose-dependent CSF SOD1 reduction, 33% in the highest group. Decreased rate of decline in ALSFRS-R	NCT02623699[93]
i.t. pump	CNS	ASO (100 mg/ over 24 or 52w)	—	Human	Sym	0%	Phase III clinical trial. Dose-dependent CSF SOD1 and plasma NFL reductions. Did not improve clinical end points	NCT02623699; NCT03070119[94]
i.v.: temporal vein; i.c.v.: lateral	Periphery and CNS	AAVrh10 (i.v.;6.8×10^{10} vg; i.c.v.: 9.75×10^{9} vg)	U7	B6SJL.*SOD1*^{G93A} mice	P1 (N)	92%	Slowed the weight loss observed at the end stage. Delayed disease onset (90%) based on the age of peak body weight	[95]
i.v.: retrobulbar/ tail; i.c.v.: lateral	Periphery and CNS	AAVrh10 (i.v.;7.3×10^{12} vg; i.c.v.: 4.6×10^{11} vg)	U7	B6SJL.*SOD1*^{G93A} mice	P50 (Pre)	58%	Preserved MNs, and myofiber area. Prevented weight loss starting at 15w. Disease onset was delayed (76.5%), but not disease duration	[95]
i.c.v.: lateral ventricle	CNS	ASO (300 μg)	—	B6SJL.*SOD1*^{G93A} mice	P50 (Pre) + P94 (LS); P63 (Pre)	22%	Delayed disease onset (30.7%) and motor performance. Preserved CMAP and NMJ. Reduction of serum p-NF. When treated at P63, CMAP decline and p-NF levels were reversed	[96]
i.t.	CNS	Two different ASO (1 mg)	—	SD *SOD1*^{G93A} rat	P65 (Pre)	32/39%	Postponed disease onset (14.8%) and motor performance decline	[96]
i.t.	CNS	ASO (4, 12, 35 mg; 5 doses)	—	NHP	N/R	N/A	Lowers *SOD1* mRNA in a dose-dependent manner and protein levels by 50% in CNS tissues	[96]

(*continued*)

Table 2. (Continued)

Delivery	Target	Vector (Dose)	Promoter	Model	T. Age[a]	Survival	Main Findings	Reference
C9ORF72								
i.c.v.: right lateral	CNS	ASO (500 mg)	—	WT c57BL/6 female mice	P40	N/A	30–40% reduction in C9orf72 RNA levels in CNS. No neuropathological or behavioral defects. TDP-43 remained nuclear, and no p-62 inclusions detected	[97]
i.c.v.: right lateral	CNS	ASO (350 mg)	—	*C9ORF72* BAC 450 repeat exp. mice	9 months. (Pre)	N/A	Decreased levels of GGGCC RNAs to 20–40% but preserved levels of WT mRNAs with reductions in RNA foci and DPR. Ameliorated behavioral deficits	[98]
i.c.v.	CNS	ASO WV4-004 (up to 100 mg)	—	*C9ORF72* BAC 450 repeat exp. mice	4–5 months. (Pre)	N/A	Dose-dependent reduction in GGGCC transcript levels and in polyGP DPRs in CNS for at least 6 months	[99]
i.c.v: lateral	CNS	ASO (1, 5, 15, 30, or 60 nmol)	—	*C9ORF72* BAC 500 repeat exp. mice	5–6 months. (Asym)	N/A	Reduced repeat-containing transcripts and tissue levels of polyGP DPRs	[100]
i.t.	CNS	ASO (afinersen)	—	Human (G_4C_2 expansion)	60 years.	N/A	Reductions of polyGP DPRs in CSF, stable ALSFRS score, no major adverse effects	[100]
i.t.	CNS	ASO (multiple)	—	Human (G_4C_2 expansion)	Sym	N/R	Phase I/II study to evaluate the safety, tolerability, PK, and PD. Failed to show clinical benefits and associated with elevated NFL levels	NCT04931862
i.t.	CNS	ASO (multiple)	—	Human (G_4C_2 expansion)	Sym	N/R	Phase I study to assess the safety, tolerability, and PK. Failed to show clinical benefits and associated with elevated NFL levels	NCT03626012[101]
FUS								
i.c.v.	CNS	ASO ION363 (20 μg)	—	*FUS* knock-in MN-P517L/Δ14 mice	P1 (N)	N/A	No MN loss, NMJ denervation, and microgliosis at 4 months. At 6 months. ASO levels decrease and microgliosis increases with no MN loss	[102]
i.t.	CNS	ASO ION363 (20–120 mg)	—	Human (FUS^{P525L})	25 years.(Sym)	N/A	First-in-human in patient with ventilatory support. Slowed rate of decline ALS-FRS-R score. Patient died because of advance state	NCT04768972[102,103]
i.t.	CNS	ASO ION363 (multiple)	—	Human	Sym	N/A	Phase I-III clinical trial to evaluate efficacy, safety, pharmacokinetics, and pharmacodynamics	NCT04768972
ATXN2								
i.c.v.: left lateral	CNS	ASO (45 μg)	—	B6SJL *TAR4/4* mice	P1 (N)	35%	Decreased *Atxn2* mRNA levels by 77%. Gait impairment score improved and slowed rate of progression	[104]
i.t.	CNS	ASO (multiple)	—	Human	Sym	N/A	Phase I/II ascending-dose trial to assess safety, tolerability, PK, and progression. No reduction in NFL levels or functional improvement	NCT04494256
TDP-43								
i.c.v.	CNS	ENA-ASO (200 mg)	—	*TDP-43* mice	P42 (Pre)	N/A	Suppresses the formation of cytosolic TDP-43 inclusions, normalizes weight, anxiety-like behavior, and locomotion but not the strength	[105]
STMN2								
i.c.v.	CNS	ASO (1,000 μg)	—	$Stmn2^{Hum\Delta UG/+}$ mice	P60 (Asym)	N/A	Corrected *Stmn2* cryptic misprocessing, restoring protein levels. Recovered axonal regeneration and lysosome trafficking in TDP-43 deficient iPSC-MNs	[106]
CRISPR/Cas								
Cas9-SOD1								
i.v.: facial vein	Broad	AAV9-2YF (4×10^{11} vg)	CMV U6-sgRNA	B6SJL.$SOD1^{G93A}$ mice	P0-P1 (N)	25%	Reduced mSOD1 protein by >2.5-fold in the spinal cord. ∼50% more MNs. Improved motor function, reduced muscle atrophy, and delayed onset (37%)	[107]
i.c.v.: lateral ventricle	CNS	AAV9 (1.2×10^{11} vg)	CMV U6-sgRNA	B6SJL.$SOD1^{G93A}$ mice	P0 (N)	22%	Delayed onset and prolonged survival, but disease duration was shortened. Hindlimb stretch and stride length were improved. Decreased $Iba1^+$ cells and hSOD1, increased spinal MNs	[108]
i.c.v.: lateral ventricles	CNS	AAV-PHP.B (2×10^{11} vg)	U6-sgRNA	B6SJL.$H11^{Cas9}$ $SOD1^{G93A}$ mice	P0 (N)	66 and 85%	Prevents muscle atrophy and NMJ and MN loss. Prevents motor function decline	[109]
i.t.	CNS	AAV-PHP.eB (1×10^{12} vg)	U6-sgRNA	B6SJL.$H11^{Cas9}$ $SOD1^{G93A}$ mice	P35 (Pre)	≥ 200%	Fully preserves neuromuscular function. Prevented body weight loss and TA CMAP decline. ∼80% SOD1 reduction in the spinal cord and cortex	[109]
i.v.	Broad	AAV-PHP.eB (1×10^{12} vg)	U6-sgRNA	B6SJL.$H11^{Cas9}$ $SOD1^{G93A}$ mice	P35 (Pre)	≥ 200%	Same favorable outcomes as i.t. administration. Animals had to be euthanized at P340	[109]
RfxCas13d-SOD1								
i.t.: lumbar	CNS	AAV9 (2×10^{11} vg)	CAG/U6-sgRNA	B6SJL.$SOD1^{G93A}$ mice	P60-65 (Pre)	65%	Strong hSOD1 depletion. Improves rotarod performance, disease duration, and body weight loss	[110]

(*continued*)

Table 2. (Continued)

Delivery	*Target*	*Vector (Dose)*	*Promoter*	*Model*	*T. Age*[a]	*Survival*	*Main Findings*	*Reference*
CBE-hSOD1								
i.t.: lumbar	Spinal cord	Dual AAV9 (8×10^{10} vg)	CAG	B6SJL.*SOD1*^{G93A} mice	P56-60 (Pre)	11%	Disease onset not delayed. Improved rotarod and grip strength. Less SOD1 inclusions and astrogliosis. Rescue of MNs and denervation of NMJ	111
Cas9-C9ORF72								
i.st.	CNS	AAV9-gRNA (7×10^{9} vg) + AAV9-Cas9 (2×10^{10} vg)	U1a/ U6-sgRNA	BAC111/Cas9, BAC112, and C9-500 mice	2–3 months (Asym)	N/A	Decreased levels of poly-GR DPRs and RNA foci number	112
i.c.v.: bilateral	CNS	AAV-PHP.eB (8×10^{9} vg)	Short CAG/ U6-sgRNA	C57BL6/J 149R and FVB/NJ C9-500 BAC mice	P0 (N)	N/A	Reduces *C9ORF72* sense and antisense repeats and polyGP and polyGA DPRs. Protects iPSC-derived MNs from excitotoxicity	113
i.c.: bilateral	MC, HP	AAV-PHP.eB (2×10^{10} vg)	CAG/ U6-sgRNA	C57BL6/J C9-BACexp mice	2 months (Asym)	N/A	Decreased expression of sense repeat containing *C9ORF72*, RNA foci, and polyGP DPRs	114
RfxCas13d-ATXN2								
i.v.: facial vein i.c.v.: lateral	CNS	AAV9 (8.4×10^{10} vg i.c.v.; 7×10^{11} vg i.v.)	CBh	B6SJL *TAR4/4* mice	P1-2 (N)	137%	Improved gait, motor coordination, body weight, and strength, while decreasing kyphosis and tremors	115
TDP-REG and TDP-43/Raver 1								
i.c.v.: left	Spinal MNs	AAV-PHP.eB ($1-3.1 \times 10^{11}$ vg)	SYN/CMV	TDP-43$^{fl/wt}$;Chat-Cre$^{+/wt}$ mice (Tardbptm1.1Pcw/J)	P0-2 (N)	N/A	TDP-REG activates gene expression in TDP-43–deficient cells using cryptic exons. Unc13A mis-splicing and splicing homeostasis were corrected via prime editing with a self-regulated TDP-43/Raver1 fusion protein	116
Proteasomal Degradation								
DD-θFx (degron fused to 14–3-3θ)								
i.c.v.: bilateral	CNS	AAV-PHP.B ($2-3 \times 10^{10}$ vg)	CAG	C57BL/6 *iTDP-43*^{A315T} mice	P0-2 (N)	N/R	Improved wire test and grip strength, reduced disinhibition and hyperactivity. Reduced TDP-43 around 75%	117
i.v.: tail	CNS	AAV-PHP.B (5×10^{12} vg)	CAG	C57BL/6 *iTDP-43*^{A315T} mice	3mo Sym	N/A	Improved disinhibition. Hyperactivity and motor deficits unchanged	117
i.v.: temporal	CNS	AAV-PHP.B (1.5×10^{10} vg)	CAG	C57BL/6 AAV-*TDP-43* mice	P0-2 (N)	N/R	Improved wire test, grip strength, and muscle mass	117
i.v.: temporal	CNS	AAV-PHP.B (1.5×10^{10} vg)	CAG	B6;C3. *rNLS8* mice	P0-2 (N)	38.5%	Delayed paralysis onset (63.9%)	117

[a]Treatment ages: asymptomatic (Asym), neonatal (N), presymptomatic (Pre), symptomatic (Sym), early symptomatic (ES), late symptomatic (LS).

ASO, antisense oligonucleotide; shRNA, short hairpin RNA; siRNA, small interfering RNA.

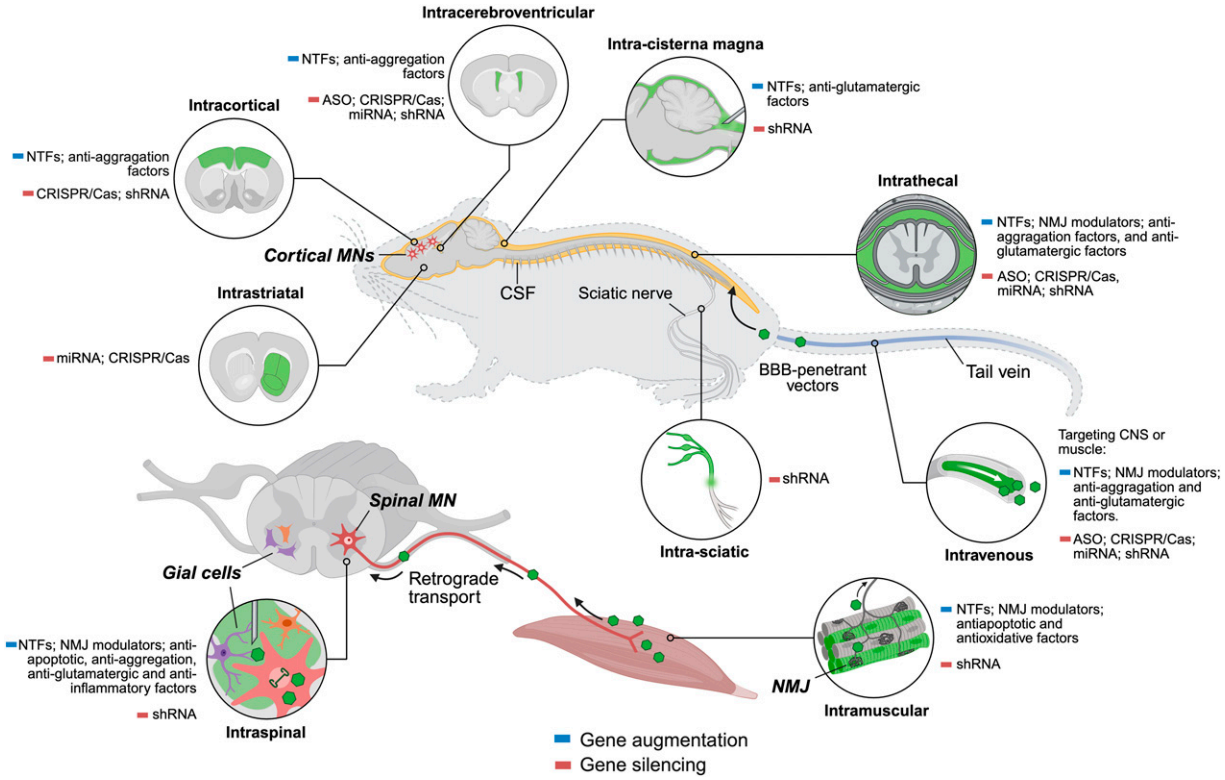

Figure 1. Gene therapy strategies tested in ALS: delivery routes and target cells and tissues. A range of gene supplementation and silencing approaches have been explored in ALS preclinical models, leveraging intraspinal, intracerebral, intrathecal, intracisternal magna, intracerebroventricular, or intramuscular delivery routes. Created in BioRender (https://BioRender.com/ynj06t6). Verdés, S. (2025). ALS, amyotrophic lateral sclerosis; ASO, antisense oligonucleotide; BBB, blood–brain barrier; CNS, central nervous system; CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein; CSF, cerebrospinal fluid; miRNA, microRNA; MN, motor neuron; NMJ, neuromuscular junction; NTFs, neurotrophic factors; shRNA, short hairpin RNA.

filamentous actin, have been identified in several familial ALS cases. *PFN1* mutations were modeled in mice (*e.g., G118V or C71G)* reproducing many key ALS features, including lower and upper MNs loss, abnormal protein ubiquitination, reduced choline acetyltransferase expression, gliosis, muscle atrophy, impaired motor performance, and reduced survival.[90,128,129]

Despite the diversity of genetic models, m*SOD1* mice containing the G93A mutation are the most used in ALS preclinical research due to their well-defined and reproducible ALS phenotype, which for other models generally only becomes apparent at an advanced age.[118,130] However, the poor translation of therapeutic efficacy from m*SOD1* mice to positive results in clinical trials has raised concerns about its representativeness. With more accumulated experience, future preclinical assays should incorporate more than one model reflecting other ALS-linked mutations to better predict clinical outcomes. Given that *SOD1* mutations account for <2% of ALS cases, most therapeutic approaches—beyond those blocking the defective *SOD1* gene—aim to be generalized to sporadic cases by demonstrating delay in motor deficits and MN degeneration that are the essential alterations in ALS and related diseases.

Mechanistically, these models have been useful to describe a wide variety of cellular pathways contributing to MN degeneration, including glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction, protein misfolding and impaired protein homeostasis, RNA metabolism defects, ER stress, deficits of axonal transport, and neuroinflammation (Fig. 2). These mechanisms may not be mutually exclusive, although it remains unclear whether a unique event triggers all these processes. This topic has been the focus of several review articles and here we only include a brief summary.[131–136]

Enhanced excitatory transmitter's input induces a massive calcium influx into the cytoplasm that damages the cells through the activation of calcium-dependent proteases, lipases, and nucleases. MNs are particularly sensitive due to low calcium buffering capacity and highly permeable α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. Elevated glutamate levels in CSF of patients with ALS, overactivity of NMDA and AMPA receptors, and reduced astrocytic glutamate uptake support this mechanism. The first drug approved for the treatment of ALS, Riluzole, mainly acts by reducing excitotoxicity. Excitotoxicity has also been linked to mutations in genes such as *GRIA2*, *DAO* (D-amino acid oxidase), and *SLC1A2* (excitatory amino acid transporter 2, EAAT2), which affect glutamate receptor function and astrocytic glutamate clearance.

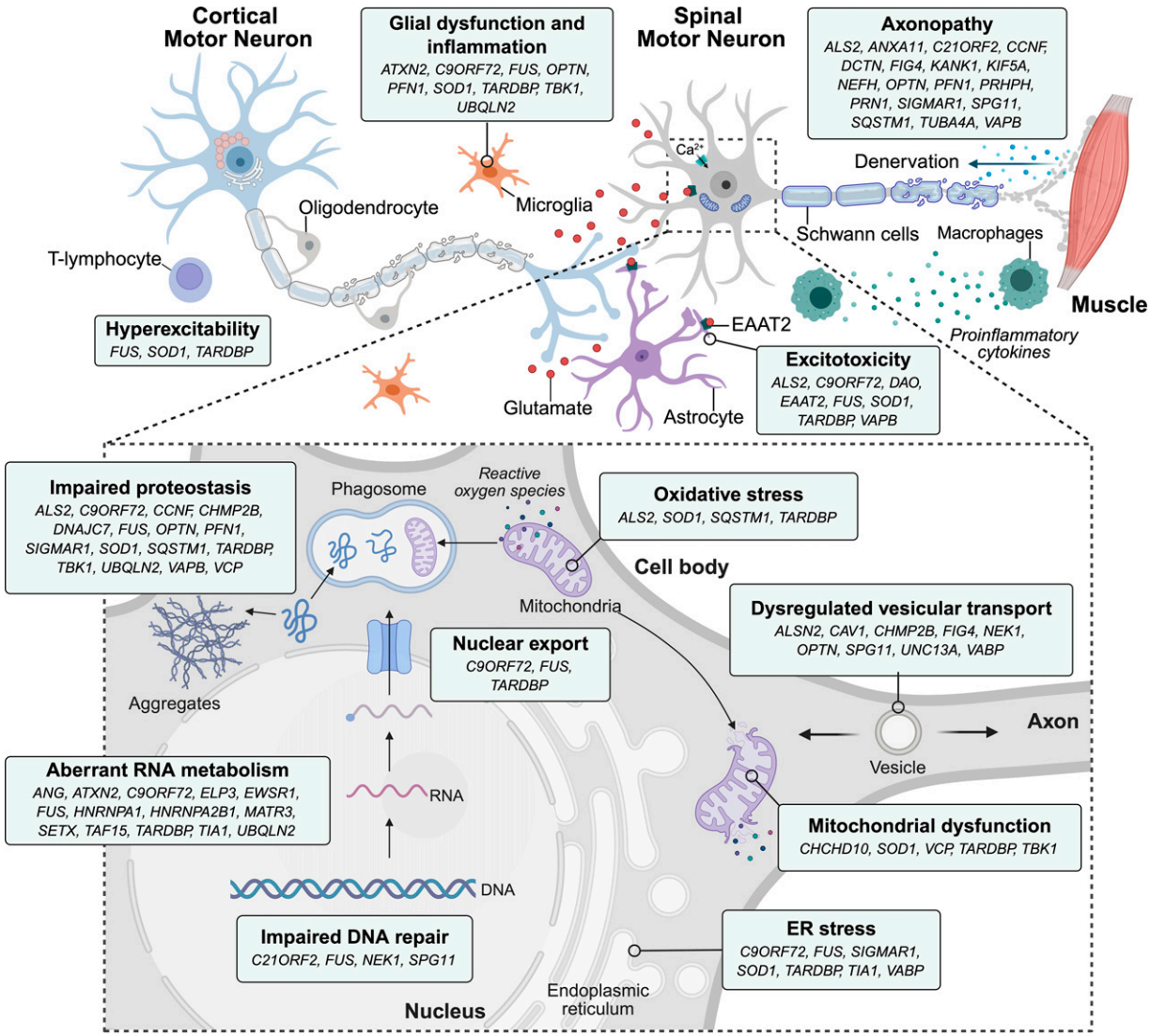

Figure 2. ALS mechanisms and associated genes. Multiple molecular and cellular pathways contribute to motor neuron degeneration in ALS, with many ALS-associated genes (*e.g., SOD1, C9ORF72, TARDBP,* and FUS) involved in overlapping mechanisms. Key pathological processes include excitotoxicity, glial dysfunction, inflammation, oxidative stress, impaired proteostasis, mitochondrial dysfunction, aberrant RNA metabolism, endoplasmic reticulum (ER) stress, DNA repair deficits, and axonopathy. Main genes linked to each mechanism are indicated in the corresponding boxes. Created in BioRender (https://BioRender.com/fusu0u5). Verdés, S. (2025).

Oxidative stress damage to proteins, lipids, and DNA also occurs in patients with ALS, as well as in ALS mouse models. Reactive oxygen species are increased in the CSF and serum of patients with ALS. Mitochondrial abnormalities—such as respiratory chain complex deficits—have been described in the spinal cord and in skeletal muscle of patients with ALS. Experiments performed in m*SOD1* mice also revealed early mitochondrial abnormalities prior to symptoms onset. DPRs in *C9ORF72*-associated models also compromise mitochondrial function and cause oxidative stress and DNA damage. Other genes such as *CHCHD10*, *OPTN*, and *VCP* have been implicated in mitochondrial dysfunction and redox imbalance, further supporting mitochondrial stress as a pathogenic driver.

The ER and the mitochondrial network are highly interconnected at the mitochondria-associated ER membrane (MAM). MAM disruption leads to an imbalanced Ca^{2+} flux into the mitochondria affecting energy production and leading to lower ATP content, and it has been found in models with mutations in *SOD1*, *TARDBP*, *FUS,* and *VAPB*. Promoting stability of the MAM, administering ligands of the Sigma1-receptor has shown therapeutic benefit in ALS mice.[137,138]

Protein aggregates or ubiquitin-positive inclusions involving TDP-43, SOD1, FUS, ubiquilin 2 (*UBQLN2*), neurofilaments, and C9ORF72-related DRPs are well-established pathological hallmarks of ALS. While their role as primary toxic species is debated, high-molecular-weight complexes preceding aggregate formation are considered likely contributors to toxicity, potentially via prion-like intercellular propagation mechanisms.

TDP-43 cytoplasmic inclusions are present in neuronal and non-neuronal cells of patients with ALS, excluding those linked to *SOD1* and *FUS* mutations. In contrast,

wild-type TDP-43 is predominantly localized in the nucleus where it plays essential roles in RNA processing, including transcriptional regulation, alternative splicing, and miRNA processing. Other ALS-linked genes involved in RNA metabolism include *FUS*, *EWSR1*, *HNRNPA1*, *HNRNPA2B1*, and *TIA1*, which are known to form stress granules and impact RNA transport and splicing. In *C9ORF72* ALS, repeat containing RNA foci sequestering RNA-binding proteins and repeat-associated non-AUG translation further perturb RNA metabolism and protein homeostasis.

The accumulation of misfolded proteins elicits the ER stress response, activating the unfolded-protein response (UPR), initially protective but becoming a maladaptive prodegenerative program under sustained stress. UPR markers are upregulated in patients with ALS as well as in mutant SOD1 mice. Interestingly, upregulation of several UPR markers occurs before muscle denervation in vulnerable large motor units but not in resistant ones, suggesting a role for ER stress in determining MNs' susceptibility to degeneration.

Axonal transport defects also contribute to pathology. Different genes associated with familial ALS, such as *PFN1*, *DCTN*, *TUBA4A*, *KIF5A,* and *NEFH*, are involved in axonal transport. Several works have demonstrated accumulation of neurofilaments in MN and abnormalities of organelle axonal trafficking in patients with ALS, suggesting impaired cytoskeletal dynamics.

Finally, neuroinflammation is a consistent pathological ALS feature and has been proposed as a therapeutic target. MN degeneration initially leads to the activation of microglia, astrocytes, and the complement system, further contributing to the disease progression. Spinal cord and CSF from ALS cases present increased microglial activation and T cell infiltration, as well as higher concentration of proinflammatory mediators. Glial cells appear to play a crucial role in MN degeneration, as selective suppression of m*SOD1* in MNs delays disease onset without affecting progression, whereas deletion in microglia and macrophages slows disease progression without altering onset. Complex signaling between CNS-resident immune cells and peripheral cells, including monocytes and T cells, has been reported. Thus, macrophages infiltrate peripheral nerves before spinal cord microgliosis in ALS mice. Astrocytes are likewise implicated in ALS pathogenesis. Astrocytes derived from both familial and sporadic patients with ALS are toxic to MNs *in vitro*, and blocking m*SOD1* expression in these cells exerts neuroprotective effects.

GENE SUPPLEMENTATION STRATEGIES

The delivery of exogenous genes and their supraphysiological expression has been mainly centered on enhancing the production of neuronal survival and neurotrophic factors (NTFs), as well as modulators of NMJs. In contrast, alternative strategies aiming to prevent apoptosis, impede protein aggregation, or promote glutamate clearance have been studied to mitigate the insidious disease progression and the selective MN degeneration. These approaches are independent of the etiology of the disease; however, they are unlikely to provide a definitive cure, but rather they hold therapeutic potential by preventing motor function loss and extending survival.

Neurotrophic factors

Neurotrophic factors are small proteins that support the growth, survival, synaptic plasticity, and differentiation of neurons. A variety of NTFs have been shown to promote survival and axonal regeneration of damaged neurons *in vitro* and in animal models. The concentration of certain NTFs and growth hormones is altered in the CSF and blood of patients with ALS, as well as in ALS animal models.[139] Supplementation with these factors as recombinant proteins can support the ailing neuromuscular function, but inadequate dosing and delivery have led to unsuccessful therapeutics with important side effects.[140] Viral-mediated delivery can finely and precisely express NTFs in the desired tissues, avoiding constant readministration.

Glial cell line-derived neurotrophic factor (GDNF) is one of the most potent neurotrophins (NT), and its delivery through different routes and targeting strategies has been experimentally assessed in several studies. In transgenic *SOD1*^{G93A} murine models, overexpression of GDNF in the spinal cord has been tested, either through systemic administration of AAV9 vectors,[14,141] through i.s. transplantation of LV-modified stem cells,[12,15,17] or direct injection of LV vectors.[11] Despite robust GDNF expression in these studies, the resulting MN preservation and improvement in motor function were modest or even negligible in LV-*GDNF*-NSC-transplanted human patients (NCT02943850).[142] Moreover, whole-body overexpression in rats following systemic AAV9 delivery was associated with slower weight gain and reduction in activity and working memory, indicating that systemic administration of some growth factors may lead to adverse effects. To minimize such side effects and with the aim to promote axon guidance and GDNF retrograde transport, several groups opted for i.m. injections of AAVs, Ad, or *ex vivo*-modified MSCs in m*SOD1* murine models. When administered into a single muscle, these approaches failed to produce clinically meaningful benefits, despite the preservation of MNs innervating the targeted muscle.[143] On the contrary, simultaneous administration into multiple limb muscles or combined with intercostal or paraspinal muscles delayed disease onset and extended survival by an average of 2 weeks.[6,8,13,144] Building on this concept, more recently we achieved widespread GDNF overexpression across all skeletal muscles through systemic administration of AAV8 vectors, using the muscle-specific desmin promoter to restrict expression to muscle tissue.

This approach effectively preserved MNs, NMJs, and the compound muscle action potential (CMAP) amplitudes, resulting in delayed disease onset and slowed disease progression.[16]

By applying analogous methods, insulin-like growth factor 1 (IGF-1) was evaluated through i.m. injections into various muscles of *SOD1*^{G93A} rodents using AAV or LV vectors. One particularly interesting study used AAV2 to retrogradely target MNs, resulting in a substantial 37-day extension of lifespan and a 31-day delay in the onset of motor deficits. However, the therapeutic benefits were markedly reduced when treatment was initiated at symptoms onset.[10] Subsequent studies employing alternative AAV serotypes failed to replicate these robust outcomes to the same extent but consistently demonstrated that IGF-1 contributed to the preservation of MNs, improved gait and rotarod performance, and modestly extended survival.[21,23,24] Similar findings were replicated with IGF-2—which signals through the same pathway—delivered i.m. with AAV9 vectors,[25] further supporting the neuroprotective role of the IGF1-PI3K-Akt pathway in MN maintenance and axonal regeneration. When focusing directly on MNs, i.s. injections of AAV2 resulted in the preservation of this particular cell type only within the treated spinal segments.[20] Notably, broader therapeutic benefits, such as delayed disease onset, slowed progression, and prolonged survival, were observed when IGF-1 was delivered i.s. to the lumbar spinal cord via AAV2[18] or systemically through i.v. administration of self-complementary (sc) AAV9 vectors.[22]

The protective effects of IGF factors have been postulated to be partly dependent on the vascular endothelial growth factor (VEGF) signaling. Azzouz and collaborators were the first to demonstrate the therapeutic potential of VEGF on ALS, using retrogradely transported LV vectors encoding *VEGF* injected into various muscles of *SOD1*^{G93A} mice.[29] This strategy resulted in a 38-day extension of lifespan and a 28-day delay in the disease onset, comparable to the outcomes reported by Kaspar *et al*. with IGF-1. In a different model, AAV-*VEGF* vectors were injected i.c.m., i.v., or i.c. into the motor cortex of limb-expression 1-deficient feline models of lower MND. Although i.c. AAV1 injection confirmed anterograde transport along the corticospinal pathway, VEGF expression in the spinal cord remained low, similar to the levels observed following i.v. scAAV9. On the contrary, i.c.m. administration of AAV1 and scAAV9 vectors yielded robust transduction of the entire spinal cord, yet no therapeutic clinical benefit was detectable by any of these strategies.[30] Conversely, in the *SOD1*^{G93A} mouse model, i.t. administration of scAAV9-VEGF fostered survival and motor performance,[31,32] but to a much lesser extent than that observed with i.m. LV-*VEGF* vectors. Interestingly, i.c.v. coadministration of IGF-1 and VEGF delayed motor decline and extended survival although not in an additive manner, further emphasizing the possibility that these NTFs may act through overlapping signaling pathways.[53]

Hepatocyte growth factor (HGF) is a multifunctional NT that has also been delivered i.t. and i.m. to *SOD1*^{G93A} mice using AAV vectors. Contrary to other growth factors, i.m. injections at three bilateral sites and retrograde transport of AAV6-*HGF* showed no effects on muscle strength and only modest signs of improvement in motor symptoms and survival.[26] This limited efficacy may be attributed to the substantially lower AAV doses in this study, 10- to 100-fold less per muscle compared with similar strategies. In contrast, i.t. administration of AAV1-*HGF* was reported to enhance rotarod, hanging wire and grip strength tests, and modestly extended lifespan.[27] More recently, the same authors investigated the underlying mechanisms of this treatment, demonstrating that i.t.-delivered AAV9-*HGF* attenuates gliosis, reduces ubiquitin accumulation, prevents vacuolization of MN apical dendrites, and mitigates upper MN loss in the motor cortex of prp*TDP-43*^{A315T} mice.[28] This supports the capacity of i.t. HGF delivery to modulate cortical pathology. Similarly, the i.s. administration of AAV1 encoding granulocyte-colony stimulating factor (*G-CSF*) to *SOD1*^{G93A} mice boosted muscle strength, delayed paresis, and increased survival by 10%, whereas i.m. delivery failed to transduce or preserve MNs.[36] Other NTFs—such as NT-3, cardiotrophin-1, and ciliary neurotrophic factor—have been less extensively studied, but they demonstrated comparable therapeutic outcomes in ALS mouse models when overexpressed in muscle. However, they were delivered using Ad vectors and administered postnatally.[33–35]

Multifactorial gene therapies for ALS have also been pursued, aiming for a more effective and holistic approach. When a combination of LV-modified MPCs populations expressing GDNF, VEGF, IGF-1, or BDNF was i.m. transplanted only into the gastrocnemius muscle (GM) muscle of SOD1^{G93A} mice, it resulted in a significant preservation of both NMJ and MN axons. This approach delayed disease onset and extended survival by 10 days.[54] A similar approach involving a lower number of GDNF- and VEGF-MPC cells injected to the tibialis anterior, triceps brachii, and longissimus thoracis of SOD1^{G93A} rats demonstrated the preservation of MNs and NMJs as well, delaying motor decline and extending survival by up to 28 days.[55]

Modulators of the NMJ

Modulation of the NMJ has emerged as a promising gene therapy strategy for ALS. This approach is based on the "dying-back" hypothesis that maintains that the progressive death of MNs in ALS is preceded by failure and detachment of NMJs and axonal retraction, depending upon defects in the interaction of motor axons with terminal Schwann cells and skeletal muscle fibers. Thus, therapeutic

strategies have been developed attempting to maintain the functional contact of the NMJ.

Several studies have implicated Neuregulin 1 (Nrg1) isoforms in neuromuscular health, with isoform I (Nrg1-I) contributing to the development and maintenance of the NMJ and isoform III (Nrg1-III) playing a role in MN preservation.[145] Loss-of-function mutations in *ErbB4*—a receptor for Nrg1—have been linked to a genetic form of ALS.[146] In a previous study, we demonstrated that i.m. injection of AAV1-*Nrg1-I* into the GM enhanced NMJ innervation and increased CMAP amplitude via collateral sprouting, without conferring protection to axons or MNs.[38] Notably, only systemic AAV8-mediated Nrg1-I overexpression across all skeletal muscles preserved MNs, neuromuscular function, and locomotor performance, ultimately delaying disease onset.[40] The i.t. infusion of AAV9-*Nrg1-III* produced similar benefits in female mice,[41] whereas the i.s. delivery of AAV1-*Nrg1-III* extended lifespan in both sexes.[39] Noteworthily, combining AAV8-*Nrg1-I* and AAV9-*Nrg1-III* along with dual-route administration (i.v. and i.t., respectively) failed to yield additive or synergistic effects.[147]

In an alternative strategy, docking protein 7 (DOK7), essential for NMJ formation, was ubiquitously expressed following AAV9 tail vein delivery. While DOK7 did not prevent MN loss, it protected from nerve terminal and muscle atrophy, clustering AChR and enlarging NMJs, resulting in a modest increase in lifespan (7.5%) and motor activity.[42] More recently, Chen *et al*. overexpressed nuclear receptor interaction protein (NRIP) in the skeletal muscles of *SOD1*^{G93A} mice.[43] NRIP, critical for sarcomere integrity and NMJ stability through AChR binding, is markedly downregulated in *SOD1*^{G93A} spinal cord and muscle, and its loss results in progressive MN degeneration. The i.m. AAVDJ/8-*NRIP* delivery to fore- and hindlimb muscles improved locomotor activity, enlarged myofibers, reduced NMJ and axonal degeneration, and preserved MNs and CMAPs but did not sustain grip strength or extend survival.

Neuroprotective strategies

Other researchers have focused on preventing MN death rather than promoting their survival. Azzouz *et al*. exemplified this approach by delivering bilateral i.s. injections of AAV2 into the lumbar spinal cord to overexpress the antiapoptotic protein Bcl-2.[44] This strategy rescued nearly 50% of the MNs, preserved CMAP amplitude, and successfully delayed disease onset. However, local Bcl-2 expression alone was insufficient to extend overall survival. In a more recent investigation, muscle overexpression of synaptotagmin 13 (SYT13)—a vesicular trafficking protein enriched in extraocular MNs and important for synapsis and vesicle metabolism—was tested in both ALS and SMA mouse models. Gene therapy with *SYT13* led to a 14% extension in lifespan in ALS mice and a remarkable 50% in the SMA model by decreasing MN apoptosis and mitigating muscle denervation.[45]

Several studies have specifically investigated strategies aimed at reducing overall toxic protein aggregation in the spinal cord of ALS mice. Among these, single-chain fragment variable (scFv) antibodies have been used to directly target aggregated forms in different preclinical models of ALS, effectively preventing their propagation and toxic effects.[46,148–150] Notably, the study by Patel *et al*. delivered scAAV1 vectors to achieve sustained expression of scFv antibodies against m*SOD1* in the spinal cords of presymptomatic adult *SOD1*^{G93A} mice, resulting in a significant 28% extension in survival and delayed disease onset.[46] Since this treatment targets the underlying cause of the disease in this model, its relevance is therefore narrowed to mutations in the *SOD1* gene. A related strategy involved the delivery of serine-rich chaperone protein 1 to the motor cortex via AAV9 vectors, which resulted in only modest reductions in SOD1 aggregates and failed to produce any survival benefit, highlighting the limitations of this chaperone and/or a suboptimal CNS coverage.[49]

Another protein with chaperone-like properties, macrophage migration inhibitory factor (MIF), has also shown promise. MIF expression is reduced in human induced pluripotent stem cells (iPSC)-derived MNs from familial patients with ALS, as well as in postmortem tissues from individuals with sporadic ALS. In an initial study, *MIF* was delivered via AAV2 vectors i.s. to postnatal day 0 (P0) *SOD1*^{G93A} mice, resulting in a significant delay in disease onset and a 20% extension in survival.[47,151] A subsequent study used the blood–brain barrier (BBB)-penetrant AAV-PHP.eB vector to achieve widespread CNS delivery of MIF in *SOD1*^{G37R} mice.[48] This approach reduced neuroinflammation, rescued MNs, and corrected dysregulated pathways, as shown through omics analyses. However, in this case, survival extension was more modest (6.8%), likely due to administration at symptoms onset. While this approach is more clinically relevant, it may limit capacity to clear pre-existing m*SOD1* aggregates. Further research would elucidate whether this strategy can be applied to other ALS models characterized by the accumulation of misfolded proteins.

Increasing autophagy through enhanced expression of optineurin (OPTN) has been proposed as a potential therapeutic strategy in ALS. Mutations in the *OPTN* gene have been reported in patients with ALS, associated with low autophagic activity. OPTN plays a role in mitophagy and in the recruitment of ubiquitinated substrates for autophagosome-mediated degradation. In ALS, however, OPTN colocalizes with ubiquitin and TDP-43-positive inclusions, suggesting sequestration and dysregulation of its function. In *SOD1*^{G93A} mice, OPTN is increased during the presymptomatic phase but declines after disease onset.[64] Two recent studies

overexpressed OPTN in the spinal cord of *SOD1*^{G93A} mice. One study delivered AAV9 vectors i.t. at a presymptomatic age, improving stride length, rotarod performance, and extending lifespan by 10.5%.[63] The second study delivered *OPTN* using LV vectors administered into the right lateral ventricle. Presymptomatic LV-*OPTN* treatment postponed disease onset and resulted in only a 7.2% increase in survival, despite increasing by 46.8% the preservation of spinal MNs at disease onset. Notably, symptomatic administration failed to produce significant effects on disease progression or survival.[64] These findings indicate that OPTN expression may be beneficial before disease onset and were attributed to enhanced autophagic and mitophagic flux, preserved mitochondrial morphology, anti-inflammatory responses, and reduced apoptosis.

Additional gene therapy strategies have aimed to counteract glutamate excitotoxicity, a key pathogenic mechanism in ALS. DAO, EAAT2, or adenosine deaminase RNA-specific B1 (ADAR2) were overexpressed with the help of AAV vectors in the CNS.[50–52] DAO metabolizes D-serine, a coagonist of NMDA receptors involved in excitatory neurotransmission; EAAT2 is exclusively expressed in astrocytes and serves as the primary transporter responsible for clearing extracellular glutamate; and ADAR2 is essential for editing the Q/R site of GluA2 mRNA, which determines the calcium permeability of AMPA receptors. Despite the rationale behind these antiglutamatergic strategies, none resulted in significant improvements in motor strength or locomotor function, and only DAO provided a modest extension of survival in m*SOD1* mice.

Using a less conventional strategy, Benkler and coauthors delivered a multifactorial cocktail i.c.m. to the CNS and i.m. to the GM muscles to address the excitotoxic and oxidative axes.[56] The mixture contained LV vectors carrying *EAAT2*, glutamate-dehydrogenase 2 (*GDH2*), and nuclear factor-related factor 2 (*NRF2*) genes. While EAAT2 is related to glutamate uptake, GDH2 metabolizes glutamate and reduces its bioavailability. NRF2, a vital regulator of antioxidant and anti-inflammatory pathways, was selected to address oxidative stress. This combination produced notably remarkable outcomes, as all three genes together but not separately prolonged survival in *SOD1*^{G93A} mice by an average of 19–22 days. This was accompanied by improvements in all neurological and motor parameters evaluated.

Collectively, gene overexpression therapies targeting skeletal muscles in m*SOD1* models have generally yielded more favorable outcomes than CNS-directed strategies, particularly when delivering NTs or using retrogradely transported viral vectors. Furthermore, some of the strategies offered cumulative effects with combinatory treatments, underscoring the significance of synergistic, multitargeted approaches in addressing the complex pathophysiology of ALS.

GENE SILENCING STRATEGIES

SOD1 silencing

A rational approach for treating ALS involves silencing genes that contribute to the disease through a gain-of-function mutation, particularly those responsible for familial forms of ALS. As the first-identified gene linked to ALS, *SOD1* has been silenced by multiple studies. In this context, the paradigm shifts toward evaluating and prioritizing the degree of silencing and survival as primary outcomes. The first two studies, concurrently published, delivered RNAi therapy against *SOD1* in *SOD1*^{G93A} mice using LV vectors. The study with more favorable outcomes targeted multiple muscles (face, tongue, diaphragm, hindlimb, and intercostal muscles) at a postnatal stage using LV based on the equine infectious anemia virus (EIAV), which is known to undergo retrograde transport following i.m. injection, resulting in up to a 77% increase in survival and a delay in disease onset of more than 100%.[65] The other study focused on bilateral i.s. injections to the lumbar spinal cord of adult mice before the onset of motor dysfunction. Although it did not significantly extend survival, it retarded onset and slowed disease progression.[66]

In line with the importance of vector tropism and transport properties, Miller et al. investigated muscle-specific toxicity by comparing AAV1 and murine LV vectors in *SOD1*^{G93A} mice.[67] As murine LV lacks retrograde transport capacity, i.m. injection restricted siRNA expression to muscle, whereas AAV1 enabled silencing in both muscle and spinal motor neurons. Only the latter improved grip strength and muscle mass. Furthermore, Cre-mediated knockdown of floxed m*SOD1*^{G37R} specifically in muscle had no impact on functional outcomes. Similarly, AAV-mediated follistatin overexpression enhanced muscle mass and strength but did not affect onset or survival, suggesting that muscle is not a major contributor to noncell-autonomous toxicity in this model.

As the i.s. injection into a single or few spots can only cover a small fraction of MNs, a different study directly infused modified small interfering RNAs (siRNAs) intrathecally at the symptoms onset using a pump-based delivery system. siRNAs efficiently knocked down *SOD1*, slowed down the progression, and slightly extended survival.[68] Interestingly, Wu and colleagues attempted to reach MNs by an intrasciatic nerve injection in an aim to cover more MN pools and use lower viral doses.[75] They demonstrated that Ad-U6-sh*SOD1* targeted lumbar MNs more efficiently than AAV2, resulting in a deceleration of body weight loss and a 6% increase in survival.

Subsequent studies have focused exclusively on AAV-mediated delivery of shRNA. Unexpectedly, and contrasting to results obtained with EIAV-LV vectors, injection of AAV6-H1-sh*SOD1* muscles across the body of *SOD1*^{G93A} mice failed to improve motor performance or extend survival.[74] It is unlikely that the discrepancy in

therapeutic outcomes can be solely attributed to differences in MNs transduction efficiency, as the initial LV study reported transduction in over 50% of MNs versus 40% in the AAV study. Conducting a thorough characterization of the lower MN axis following vector transduction would help to determine whether a threshold of MN transduction is required for therapeutic benefits via i.m. delivery and retrograde transport.

Initially, i.v. delivery of AAV-sh*SOD1* vectors conferred no clinical benefits,[73] probably attributed to the use of the AAV6 serotype, which does not cross the BBB efficiently. Conversely, i.v. administration of slightly higher doses of AAV9-sh*SOD1* improved motor function, retained muscle mass, and significantly extended survival in *SOD1* transgenic mice. This effect was age-dependent, ranging from 22% to 39% across the postnatal age to the diseased age spectrum. Interestingly, only mice treated at P1 exhibited delayed disease onset due to more effective MN *SOD1* silencing, whereas those treated at P21 displayed slower disease progression, linked to enhanced transduction of non-neuronal cells.[72] Intracisterna magna or intracortical delivery of AAV9-sh*SOD1* resulted in a less effective correction of motor symptoms, disease onset, and survival (12–13%).[69,71] Finally, a more recent study described a novel subpial injection technique for smoothly delivering AAV9 through all the spinal cord and brain motor centers of mice, pigs, and nonhuman primates (NHPs). Among all *SOD1*-silencing strategies reported to date, this study has shown the most promising outcomes. The authors successfully suppressed MND, achieved near complete preservation of MNs and muscle innervation, with a full correction of survival, and a block in progression when administered after symptoms onset.[70]

miRNAs are part of the cell natural regulatory machinery and, rather than completely silencing, can partly reduce mRNA levels. In comparison with shRNA, they offer a more precise targeting, endogenous regulation, and lower immunogenicity and can be combined with specific promoters, all lowering off-target effects.[152] The intra-CSF AAV-miR*SOD1* delivery at postnatal stages to *SOD1*^{G93A} mice sustains survival to varying degrees, depending on the serotype and dose administered.[82,83,86] At that age, specifically targeting astrocytes extended survival and rescued neuromuscular function, although not to the same extent as when targeting MNs, and it did not result in a delay in the disease onset.[83] Conversely, i.t. injections in adult nonsymptomatic mice induced *SOD1* silencing and enhanced locomotor function but provided scarce to no extension of lifespan.[79–81,83] A phase I/II clinical trial is currently ongoing using AAVrh10 delivered i.t. (NCT06100276).

ASOs have been thoroughly investigated in neurodegenerative diseases and even progressed to clinical trials. Typically delivered without vectors, they offer advantages including an improved safety profile, easy administration, high tissue penetration, and rapid onset of action. The first study evaluating ASO therapy in SOD1^{G93A} rats employed a modified ASO targeting *SOD1*, continuously infused intraventricularly. This approach demonstrated the feasibility of reducing *SOD1* mRNA and protein levels in both the spinal cord and brain. Although treatment was initiated near disease onset and did not delay it, it significantly slowed disease progression and modestly extended survival by 8%.[91] These findings led to the first-in-human phase I clinical trial of i.t. ASO delivery, which reported no dose-limiting toxicities, tolerability of redosing and validated the i.t. route for CSF administrations (NCT01041222).[92] Subsequent studies in *SOD1*^{G93A} rats using next-generation ASOs at higher doses, administered via i.c.v. or i.t. routes, showed improved outcomes. These included increased survival rates (22% and 32%–39%), delayed disease onset (by 30.7% and 14.8%), improved locomotor function, and reversal of the initial CMAP decline.[96] This paved the way for the next-generation compound, BIIB067 (Tofersen), which was evaluated in a follow-up phase I/II clinical trial (NCT02623699) involving repeated i.t. dosing at 20–100 mg over a 12-week period via lumbar injections. The trial established the safety of higher dosing regimens and demonstrated a 33% reduction in CSF SOD1 protein levels in the highest-dose cohort, along with preliminary evidence of slowed functional decline.[93] Most recently, the phase III trial concluded, showcasing both reduced CSF SOD1 concentrations and neurofilament light chain (NFL) plasma levels. Although the primary clinical endpoints were not met and the therapy showed adverse events, Tofersen was approved by regulatory agencies as an investigational drug under the name Qalsody.[94] The trial included an open-label extension phase (NCT03070119), enabling a delayed-start comparison between participants who began Tofersen at trial entry and those who switched from placebo after 28 weeks. This phase also supported long-term evaluation of BIIB067, providing data for up to 1 year. Results suggested a slower rate of functional decline in those who initiated therapy earlier. However, clinical interpretations are limited by the unblinded and uncontrolled design of the extension phase. Given these results, a confirmatory phase III trial (ATLAS, NCT04856982) is currently ongoing in presymptomatic *SOD1* mutation carriers, aiming to determine whether early initiation of Tofersen can delay disease onset and progression.

In parallel to ASO-based strategies, a gene therapy approach using AAVrh10 vectors has also shown strong efficacy in the *SOD1*^{G93A} mouse model. This approach mediated exon skipping of the *SOD1* pre-mRNA by delivering exon 2-targeted antisense sequences embedded in a modified U7 small nuclear RNA (AAV10-U7-h*SOD1*). Exon 2 skipping resulted in the introduction of a premature termination codon, effectively disrupting SOD1 protein production. A single administration of AAV10-U7-h*SOD1*

via combined i.c.v. and i.v. routes at P1 resulted in a notable 92% extension in survival and a substantial 90% delay in onset, whereas treatment at P50 still led to a significant 58% increase in survival and a 76.5% delay in onset.[95] These findings highlight the strong therapeutic potential of early, systemic gene therapy targeting *SOD1* expression as a powerful alternative or complementary approach to ASO-based interventions.

The emerging field of genome editing has also been explored in the context of ALS. The first clustered regularly interspaced short palindromic repeats (CRISPR)-mediated editing of *SOD1* and delivery of *Staphylococcus aureus*-derived Cas9 (SaCas9) and single-guide (gRNA) against *SOD1* was published in 2017.[107] Gaj and collaborators used the modified AAV9-2YF vector administered via the facial vein in *SOD1*^{G93A} neonatal pups. The approach yielded a ≥2.5-fold decrease in SOD1 levels in the spinal cord, resulting in improved motor function and reduced muscle atrophy. CRISPR-edited mice had 50% more surviving MNs at end stage, displayed a 37% delay in disease onset, and a 25% increase in survival. A subsequent similar study using i.c.v. delivery of AAV9-SaCas9-sgRNA at a lower dose in neonatal mice achieved comparable results, including a 54.6% increase in lifespan.[108] More recently, the BBB-penetrating AAV-PHP.B and PHP.eB vectors encoding a sgRNA were tested for *SOD1* silencing in bigenic mice harboring the human *SOD1*^{G93A} and Cas9 transgenes. The i.c.v. administration of AAV-PHP.B immediately after birth reduced spinal MNs loss, denervation of NMJ, and muscle atrophy. This therapy also diminished axonal damage and preserved CMAP through animals' lifespan, which was extended between 66% and 85% depending on the cohort. Importantly, i.v. or i.t. injections of AAV-PHP.eB in young adult mice immediately before symptom onset fully preserved neuromuscular and motor function, correcting survival (by ≥200%) at least until all animals had to be euthanized due to ethical endpoints rather than disease progression. The treatment in adult mice rendered a higher degree of mSOD1 protein reduction and stronger therapeutic benefits and may reflect the superior CNS transduction efficiency of AAV.PHP.eB versus AAV.PHP.B vectors as well as differences in the route of administration.[109]

Finally, *in vivo* base editing, which enables single-nucleotide changes without introducing double-strand breaks, was explored in a study via i.t. injection of dual AAV particles encoding a split-intein cytidine base editor engineered to introduce a nonsense mutation into the m*SOD1* gene. Treated adult *SOD1*^{G93A} animals had a prolonged survival (11%) and a marked slowed disease progression, with a reduced rate of muscle atrophy and denervation, improved neuromuscular function, and up to 40% fewer SOD1 immunoreactive inclusions.[111] This approach offers high target specificity while preserving genomic integrity. However, its overall efficiency is limited by the need of successful cotransduction of target cells with both AAV vectors, followed by effective trans-splicing to reconstitute the functional base editor.

C9ORF72 silencing

Silencing strategies for *C9ORF72*-linked ALS have been more challenging compared with SOD1-related ALS. The pathogenic expansion in *C9ORF72* involves a long and complex hexanucleotide repeat in contrast to the point mutations observed in *SOD1* ALS. Identifying optimal target sites is laborious due to its repetitive nature, and neutralizing the toxic RNA without compromising the normal *C9ORF72* gene function poses a significant difficulty. Furthermore, *C9ORF72* ALS exhibits a high degree of genetic and phenotypic heterogeneity, complicating the development of a one-size-fits-all silencing therapy.[153] Tailoring therapies for different variants and disease manifestations is a more intricate task, which adds to the limitation of animal models that do not accurately replicate this disease phenotype.

The first study investigating *C9ORF72* silencing was conducted on patient-derived fibroblasts and demonstrated that effective reduction of nuclear RNA foci requires targeting both the sense and antisense strand repeat-containing RNAs with ASOs, as abundant antisense RNA foci (GGCCCC) were also observed. In contrast, siRNAs failed to reduce nuclear RNA foci despite markedly reducing overall *C9ORF72* RNA levels, consistent with the cytoplasmic localization of the RISC complex and the primary site of siRNA activity. The i.c.v. administration of ASOs in wild-type mice reduced 30–40% the mRNA levels of *C9orf72*, without inducing neuropathological or behavioral abnormalities.[97] In a subsequent study, sense-targeting ASOs were administered i.c.v to *C9ORF72* BAC transgenic mice carrying 450 repeats, achieving a 20–40% reduction in the cortex and spinal cord, while sparing wild-type *C9orf72* mRNA levels. This treatment also reduced DRP inclusions and ameliorated age-related anxiety and cognitive deficits when administered at later stages of disease progression.[98] These promising results paved the way for advancing this treatment (BIIB078) into a phase I/II clinical trial (NCT03626012).

A separate study by Tran *et al*. provided the first clinical evidence of successful *C9ORF72* suppression using a chemically modified ASO (afinersen) targeting the intronic region surrounding the repeat expansion. The study demonstrated target engagement and polyGP reduction in the CSF following repeated i.t. administration in a single human subject harboring approximately 2,400 G_4C_2 repeats. The patient's ALS Functional Rating Scale score remained largely stable, with no meaningful clinical improvement or decline. To date, no clinical trial has yet been initiated based on this ASO program.[100]

In another study, a different *C9ORF72* BAC transgenic mouse harboring 800 G_4C_2 repeats was used to evaluate the efficacy of an experimental stereopure ASO (WVE-004). This ASO selectively and dose-dependently reduced sense repeat-containing transcripts, achieving CNS reductions of 59–84% following multiple i.c.v. administrations and 65% in iPSC-derived MN. The effects were long-lasting, persistently reducing transcripts and polyGP DPRs for at least 6 months.[99] As a result, WVE-004 progressed to clinical evaluation for patients with the *C9ORF72* G_4C_2 expansion (NCT04931862). Despite encouraging preclinical results, both clinical trials, BIIB078 and WVE-004, failed to show clinical benefits and were associated with elevated NFL levels. These outcomes have raised concerns about potential *C9ORF72* loss-of-function, contributions from antisense repeat transcripts, DNA-level toxicity, and/or inadequate target engagement.[101,154]

Suboptimal target engagement may be attributed by timing of therapeutic administration or low biodistribution to neurons, despite achieving approximately 50% reductions in polyGP levels. Clinical benefit could emerge with earlier treatment or longer dosing durations, as seen for the open-label extension of the Tofersen trial for SOD1-related ALS.[94] However, the observed increases in NFL levels in both investigational arms raise the possibility of ASO-associated neurotoxicity when delivered in the CSF. While current clinical data do not provide clear evidence of ASO-induced neurotoxicity in humans, some preclinical studies have reported neuronal toxicity in mice, characterized by seizure-like phenotypes and dose-dependent reductions in consciousness and locomotor function, potentially linked to disruptions in intracellular calcium homeostasis.[100,155–157]

Moreover, these studies primarily focused on polyGP and polyGA levels, while overlooking the most toxic, insoluble aggregates formed by polyGR or omitting the measure of antisense-derived DPRs. Some evidence suggests that ASOs targeting the sense intronic region may exert an inhibitory bystander effect on antisense RNA transcription.[158–160] Thus, it would be valuable to assess patient samples from these clinical trials for the levels of polyGR and polyPR to better understand how the spectrum of DPR suppression may contribute to therapeutical efficacy.

In contrast, although *C9orf72* knockout (KO) mice exhibit immune abnormalities, they do not develop MN degeneration or ALS-like motor deficits. However, suppression of the endogenous wild-type allele in G_4C_2 repeat-overexpressing mice impairs autophagy, alters microglial function, and exacerbates disease progression. These findings highlight that C9orf72 loss-of-function should be considered a relevant contributor to disease pathogenesis and a potential limitation in gene-silencing therapeutic strategies.[161–163]

In a different approach, Martier *et al*. delivered AAV5 vectors coding concatenated miRNA hairpins through intrastriatal (i.s.t.) injections to Tg(*C9orf72*_3) line 112 mice (BAC112), a strain not displaying neurodegeneration but rather RNA foci and polyGP proteins.[87] They achieved a 20–40% reduction in *C9orf72* mRNA and 20% fewer cells with RNA foci. Another study also targeted the striatum of BAC112 mice with artificial miRNAs delivered via AAV9 vectors, resulting in approximately 50% reduction in total *C9orf72* transcripts and polyGP DPRs.[88] However, despite the stable expression from viral vectors, their reliance on cytoplasmic RNA processing pathways may limit therapeutic potential, given that pathogenic repeat RNAs are predominantly localized in the nucleus.

The failure of ASO clinical trials highlights the need for therapeutic strategies that can target *C9ORF72* antisense repeat RNA transcripts and intervene at the DNA level. Genome editing approaches aimed at suppressing pathological *C9orf72* expression have been investigated in only a few *in vivo* studies, most lacking functional outcome analyses. A first study demonstrated that delivery of AAV9-mediated CRISPR/Cas9 could excise the hexanucleotide repeat expansion from its genomic locus using gRNA flanking the repeat in three different C9BAC models (BAC111/Cas9, BAC112, and C9-500 mice).[112] More recently, two back-to-back studies employed a high-fidelity CRISPR-Cas13 system, which cleaves RNA through its intrinsic RNase activity—offering a reversible and potentially safer approach for modulating gene expression—and is compact enough for packaging into AAV vectors.[113,114] Both studies targeted sequences upstream of the G_4C_2 repeat to minimize off-target effects associated with the repeat's high GC content and its presence elsewhere in the genome. This strategy enabled selective targeting of *C9orf72* transcript variants 1 and 3, while sparing variant 2, the predominant isoform in the brain, which initiates transcription downstream of the repeat, thereby reducing the risk of *C9orf72* loss of function. Notably, the study by Kempthorne *et al*. leveraged CasRx variant ability to process multiple guide RNAs from a single construct, enabling concurrent reduction of sense and antisense transcripts. Their approach was validated in two different mouse models and also demonstrated neuroprotection against glutamate-induced excitotoxicity in iPSC-derived MNs.[113]

FUS silencing

In an initial proof-of-concept study, the *FUS* gene was suppressed in the common marmoset by delivering shRNA through AAV9, directly injected into the frontal cortex. This approach resulted in robust *FUS* silencing (approximately 70–80%) across various cell types, provoking an increased proliferation of astrocytes and

microglia.[76] However, no additional shRNA-based approaches have been reported to date.

More recently, ASOs against *FUS* were introduced i.c.v. in newborn *FUS* knock-in MN-P517L/Δ14 mice, demonstrating protective effects against MN loss and denervation for up to 4 months.[102] As ASO levels gradually decreased, microgliosis intensified but without subsequent MN degeneration. This ASO (ION363 or Ulefnersen) obtained the Food and Drug Administration's approval for experimental compassionate use in a young woman reliant on ventilatory support. Administered i.t. and initiated 6 months after clinical onset, the ALS-FRS-R score decline rate slowed during the treatment course.[102] Unfortunately, the patient's condition later deteriorated, particularly in ventilatory and bulbar function, and ultimately passed away due to complications. The therapy, named jacifusen in her honor, is currently undergoing recruitment in a phase III clinical trial (NCT04768972). Previously, a series of single-patient investigational new drug applications has suggested the safety and possible efficacy of jacifusen for treating *FUS*-ALS.[103]

Targeting TDP-43 pathology

TDP-43 aggregation is a pathological hallmark of nearly all ALS cases; however, effective therapies directly targeting TDP-43 for ALS/FTD and related TDP-43 proteinopathies remain elusive. Given the essential role of TDP-43 in RNA metabolism and cellular homeostasis, direct silencing of TDP-43 may be unsuitable as a therapeutic strategy. As an alternative, Becker *et al*. proposed targeting ataxin-2 (*ATXN2*) to disrupt TDP-43 aggregation.[104] Intermediate-length trinucleotide repeat expansions in the *ATXN2* gene are a known genetic risk factor for familial ALS and have been implicated in stress granule formation, promoting aberrant TDP-43 cleavage and its mislocalization to stress granules.[164–166] In a proof-of-concept study, newborn TAR4/4 mouse pups were treated i.c.v with ASOs against *Atxn2*, resulting in a 35% extended lifespan in this severely affected model, which typically reaches humane endpoints around day 21. Beyond prolonging survival, reducing Atxn2 improved gait scores and slowed disease progression. These data supported the advancement of this ASO, BIIB105 (also known as ION541), into a phase I/II clinical trial (NCT04494256). The trial involved i.t. administration to patients with ALS with or without *ATXN2* repeat expansions. However, the study was recently terminated after reporting no reduction in NFL levels or improvement in functional, respiratory, or strength-related outcomes over a follow-up period of more than 40 weeks, despite a reduction in ATXN2 protein levels in CSF.

In a separate study, a Cas13-based gene silencing approach targeting *Atxn2* system was delivered to neonatal TAR4/4 pups via AAV9 administered through combined i.c.v. and i.v. routes.[115] This approach led to significant therapeutic benefits, including improved gait, reduced kyphosis and tremors, increased body weight, and a 137% increase in survival, extending lifespan by 35 days. In the same mouse model, AAV-mediated RNAi delivery using a novel capsid (PM-AAV9) administered i.c.v. at P1 also extended survival, albeit to a lesser extent, consistent with its knockdown mechanism.[77] RNAi treatment resulted in a 45.5% increase in median survival, suggesting that while both approaches are beneficial, Cas13-mediated KO may confer a more robust therapeutic effect. To assess the therapeutic potential of Atxn2 reduction in a non-TDP-43 overexpressing ALS mouse, Hawley *et al*. developed a miRNA-based approach targeting *Atxn2* in the *PFN1*^{C17G} model, which exhibits strong TDP-43 pathology.[90] Mice were injected i.t. with AAV-PHP.eB-m*Atxn2*-amiR at 6–9 weeks of age, coinciding with the emergence of insoluble pTDP-43 accumulation. This intervention delayed the rise of serum NFL levels and was associated with preserved body weight gain, motor function, and muscle strength. However, effects on survival were not assessed.

Directly targeted TDP-43 has also been assessed with ASO-based therapies. Takeuchi *et al*. employed gapmer-type ASOs incorporating 2′-O,4′-C-ethylene-bridged nucleic acids (ENAs) to induce a transient and specific reduction of human TDP-43 expression. These highly stable, ENA-modified ASOs were administered i.c.v. to presymptomatic 6-week-old mice. A single injection effectively lowered TDP-43 levels throughout the CNS, resulting in 12 weeks of sustained suppression of cytoplasmic TDP-43 aggregation and lasting improvement in behavioral and locomotor deficits, even after pathological TDP-43 levels returned.[105] However, the study did not assess potential toxicities from suppressing endogenous TDP-43, as mouse TDP-43 was not targeted by gapmer ASOs, a critical consideration for clinical translation.

Along a similar line, Ke *et al*. leveraged their discovery of a noncanonical interaction between TDP-43 and 14-3-3θ to specifically target the aberrant accumulation of TDP-43 without affecting the expression or function of endogenous mRNA.[117] The chaperone-like protein 14-3-3θ selectively binds to pathological TDP-43 variants, exacerbating the disease. They engineered a gene therapy construct, DD-θFx, which fuses a degron to the α6 helix of 14-3-3θ. This modification enables proteasomal degradation of misfolded TDP-43. AAV-mediated delivery of DD-θFx effectively reduced toxic TDP-43 accumulation in three ALS mouse models: *TDP-43*^{A315T} model, AAV-induced human TDP-43 overexpressing mice, and the aggressive rNLS8 model. In the TDP-43^{A315T} and AAV-hTDP-43 models, DD-θFx improved behavioral and motor deficits. In the rNLS8 model, DD-θFx treatment significantly delayed paralysis onset and extended survival by 38.5%. This study

underscores the therapeutic potential of disrupting the pathological 14-3-3θ/TDP-43 interaction as a targeted strategy to reduce TDP-43 aggregation and mitigate neurodegeneration in ALS.

TDP-43 loss drives disease through splicing dysregulation in two critical neuronal genes: *STMN2* and *UNC13A* by reducing the expression of proteins essential for neuronal function. Loss of function in *STMN2* leads to motor neuropathy and denervation of the NMJ in mice.[167] In patients with ALS, nuclear TDP-43 depletion causes addition of a premature polyA tail in *STMN2*, reducing its protein function. In cellular models and in humanized mice carrying the cryptic intronic sequence of *STMN2*, gene editing using dCasRx—a catalytically inactive Cas13 guided to the splice site—or i.t. administration of ASOs restored proper splicing. This correction led to normalized STMN2 mRNA and protein levels, improved axonal regeneration following injury, and rescued lysosomal trafficking.[106] Notably, the ANQUR phase I clinical trial (NCT05633459) is evaluating the ASO QRL-201 to restore STMN2 expression in patients with ALS lacking *SOD1* and *FUS* mutations. In contrast, *UNC13A* is a gene essential for synaptic vesicle fusion. When TDP-43 is lost from the nucleus, a cryptic exon is included in the *UNC13A* transcript, introducing a premature stop codon and triggering degradation via nonsense-mediated decay. In patients with ALS, higher levels of cryptic splicing and more severe reduction of *UNC13A* expression are strongly associated with faster disease progression.[168,169] In a more sophisticated approach, Wilkins and colleagues developed a novel gene therapy platform called TDP-REG, which harnesses cytotoxic cryptic splicing induced by TDP-43 loss-of-function, to control therapeutic gene expression precisely in diseased cells, when the cryptic exon is inserted upstream of their constructs. They used a TDP-43/Raver1 fusion protein that acts as a splicing repressor, restoring normal splicing for *UNC13A* and *STMN2* both in patients' iPSC-derived MNs and in TDP-43 conditional KO mice.[116]

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Gene therapy has emerged as a promising approach for ALS, enabling molecularly targeted interventions in a disease with multifactorial origin and unmet medical needs. Preclinical studies—particularly in *SOD1*-related mouse models—have shown that AAV-based delivery of NTFs can delay disease onset, preserve MNs and NMJs, and moderately extend survival, especially when administered early. Multifactorial strategies combining neurotrophic, anti-inflammatory, and antioxidant genes have demonstrated additive effects while targeting NMJ stability and maintaining muscle–nerve connectivity. In contrast, muscle-targeted delivery can preserve NMJs and delay MN death by synaptic contacts. Moreover, combining peripheral and central approaches may enhance efficacy.

Gene silencing has demonstrated even greater therapeutic potential, particularly in the context of SOD1 mutations, achieving near-complete preservation of MNs and normalization of survival in preclinical models. As a result, ASO-based therapies have progressed to clinical trials, leading to the conditional approval of Qalsody for a small subset of patients. Despite this milestone, clinical outcomes have shown mixed efficacy across key endpoints. It remains critical to assess the therapeutic benefit of this treatment in a broader patient population, especially considering that, to date, clinical trials in ALS have largely failed to yield consistently positive results.

Although new therapeutic platforms have shown great promise for treating neurological diseases such as ALS, their safety profiles remain a key concern. In the case of AAV vectors, although the CNS is relatively immune-privileged and usually only a single administration is needed, high-dose i.v., or even i.t. or i.c.m. delivery has been associated with dorsal root ganglia toxicity, inflammation, and neuronal degeneration in both preclinical and clinical settings.[170] Moreover, pre-existing neutralizing antibodies against AAV capsids and immune responses to the transgene product can compromise efficacy and induce adverse effects.[171] To mitigate these risks, strategies include using lower doses, neuron-specific or endogenous promoters, immunosuppressive regimens, and novel capsids with enhanced CNS tropism and reduced immunogenicity.[172,173]

ASOs are administered intrathecally to bypass the BBB. Their safety depends largely on the sequence, chemistry, and dosing regimen (usually weekly or monthly for the duration of the patient's life). Some ASOs have been associated with neurotoxicity, motor dysfunction, or inflammatory responses in the spinal cord and brain in animal models, often influenced by the physiological importance of the wild-type function of the targeted protein.[174] However, Tofersen has shown acceptable safety profiles, with the most common side effects related to lumbar puncture.[175]

Despite its revolutionary potential, CRISPR gene editing still poses significant safety concerns, especially in the context of permanent changes to the genome. One of the most critical risks is off-target editing, where the CRISPR-Cas system may cut DNA at unintended sites, potentially disrupting essential genes or activating oncogenes.[176,177] Moreover, delivery remains a critical factor: often AAV vectors are still necessary to deliver CRISPR tools into the CNS, leading to persistent expression of Cas9, increasing the window for genomic damage. Immunogenicity is another issue: the bacterial origin of Cas proteins can provoke immune responses that lead to inflammation or clearance of edited cells.[178] Alternative systems such as high-fidelity Cas9

variants, DNA break-free editing systems, as well as editing approaches that avoid double-strand breaks—such as base editors and prime editors—are being studied to increased CIRSPR gene editing.[179,180]

Collectively, the reviewed data highlight the difficulty of treating this pathology, the importance of early intervention, and combinatorial targeting. Still, challenges remain—immunogenicity, delivery efficiency, and variability across ALS subtypes must be addressed. Translation to human therapy will require precise patient stratification, early biomarker development to identify the disease before its onset, and long-term studies.

Future directions include refining viral vectors, combining systemic and CNS-targeted approaches, using disease models beyond *SOD1* mutants, including patient-derived and additional animal models, and expanding genome editing strategies. While no single treatment is expected to cure ALS, a synergistic, multitargeted approach may ultimately convert this devastating disease into a manageable chronic condition.

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REFERENCES

1. Bensimon G, Lacomblez L, Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 1994;330(9):585–591; doi: 10.1056/NEJM199403033300901
2. Doble A. The pharmacology and mechanism of action of riluzole. Neurology 1996;47(6 (Suppl 4)): S233–S241; doi: 10.1212/WNL.47.6_Suppl_4.233S
3. Abe K, Aoki M, Tsuji S, et al. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: A randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017;16(7):505–512; doi: 10.1016/S1474-4422(17)30115-1
4. Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium phenylbutyrate–taurursodiol for amyotrophic lateral sclerosis. N Engl J Med 2020; 383(10):919–930; doi: 10.1056/NEJMoa1916945
5. Paganoni S, Hendrix S, Dickson SP, et al. Long-term survival of participants in the CENTAUR trial of sodium phenylbutyrate-taurursodiol in amyotrophic lateral sclerosis. Muscle Nerve 2021;63(1):31–39; doi: 10.1002/mus.27091
6. Mohajeri MH, Figlewicz DA, Bohn MC. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. Hum Gene Ther 1999;10(11): 1853–1866; doi: 10.1089/10430349950017536
7. Wang L-J, Lu Y-Y, Muramatsu S-I, et al. Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. J Neurosci 2002;22(16):6920–6928; doi: 10.1523/JNEUROSCI.22-16-06920.2002
8. Acsadi G, Anguelov RA, Yang H, et al. Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. Hum Gene Ther 2002;13(9):1047–1059; doi: 10.1089/104303402753812458
9. Manabe Y, Nagano I, Gazi A, et al. Glial cell line-derived neurotrophic factor protein prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. Neurol Res 2003;25(2)5: 195–200; doi: 10.1179/016164103101201193
10. Kaspar BK, Lladó J, Sherkat N, et al. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science 2003;301(5634): 839–842; doi: 10.1126/science.1086137
11. Guillot S, Azzouz M, Déglon N, et al. Local GDNF expression mediated by lentiviral vector protects facial nerve motoneurons but not spinal motoneurons in SOD1G93A transgenic mice. Neurobiol Dis 2004;16(1):139–149; doi: 10.1016/j.nbd.2004.01.017
12. Klein SM, Behrstock S, McHugh J, et al. GDNF delivery using human neural progenitor cells in a rat model of ALS. Hum Gene Ther 2005; 16(4):509–521; doi: 10.1089/hum.2005.16.509
13. Suzuki M, McHugh J, Tork C, et al. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. Mol Ther 2008;16(12):2002–2010; doi: 10.1038/mt.2008.197
14. Thomsen GM, Alkaslasi M, Vit J-P, et al. Systemic injection of AAV9-GDNF provides modest functional improvements in the SOD1G93A ALS rat but has adverse side effects. Gene Ther 2017;24(4):245–252; doi: 10.1038/gt.2017.9
15. Thomsen GM, Avalos P, Ma AA, et al. Transplantation of neural progenitor cells expressing glial cell line-derived neurotrophic factor into the motor cortex as a strategy to treat amyotrophic lateral sclerosis. Stem Cells 2018;36(7):1122–1131; doi: 10.1002/stem.2825
16. Mòdol-Caballero G, García-Lareu B, Herrando-Grabulosa M, et al. Specific expression of glial-derived neurotrophic factor in muscles as gene therapy strategy for amyotrophic lateral sclerosis. Neurotherapeutics 2021;18(2):1113–1126; doi: 10.1007/s13311-021-01025-6
17. Laperle AH, Moser VA, Avalos P, et al. Human iPSC-derived neural progenitor cells secreting GDNF provide protection in rodent models of ALS and retinal degeneration. Stem Cell Reports 2023;18(8):1629–1642; doi: 10.1016/j.stemcr.2023.03.016
18. Lepore AC, Haenggeli C, Gasmi M, et al. Intraparenchymal spinal cord delivery of adeno-associated virus IGF-1 is protective in the SOD1G93A model of ALS. Brain Res 2007; 1185:256–265; doi: 10.1016/j.brainres.2007.09.034

19. Dodge JC, Haidet AM, Yang W, et al. Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. Mol Ther 2008;16(6):1056–1064; doi: 10.1038/mt.2008.60

20. Franz CK, Federici T, Yang J, et al. Intraspinal cord delivery of IGF-I mediated by adeno-associated virus 2 is neuroprotective in a rat model of familial ALS. Neurobiol Dis 2009; 33(3):473–481; doi: 10.1016/j.nbd.2008.12.003

21. Eleftheriadou I, Manolaras I, Irvine EE, et al. α CAR IGF-1 vector targeting of motor neurons ameliorates disease progression in ALS mice. Ann Clin Transl Neurol 2016;3(10):752–768; doi: 10.1002/acn3.335

22. Wang W, Wen D, Duan W, et al. Systemic administration of scAAV9-IGF1 extends survival in SOD1 G93A ALS mice via inhibiting p38 MAPK and the JNK-mediated apoptosis pathway. Brain Res Bull 2018;139:203–210; doi: 10.1016/j.brainresbull.2018.02.015

23. Lin HQ, Hu HJ, Duan WS, et al. Intramuscular delivery of scAAV9-hIGF1 prolongs survival in the hSOD1G93A ALS mouse model via upregulation of D-amino acid oxidase. Mol Neurobiol 2018;55(1):682–695; doi: 10.1007/s12035-016-0335-z

24. Wen D, Cui C, Duan W, et al. The role of insulin-like growth factor 1 in ALS cell and mouse models: A mitochondrial protector. Brain Res Bull 2019;144:1–13; doi: 10.1016/j.brainresbull.2018.09.015

25. Allodi I, Comley L, Nichterwitz S, et al. Differential neuronal vulnerability identifies IGF-2 as a protective factor in ALS. Sci Rep 2016;6(1): 25960; doi: 10.1038/srep25960

26. Lee SH, Lee N, Kim S, et al. Intramuscular delivery of HGF-expressing recombinant AAV improves muscle integrity and alleviates neurological symptoms in the nerve crush and SOD1-G93A transgenic mouse models. Biochem Biophys Res Commun 2019;517(3):452–457; doi: 10.1016/j.bbrc.2019.07.105

27. Lee SH, Kim S, Lee N, et al. Intrathecal delivery of recombinant AAV1 encoding hepatocyte growth factor improves motor functions and protects neuromuscular system in the nerve crush and SOD1-G93A transgenic mouse models. Acta Neuropathol Commun 2019;7(1):96; doi: 10.1186/s40478-019-0737-z

28. Genç B, Nho B, Seung H, et al. Novel rAAV vector mediated intrathecal HGF delivery has an impact on neuroimmune modulation in the ALS motor cortex with TDP-43 pathology. Gene Ther 2023;30(7–8):560–574; doi: 10.1038/s41434-023-00383-4

29. Azzouz M, Ralph GS, Storkebaum E, et al. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 2004;429(6990):413–417; doi: 10.1038/nature02544

30. Bucher T, Colle MA, Wakeling E, et al. ScAAV9 intracisternal delivery results in efficient gene transfer to the central nervous system of a feline model of motor neuron disease. Hum Gene Ther 2013;24(7):670–682; doi: 10.1089/hum.2012.218

31. Wang Y, Duan W, Wang W, et al. scAAV9-VEGF prolongs the survival of transgenic ALS mice by promoting activation of M2 microglia and the PI3K/Akt pathway. Brain Res 2016; 1648(Pt A):1–10; doi: 10.1016/j.brainres.2016.06.043

32. Wang Y, Sun S, Zhai J, et al. scAAV9-VEGF alleviates symptoms of amyotrophic lateral sclerosis (ALS) mice through regulating aromatase. Exp Brain Res 2023;241(11–12):2817–2827; doi: 10.1007/s00221-023-06721-7

33. Haase G, Kennel P, Pettmann B, et al. Gene therapy of murine motor neuron disease using adenoviral vectors for neurotrophic factors. Nat Med 1997;3(4):429–436; doi: 10.1038/nm0497-429

34. Haase G, Pettmann B, Vigne E, et al. Adenovirus-mediated transfer of the neurotrophin-3 gene into skeletal muscle of PMN mice: Therapeutic effects and mechanisms of action. J Neurol Sci 1998;160 (Suppl 1):S97–S105; doi: 10.1016/S0022-510X(98)00207-X

35. Bordet T, Lesbordes J-C, Rouhani S, et al. Protective effects of cardiotrophin-1 adenoviral gene transfer on neuromuscular degeneration in transgenic ALS mice. Hum Mol Genet 2001; 10(18):1925–1933; doi: 10.1093/hmg/10.18.1925

36. Henriques A, Pitzer C, Dittgen T, et al. CNS-targeted viral delivery of G-CSF in an animal model for ALS: Improved efficacy and preservation of the neuromuscular unit. Mol Ther 2011;19(2):284–292; doi: 10.1038/mt.2010.271

37. Cheng W, Huang J, Fu X-Q, et al. Intrathecal delivery of AAV-NDNF ameliorates disease progression of ALS mice. Mol Ther 2023; 31(11):3277–3289; doi: 10.1016/j.ymthe.2023.09.018

38. Mancuso R, Martínez-Muriana A, Leiva T, et al. Neuregulin-1 promotes functional improvement by enhancing collateral sprouting in SOD1G93A ALS mice and after partial muscle denervation. Neurobiol Dis 2016;95:168–178; doi: 10.1016/j.nbd.2016.07.023

39. Lasiene J, Komine O, Fujimori-Tonou N, et al. Neuregulin 1 confers neuroprotection in SOD1-linked amyotrophic lateral sclerosis mice via restoration of C-boutons of spinal motor neurons. Acta Neuropathol Commun 2016;4:15; doi: 10.1186/s40478-016-0286-7

40. Mòdol-Caballero G, Herrando-Grabulosa M, García-Lareu B, et al. Gene therapy for overexpressing Neuregulin 1 type I in skeletal muscles promotes functional improvement in the SOD1G939 ALS mice. Neurobiol Dis 2020; 137:104793; doi: 10.1016/j.nbd.2020.104793

41. Mòdol-Caballero G, García-Lareu B, Verdés S, et al. Therapeutic role of neuregulin 1 type III in SOD1-linked amyotrophic lateral sclerosis. Neurotherapeutics 2020;17(3):1048–1060; doi: 10.1007/s13311-019-00811-7

42. Miyoshi S, Tezuka T, Arimura S, et al. DOK7 gene therapy enhances motor activity and life span in ALS model mice. EMBO Mol Med 2017; 9(7):880–889; doi: 10.15252/emmm.201607298

43. Chen H-H, Yeo H-T, Huang Y-H, et al. AAV-NRIP gene therapy ameliorates motor neuron degeneration and muscle atrophy in ALS model mice. Skelet Muscle 2024;14(1):17; doi: 10.1186/s13395-024-00349-z

44. Azzouz M, Hottinger A, Paterna JC, et al. Increased motoneuron survival and improved neuromuscular function in transgenic ALS mice after intraspinal injection of an adeno-associated virus encoding Bcl-2. Hum Mol Genet 2000;9(5):803–811; doi: 10.1093/hmg/9.5.803

45. Nizzardo M, Taiana M, Rizzo F, et al. Synaptotagmin 13 is neuroprotective across motor neuron diseases. Acta Neuropathol 2020;139(5): 837–853; doi: 10.1007/s00401-020-02133-x

46. Patel P, Kriz J, Gravel M, et al. Adeno-associated virus-mediated delivery of a recombinant single-chain antibody against misfolded superoxide dismutase for treatment of amyotrophic lateral sclerosis. Mol Ther 2014;22(3):498–510; doi: 10.1038/mt.2013.239

47. Leyton-Jaimes MF, Kahn J, Israelson A. AAV2/9-mediated overexpression of MIF inhibits SOD1 misfolding, delays disease onset, and extends survival in mouse models of ALS. Proc Natl Acad Sci USA 2019;116(29): 14755–14760; doi: 10.1073/pnas.1904665116

48. Alfahel L, Gschwendtberger T, Kozareva V, et al. Targeting low levels of MIF expression as a potential therapeutic strategy for ALS. Cell Rep Med 2024;5(5):101546; doi: 10.1016/j.xcrm.2024.101546

49. Luecke IW, Lin G, Santarriaga S, et al. Viral vector gene delivery of the novel chaperone protein SRCP1 to modify insoluble protein in in vitro and in vivo models of ALS. Gene Ther 2023;30(6):528–533; doi: 10.1038/s41434-021-00276-4

50. Yamashita T, Chai HL, Teramoto S, et al. Rescue of amyotrophic lateral sclerosis phenotype in a mouse model by intravenous AAV9-ADAR2 delivery to motor neurons. EMBO Mol Med 2013;5(11):1710–1719; doi: 10.1002/emmm.201302935

51. Li K, Hala TJ, Seetharam S, et al. GLT1 overexpression in SOD1G93A mouse cervical spinal cord does not preserve diaphragm function or extend disease. Neurobiol Dis 2015;78:12–23; doi: 10.1016/j.nbd.2015.03.010

52. Wang W, Duan W, Wang Y, et al. Intrathecal delivery of ssAAV9-DAO extends survival in

SOD1G93A ALS Mice. Neurochem Res 2017; 42(4):986–996; doi: 10.1007/s11064-016-2131-6

53. Dodge JC, Treleaven CM, Fidler JA, et al. AAV4-mediated expression of IGF-1 and VEGF within cellular components of the ventricular system improves survival outcome in familial ALS mice. Mol Ther 2010;18(12):2075–2084; doi: 10.1038/mt.2010.206

54. Dadon-Nachum M, Ben-Yaacov K, Ben-Zur T, et al. Transplanted modified muscle progenitor cells expressing a mixture of neurotrophic factors delay disease onset and enhance survival in the SOD1 mouse model of ALS. J Mol Neurosci 2015;55(3):788–797; doi: 10.1007/s12031-014-0426-0

55. Krakora D, Mulcrone P, Meyer M, et al. Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. Mol Ther 2013;21(8):1602–1610; doi: 10.1038/mt.2013.108

56. Benkler C, Barhum Y, Ben-Zur T, et al. Multifactorial gene therapy enhancing the glutamate uptake system and reducing oxidative stress delays symptom onset and prolongs survival in the SOD1-G93A ALS Mouse Model. J Mol Neurosci 2016;58(1):46–58; doi: 10.1007/s12031-015-0695-2

57. Baird MC, Likhite SB, Vetter TA, et al. Combination AAV therapy with galectin-1 and SOD1 downregulation demonstrates superior therapeutic effect in a severe ALS mouse model. Mol Ther Methods Clin Dev 2024;32(3): 101312; doi: 10.1016/j.omtm.2024.101312

58. Nanou A, Higginbottom A, Valori CF, et al. Viral delivery of antioxidant genes as a therapeutic strategy in experimental models of amyotrophic lateral sclerosis. Mol Ther 2013; 21(8):1486–1496; doi: 10.1038/mt.2013.115

59. Ayers JI, Fromholt S, Sinyavskaya O, et al. Widespread and efficient transduction of spinal cord and brain following neonatal AAV injection and potential disease modifying effect in ALS mice. Mol Ther 2015;23(1):53–62; doi: 10.1038/mt.2014.180

60. Trolese MC, Scarpa C, Melfi V, et al. Boosting the peripheral immune response in the skeletal muscles improved motor function in ALS transgenic mice. Mol Ther 2022;30(8): 2760–2784; doi: 10.1016/j.ymthe.2022.04.018

61. Wang S, Ichinomiya T, Savchenko P, et al. Subpial delivery of adeno-associated virus 9-synapsincaveolin- 1 (AAV9-SynCav1) preserves motor neuron and neuromuscular junction morphology, motor function, delays disease onset, and extends survival in hSOD1G93A mice. Theranostics 2022;12(12):5389–5403; doi: 10.7150/thno.72614

62. Magrì A, Lipari CLR, Caccamo A, et al. AAV-mediated upregulation of VDAC1 rescues the mitochondrial respiration and sirtuins expression in a SOD1 mouse model of inherited ALS. Cell Death Discov 2024;10(1):178; doi: 10.1038/s41420-024-01949-w

63. Wen D, Ji Y, Li Y, et al. *OPTN* gene therapy increases autophagy and protects mitochondria in *SOD1* -G93A-expressing transgenic mice and cells. Febs J 2024;291(4):795–813; doi: 10.1111/febs.17009

64. Zhao S, Chen R, An Y, et al. Optineurin overexpression ameliorates neurodegeneration through regulating neuroinflammation and mitochondrial quality in a murine model of amyotrophic lateral sclerosis. Front Aging Neurosci 2025;17:1522073; doi: 10.3389/fnagi.2025.1522073

65. Ralph GS, Radcliffe PA, Day DM, et al. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat Med 2005;11(4):429–433; doi: 10.1038/nm1205

66. Raoul C, Abbas-Terki T, Bensadoun JC, et al. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. Nat Med 2005;11(4):423–428; doi: 10.1038/nm1207

67. Miller TM, Kim SH, Yamanaka K, et al. Gene transfer demonstrates that muscle is not a primary target for non-cell-autonomous toxicity in familial amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 2006;103(51):19546–19551; doi: 10.1073/pnas.0609411103

68. Wang H, Ghosh A, Baigude H, et al. Therapeutic gene silencing delivered by a chemically modified small interfering RNA against mutant SOD1 slows amyotrophic lateral sclerosis progression. J Biol Chem 2008;283(23):15845–15852; doi: 10.1074/jbc.M800834200

69. Thomsen GM, Gowing G, Latter J, et al. 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 2014; 34(47):15587–15600; doi: 10.1523/JNEUROSCI.2037-14.2014

70. Bravo-Hernandez M, Tadokoro T, Navarro MR, et al. Spinal subpial delivery of AAV9 enables widespread gene silencing and blocks motoneuron degeneration in ALS. Nat Med 2020; 26(1):118–130; doi: 10.1038/s41591-019-0674-1

71. Iannitti T, Scarrott JM, Likhite S, et al. Translating SOD1 gene silencing toward the clinic: A highly efficacious, off-target-free, and biomarker-supported strategy for fALS. Mol Ther Nucleic Acids 2018;12:75–88; doi: 10.1016/j.omtn.2018.04.015

72. Foust KD, Salazar DL, Likhite S, et al. Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS. Mol Ther 2013; 21(12):2148–2159; doi: 10.1038/mt.2013.211

73. Towne C, Raoul C, Schneider BL, et al. Systemic AAV6 delivery mediating RNA interference against SOD1: Neuromuscular transduction does not alter disease progression in fALS mice. Mol Ther 2008;16(6):1018–1025; doi: 10.1038/mt.2008.73

74. Towne C, Setola V, Schneider BL, et al. Neuroprotection by gene therapy targeting mutant SOD1 in individual pools of motor neurons does not translate into therapeutic benefit in fALS mice. Mol Ther 2011;19(2):274–283; doi: 10.1038/mt.2010.260

75. Wu R, Wang H, Xia X, et al. Nerve injection of viral vectors efficiently transfers transgenes into motor neurons and delivers RNAi therapy against ALS. Antioxid Redox Signal 2009;11(7): 1523–1534; doi: 10.1089/ars.2009.2618

76. Endo K, Ishigaki S, Masamizu Y, et al. Silencing of FUS in the common marmoset (Callithrix jacchus) brain via stereotaxic injection of an adeno-associated virus encoding shRNA. Neurosci Res 2018;130:56–64; doi: 10.1016/j.neures.2017.08.006

77. Amado DA, Robbins AB, Whiteman KR, et al. AAV-based delivery of RNAi targeting ataxin-2 improves survival and pathology in TDP-43 mice. Nat Commun 2025;16(1):5334; doi: 10.1038/s41467-025-60497-8

78. Yen Y-P, Lung T-H, Liau ES, et al. The motor neuron m6A repertoire governs neuronal homeostasis and FTO inhibition mitigates ALS symptom manifestation. Nat Commun 2025; 16(1):4063; doi: 10.1038/s41467-025-59117-2

79. Borel F, Gernoux G, Cardozo B, et al. Therapeutic rAAVrh10 mediated SOD1 silencing in adult SOD1(G93A) mice and nonhuman primates. Hum Gene Ther 2016;27(1):19–31; doi: 10.1089/hum.2015.122

80. Borel F, Gernoux G, Sun H, et al. Safe and effective superoxide dismutase 1 silencing using artificial microRNA in macaques. Sci Transl Med 2018;10(465):eaau6414; doi: 10.1126/scitranslmed.aau6414

81. Li D, Liu C, Yang C, et al. Slow intrathecal injection of rAAVrh10 enhances its transduction of spinal cord and therapeutic efficacy in a mutant SOD1 model of ALS. Neuroscience 2017;365: 192–205; doi: 10.1016/j.neuroscience.2017.10.001

82. Stoica L, Todeasa SH, Cabrera GT, et al. Adeno-associated virus-delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model. Ann Neurol 2016;79(4):687–700; doi: 10.1002/ana.24618

83. Dirren E, Aebischer J, Rochat C, et al. SOD1 silencing in motoneurons or glia rescues neuromuscular function in ALS mice. Ann Clin Transl Neurol 2015;2(2):167–184; doi: 10.1002/acn3.162

84. Wang H, Yang B, Qiu L, et al. Widespread spinal cord transduction by intrathecal injection of rAAV delivers efficacious RNAi therapy for amyotrophic lateral sclerosis. Hum Mol Genet 2014;23(3):668–681; doi: 10.1093/hmg/ddt454

85. Rochat C, Bernard-Marissal N, Källstig E, et al. Astrocyte-targeting RNA interference against

mutated superoxide dismutase 1 induces motoneuron plasticity and protects fast-fatigable motor units in a mouse model of amyotrophic lateral sclerosis. Glia 2022;70(5): 842–857; doi: 10.1002/glia.24140

86. Chen SK, Hawley ZCE, Zavodszky MI, et al. Efficacy and safety of a SOD1-targeting artificial miRNA delivered by AAV9 in mice are impacted by miRNA scaffold selection. Mol Ther Nucleic Acids 2023;34:102057; doi: 10 .1016/j.omtn.2023.102057

87. Martier R, Liefhebber JM, Miniarikova J, et al. Artificial MicroRNAs targeting C9orf72 can reduce accumulation of intra-nuclear transcripts in ALS and FTD patients. Mol Ther Nucleic Acids 2019;14:593–608; doi: 10.1016/j .omtn.2019.01.010

88. Cabrera GT, Meijboom KE, Abdallah A, et al. Artificial microRNA suppresses C9ORF72 variants and decreases toxic dipeptide repeat proteins in vivo. Gene Ther 2024;31(3–4): 105–118; doi: 10.1038/s41434-023-00418-w

89. Tung Y-T, Peng K-C, Chen Y-C, et al. Mir-17∼92 confers motor neuron subtype differential resistance to ALS-associated degeneration. Cell Stem Cell 2019;25(2):193–209; doi: 10 .1016/j.stem.2019.04.016

90. Hawley ZCE, Li X, Bodnar D, et al. Viral-mediated knockdown of Atxn2 attenuates TDP-43 pathology and muscle dysfunction in the PFN1C71G ALS mouse model. Acta Neuropathol Commun 2025;13(1):116; doi: 10.1186/ s40478-025-02005-z

91. Smith RA, Miller TM, Yamanaka K, et al. Antisense oligonucleotide therapy for neurodegenerative disease. J Clin Invest 2006;116(8): 2290–2296; doi: 10.1172/JCI25424

92. Miller TM, Pestronk A, David W, et al. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: A phase 1, randomised, first-in-man study. Lancet Neurol 2013;12(5): 435–442; doi: 10.1016/S1474-4422(13)70061-9

93. Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1–2 trial of antisense oligonucleotide tofersen for *SOD1* ALS. N Engl J Med 2020;383(2): 109–119; doi: 10.1056/NEJMoa2003715

94. Miller TM, Cudkowicz ME, Genge A, et al.; VALOR and OLE Working Group. Trial of antisense oligonucleotide tofersen for SOD1 ALS. N Engl J Med 2022;387(12):1099–1110; doi: 10.1056/NEJMoa2204705

95. Biferi MG, Cohen-Tannoudji M, Cappelletto A, et al. A new AAV10-U7-mediated gene therapy prolongs survival and restores function in an ALS mouse model. Mol Ther 2017;25(9): 2038–2052; doi: 10.1016/j.ymthe.2017.05.017

96. McCampbell A, Cole T, Wegener AJ, et al. Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models. J Clin Invest 2018;128(8):3558–3567; doi: 10.1172/JCI99081

97. Lagier-Tourenne C, Baughn M, Rigo F, et al. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci USA 2013;110(47); doi: 10.1073/pnas.1318835110

98. Jiang J, Zhu Q, Gendron TF, et al. Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. Neuron 2016;90(3):535–550; doi: 10 .1016/j.neuron.2016.04.006

99. Liu Y, Andreucci A, Iwamoto N, et al. Preclinical evaluation of WVE-004, an investigational stereopure oligonucleotide for the treatment of C9orf72-associated ALS or FTD. Mol Ther Nucleic Acids 2022;28:558–570; doi: 10.1016/j .omtn.2022.04.007

100. Tran H, Moazami MP, Yang H, et al. Suppression of mutant C9orf72 expression by a potent mixed backbone antisense oligonucleotide. Nat Med 2022;28(1):117–124; doi: 10.1038/ s41591-021-01557-6

101. van den Berg LH, Rothstein JD, Shaw PJ, et al. Safety, tolerability, and pharmacokinetics of antisense oligonucleotide BIIB078 in adults with C9orf72-associated amyotrophic lateral sclerosis: A phase 1, randomised, double blinded, placebo-controlled, multiple ascending dose study. Lancet Neurol 2024;23(9):901–912; doi: 10.1016/ S1474-4422(24)00216-3

102. Korobeynikov VA, Lyashchenko AK, Blanco-Redondo B, et al. Antisense oligonucleotide silencing of FUS expression as a therapeutic approach in amyotrophic lateral sclerosis. Nat Med 2022; 28(1):104–116; doi: 10.1038/s41591-021-01615-z

103. Shneider NA, Harms MB, Korobeynikov VA, et al. Antisense oligonucleotide jacifusen for FUS-ALS: An investigator-initiated, multicentre, open-label case series. Lancet 2025; 405(10494):2075–2086; doi: 10.1016/S0140-6736(25)00513-6

104. Becker LA, Huang B, Bieri G, et al. Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature 2017; 544(7650):367–371; doi: 10.1038/nature22038

105. Takeuchi T, Maeta K, Ding X, et al. Sustained therapeutic benefits by transient reduction of TDP-43 using ENA-modified antisense oligonucleotides in ALS/FTD mice. Mol Ther Nucleic Acids 2023;31:353–366; doi: 10.1016/j.omtn .2023.01.006

106. Baughn MW, Melamed Z, López-Erauskin J, et al. Mechanism of *STMN2* cryptic splice-polyadenylation and its correction for TDP-43 proteinopathies. Science 2023;379(6637): 1140–1149; doi: 10.1126/science.abq5622

107. Gaj T, Ojala DS, Ekman FK, et al. In vivo genome editing improves motor function and extends survival in a mouse model of ALS. Sci Adv 2017;3(12):eaar3952; doi: 10.1126/sciadv .aar3952

108. Duan W, Guo M, Yi L, et al. The deletion of mutant SOD1 via CRISPR/Cas9/sgRNA prolongs survival in an amyotrophic lateral sclerosis mouse model. Gene Ther 2020;27(3–4): 157–169; doi: 10.1038/s41434-019-0116-1

109. Chen YA, Kankel MW, Hana S, et al. In vivo genome editing using novel AAV-PHP variants rescues motor function deficits and extends survival in a SOD1-ALS mouse model. Gene Ther 2023;30(5):443–454; doi: 10.1038/s41434-022-00375-w

110. Powell JE, Lim CKW, Krishnan R, et al. Targeted gene silencing in the nervous system with CRISPR-Cas13. Sci Adv 2022;8(3): eabk2485; doi: 10.1126/sciadv.abk2485

111. Lim CKW, Gapinske M, Brooks AK, et al. Treatment of a mouse model of ALS by in vivo base editing. Mol Ther 2020;28(4):1177–1189; doi: 10.1016/j.ymthe.2020.01.005

112. Meijboom KE, Abdallah A, Fordham NP, et al. CRISPR/Cas9-mediated excision of ALS/FTD-causing hexanucleotide repeat expansion in C9ORF72 rescues major disease mechanisms in vivo and in vitro. Nat Commun 2022;13(1): 6286; doi: 10.1038/s41467-022-33332-7

113. Kempthorne L, Vaizoglu D, Cammack AJ, et al. Dual-targeting CRISPR-CasRx reduces C9orf72 ALS/FTD sense and antisense repeat RNAs in vitro and in vivo. Nat Commun 2025;16(1):459; doi: 10.1038/s41467-024-55550-x

114. McCallister TX, Lim CKW, Singh M, et al. A high-fidelity CRISPR-Cas13 system improves abnormalities associated with C9ORF72-linked ALS/FTD. Nat Commun 2025;16(1):460; doi: 10 .1038/s41467-024-55548-5

115. Zeballos C MA, Moore HJ, Smith TJ, et al. Mitigating a TDP-43 proteinopathy by targeting ataxin-2 using RNA-targeting CRISPR effector proteins. Nat Commun 2023;14(1): 6492; doi: 10.1038/s41467-023-42147-z

116. Wilkins OG, Chien MZYJ, Wlaschin JJ, et al. Creation of de novo cryptic splicing for ALS and FTD precision medicine. Science 2024;386(6717): 61–69; doi: 10.1126/science.adk2539

117. Ke YD, van Hummel A, Au C, et al. Targeting 14-3-3θ-mediated TDP-43 pathology in amyotrophic lateral sclerosis and frontotemporal dementia mice. Neuron 2024;112(8):1249–1264; doi: 10 .1016/j.neuron.2024.01.022

118. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362(6415):59–62; doi: 10.1038/362059a0

119. Ripps ME, Huntley GW, Hof PR, et al. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 1995;92(3):689–693; doi: 10.1073/pnas.92.3.689

120. Mancuso R, Santos-Nogueira E, Osta R, et al. Electrophysiological analysis of a murine model of motoneuron disease. Clin Neurophysiol 2011;122(8):1660–1670; doi: 10.1016/j.clinph.2011.01.045

121. Bosco DA, Morfini G, Karabacak NM, et al. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci 2010;13(11):1396–1403; doi: 10.1038/nn.2660

122. Wegorzewska I, Baloh RH. TDP-43-based animal models of neurodegeneration: New insights into ALS pathology and pathophysiology. Neurodegener Dis 2011;8(4):262–274; doi: 10.1159/000321547

123. Watkins JA, Alix JJP, Shaw PJ, et al. Extensive phenotypic characterisation of a human TDP-43Q331K transgenic mouse model of amyotrophic lateral sclerosis (ALS). Sci Rep 2021;11(1):16659; doi: 10.1038/s41598-021-96122-z

124. Batra R, Lee CW. Mouse models of C9orf72 hexanucleotide repeat expansion in amyotrophic lateral sclerosis/frontotemporal dementia. Front Cell Neurosci 2017;11:196; doi: 10.3389/fncel.2017.00196

125. O'Rourke JG, Bogdanik L, Muhammad AKMG, et al. C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. Neuron 2015;88(5):892–901; doi: 10.1016/j.neuron.2015.10.027

126. Verdone BM, Cicardi ME, Wen X, et al. A mouse model with widespread expression of the C9orf72-linked glycine–arginine dipeptide displays non-lethal ALS/FTD-like phenotypes. Sci Rep 2022;12(1):5644; doi: 10.1038/s41598-022-09593-z

127. Liu Y, Pattamatta A, Zu T, et al. C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. Neuron 2016;90(3):521–534; doi: 10.1016/j.neuron.2016.04.005

128. Fil D, DeLoach A, Yadav S, et al. Mutant Profilin1 transgenic mice recapitulate cardinal features of motor neuron disease. Hum Mol Genet 2017;26(4):686–701; doi: 10.1093/hmg/ddw429

129. Mueller KA, Suneby EG, Ferola MH, et al. Comprehensive characterization and validation of the Prp-hPFN1G118V mouse model: Guidelines for preclinical therapeutic testing for ALS. Neurobiol Dis 2025;212:106975; doi: 10.1016/j.nbd.2025.106975

130. Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. Prog Neurobiol 2008;85(1):94–134; doi: 10.1016/j.pneurobio.2008.01.001

131. Ferraiuolo L, Kirby J, Grierson AJ, et al. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. Nat Rev Neurol 2011;7(11):616–630; doi: 10.1038/nrneurol.2011.152

132. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 2013;14(4):248–264; doi: 10.1038/nrn3430

133. Mancuso R, Navarro X. Amyotrophic lateral sclerosis: Current perspectives from basic research to the clinic. Prog Neurobiol 2015;133:1–26; doi: 10.1016/j.pneurobio.2015.07.004

134. Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. Nat Rev Dis Primers 2017;3:17085; doi: 10.1038/nrdp.2017.71

135. Ragagnin AMG, Shadfar S, Vidal M, et al. Motor neuron susceptibility in ALS/FTD. Front Neurosci 2019;13:532; doi: 10.3389/fnins.2019.00532

136. Genge A, Wainwright S, Vande Velde C. Amyotrophic lateral sclerosis: Exploring pathophysiology in the context of treatment. Amyotroph Lateral Scler Frontotemporal Degener 2024;25(3–4):225–236; doi: 10.1080/21678421.2023.2278503

137. Mancuso R, Oliván S, Rando A, et al. Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. Neurotherapeutics 2012;9(4):814–826; doi: 10.1007/s13311-012-0140-y

138. Gaja-Capdevila N, Hernández N, Navarro X, et al. Sigma-1 receptor is a pharmacological target to promote neuroprotection in the SOD1G93A ALS mice. Front Pharmacol 2021;12:780588; doi: 10.3389/fphar.2021.780588

139. Xiao N, Le Q-T. Neurotrophic factors and their potential applications in tissue regeneration. Arch Immunol Ther Exp (Warsz) 2016;64(2):89–99; doi: 10.1007/s00005-015-0376-4

140. Bartus RT, Johnson EM. Clinical tests of neurotrophic factors for human neurodegenerative diseases, part 1: Where have we been and what have we learned? Neurobiol Dis 2017;97(Pt B):156–168; doi: 10.1016/j.nbd.2016.03.027

141. Foust KD, Nurre E, Montgomery CL, et al. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 2009;27(1):59–65; doi: 10.1038/nbt.1515

142. Baloh RH, Johnson JP, Avalos P, et al. Transplantation of human neural progenitor cells secreting GDNF into the spinal cord of patients with ALS: A phase 1/2a trial. Nat Med 2022;28(9):1813–1822; doi: 10.1038/s41591-022-01956-3

143. Manabe Y, Nagano IA, Gazi MS, et al. Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. Apoptosis 2002;7(4):329–334; doi: 10.1023/a:1016123413038

144. Lu Y-Y, Wang L-J, Muramatsu S, et al. Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal motoneurons by retrograde transport. Neurosci Res 2003;45(1):33–40; doi: 10.1016/s0168-0102(02)00195-5

145. Song F, Chiang P, Wang J, et al. Aberrant neuregulin 1 signaling in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2012;71(2):104–115; doi: 10.1097/NEN.0b013e3182423c43

146. Takahashi Y, Fukuda Y, Yoshimura J, et al.; JaCALS. ERBB4 mutations that disrupt the neuregulin-ErbB4 pathway cause amyotrophic lateral sclerosis type 19. Am J Hum Genet 2013;93(5):900–905; doi: 10.1016/j.ajhg.2013.09.008

147. Mòdol-Caballero G, Herrando-Grabulosa M, Verdés S, et al. Gene therapy overexpressing neuregulin 1 type I in combination with neuregulin 1 type III promotes functional improvement in the SOD1G93A ALS mice. Front Neurol 2021;12:693309; doi: 10.3389/fneur.2021.693309

148. Gomes-Duarte A, Pascoal S, Haselberg R, et al. Targeting oxidized phosphatidylcholines in SOD1-associated ALS: Therapeutic potential of PC-OxPL-VecTab®. Front Neurosci 2025;19:1620181; doi: 10.3389/fnins.2025.1620181

149. Pozzi S, Thammisetty SS, Codron P, et al. Virus-mediated delivery of antibody targeting TAR DNA-binding protein-43 mitigates associated neuropathology. J Clin Invest 2019;129(4):1581–1595; doi: 10.1172/JCI123931

150. Ghadge GD, Kay BK, Drigotas C, et al. Single chain variable fragment antibodies directed against SOD1 ameliorate disease in mutant SOD1 transgenic mice. Neurobiol Dis 2019;121:131–137; doi: 10.1016/j.nbd.2018.08.021

151. Leyton-Jaimes MF, Benaim C, Abu-Hamad S, et al. Endogenous macrophage migration inhibitory factor reduces the accumulation and toxicity of misfolded SOD1 in a mouse model of ALS. Proc Natl Acad Sci USA 2016;113(36):10198–10203; doi: 10.1073/pnas.1604600113

152. Lam JKW, Chow MYT, Zhang Y, et al. siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Nucleic Acids 2015;4(9):e252; doi: 10.1038/mtna.2015.23

153. Hu J, Liu J, Li L, et al. Engineering duplex RNAs for challenging targets: Recognition of GGGGCC/CCCCGG Repeats at the ALS/FTD C9orf72 Locus. Chem Biol 2015;22(11):1505–1511; doi: 10.1016/j.chembiol.2015.09.016

154. Cammack AJ, Balendra R, Isaacs AM. Failure of *C9orf72* sense repeat-targeting antisense oligonucleotides: Lessons learned and the path forward. Brain 2024;147(8):2607–2609; doi: 10.1093/brain/awae168

155. Jia C, Lei Mon SS, Yang Y, et al. Change of intracellular calcium level causes acute neurotoxicity by antisense oligonucleotides via CSF route. Mol Ther Nucleic Acids 2023;31:182–196; doi: 10.1016/j.omtn.2022.12.010

156. Miller R, Paquette J, Barker A, et al. Preventing acute neurotoxicity of CNS therapeutic oligonucleotides with the addition of Ca2+ and Mg2+ in the formulation. Mol Ther Nucleic Acids 2024;35(4):102359; doi: 10.1016/j.omtn.2024.102359

157. Moazami MP, Rembetsy-Brown JM, Sarli SL, et al. Quantifying and mitigating motor phenotypes induced by antisense oligonucleotides in the central nervous system. Mol Ther 2024;32(12):4401–4417; doi: 10.1016/j.ymthe.2024.10.024

158. Lai F, Damle SS, Ling KK, et al. Directed RNase H cleavage of nascent transcripts causes transcription termination. Mol Cell 2020;77(5):1032–1043; doi: 10.1016/j.molcel.2019.12.029

159. Lee J-S, Mendell JT. Antisense-mediated transcript knockdown triggers premature transcription termination. Mol Cell 2020;77(5):1044–1054; doi: 10.1016/j.molcel.2019.12.011

160. Marasco LE, Dujardin G, Sousa-Luís R, et al. Counteracting chromatin effects of a splicing-correcting antisense oligonucleotide improves its therapeutic efficacy in spinal muscular atrophy. Cell 2022;185(12):2057–2070; doi: 10.1016/j.cell.2022.04.031

161. Zhu Q, Jiang J, Gendron TF, et al. Reduced C9ORF72 function exacerbates gain of toxicity from ALS/FTD-causing repeat expansion in C9orf72. Nat Neurosci 2020;23(5):615–624; doi: 10.1038/s41593-020-0619-5

162. O'Rourke JG, Bogdanik L, Yáñez A, et al. *C9orf72* is required for proper macrophage and microglial function in mice. Science 2016;351(6279):1324–1329; doi: 10.1126/science.aaf1064

163. Sudria-Lopez E, Koppers M, de Wit M, et al. Full ablation of C9orf72 in mice causes immune system-related pathology and neoplastic events but no motor neuron defects. Acta Neuropathol 2016;132(1):145–147; doi: 10.1007/s00401-016-1581-x

164. Elden AC, Kim H-J, Hart MP, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010;466(7310):1069–1075; doi: 10.1038/nature09320

165. Zhang K, Daigle JG, Cunningham KM, et al. Stress granule assembly disrupts nucleocytoplasmic transport. Cell 2018;173(4):958–971; doi: 10.1016/j.cell.2018.03.025

166. Hart MP, Gitler AD. ALS-associated Ataxin 2 PolyQ expansions enhance stress-induced caspase 3 activation and increase TDP-43 pathological modifications. J Neurosci 2012;32(27):9133–9142; doi: 10.1523/JNEUROSCI.0996-12.2012

167. Krus KL, Strickland A, Yamada Y, et al. Loss of Stathmin-2, a hallmark of TDP-43-associated ALS, causes motor neuropathy. Cell Rep 2022;39(13):111001; doi: 10.1016/j.celrep.2022.111001

168. Brown A-L, Wilkins OG, Keuss MJ, et al.; NYGC ALS Consortium. TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of UNC13A. Nature 2022;603(7899):131–137; doi: 10.1038/s41586-022-04436-3

169. Ma XR, Prudencio M, Koike Y, et al. TDP-43 represses cryptic exon inclusion in the FTD-ALS gene UNC13A. Nature 2022;603(7899):124–130; doi: 10.1038/s41586-022-04424-7

170. Hinderer C, Katz N, Buza EL, et al. Severe toxicity in nonhuman primates and piglets following high-dose intravenous administration of an adeno-associated virus vector expressing human SMN. Hum Gene Ther 2018;29(3):285–298; doi: 10.1089/hum.2018.015

171. Shen W, Liu S, Ou L. rAAV immunogenicity, toxicity, and durability in 255 clinical trials: A meta-analysis. Front Immunol 2022;13:1104646; doi: 10.3389/fimmu.2022.1001263

172. Moyer TC, Hoffman BA, Chen W, et al. Highly conserved brain vascular receptor ALPL mediates transport of engineered AAV vectors across the blood-brain barrier. Mol Ther 2025;33(8):3902–3916; doi: 10.1016/j.ymthe.2025.04.046

173. Xie Q, Chen X, Ma H, et al. Improved gene therapy for spinal muscular atrophy in mice using codon-optimized hSMN1 transgene and hSMN1 gene-derived promotor. EMBO Mol Med 2024;16(4):945–965; doi: 10.1038/s44321-024-00037-x

174. Dietrich P, Johnson IM, Alli S, et al. Elimination of huntingtin in the adult mouse leads to progressive behavioral deficits, bilateral thalamic calcification, and altered brain iron homeostasis. PLoS Genet 2017;13(7):e1006846; doi: 10.1371/journal.pgen.1006846

175. Wiesenfarth M, Dorst J, Brenner D, et al. Effects of tofersen treatment in patients with SOD1-ALS in a "real-world" setting – a 12-month multicenter cohort study from the German early access program. EClinicalMedicine 2024;69:102495; doi: 10.1016/j.eclinm.2024.102495

176. Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. Nat Commun 2018;9(1):3497; doi: 10.1038/s41467-018-05843-9

177. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol 2013;31(9):822–826; doi: 10.1038/nbt.2623

178. Charlesworth CT, Deshpande PS, Dever DP, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med 2019;25(2):249–254; doi: 10.1038/s41591-018-0326-x

179. Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 2019;576(7785):149–157; doi: 10.1038/s41586-019-1711-4

180. Komor AC, Kim YB, Packer MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 2016;533(7603):420–424; doi: 10.1038/nature17946

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