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REVIEW

Research advances in gene therapy approaches for the treatment of amyotrophic lateral sclerosis

Monica Nizzardo · Chiara Simone · Marianna Falcone · Giulietta Riboldi · Federica Rizzo · Francesca Magri · Nereo Bresolin · Giacomo P. Comi · Stefania Corti

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Abstract Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease of motor neurons that causes progressive muscle weakness, paralysis, and premature death. No effective therapy is available. Research in the motor neuron field continues to grow, and recent breakthroughs have demonstrated the possibility of completely achieving rescue in animal models of spinal muscular atrophy, a genetic motor neuron disease. With adeno-associated virus (AAV) vectors, gene transfer can be achieved with systemic non-invasive injection and minimal toxicity. In the context of this success, we review gene therapy approaches for ALS, considering what has been done and the possible future directions for effective application of the latest generation of vectors for clinical translation. We focus on recent developments in the areas of RNA/antisense-mediated silencing of specific ALS causative genes like superoxide dismutase-1 and other molecular pathogenetic targets, as well as the administration of neuroprotective factors with viral vectors. We argue that gene therapy offers new opportunities to open the path for clinical progress in treating ALS.

Keywords Amyotrophic lateral sclerosis · Gene therapy · RNA interference · Adeno-associated vectors

M. Nizzardo (✉) · C. Simone · M. Falcone · G. Riboldi · F. Rizzo · F. Magri · N. Bresolin · G. P. Comi · S. Corti
Department of Neurological Sciences, Dino Ferrari Centre, University of Milan, IRCCS' Foundation Ca' Granda, Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milan, Italy
e-mail: monica.nizzardo@unimi.it

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease and the most common adult-onset motor neuron disease for which no effective cure is available. ALS is a multifactorial disease, with etiological heterogeneity and a high variability of clinical presentation. It is characterized by a selective degeneration and death of upper and lower motor neurons, initiating in mid-adult life and progressing to paralysis and death in 1–5 years [1, 2]. The clinical manifestations reflect the involvement of both upper and lower motor neurons. Upper motor neuron dysfunction clinically leads to limb spasticity, weakness, and brisk deep tendon reflexes. On the other hand, lower motor neuron involvement causes fasciculations, muscle wasting, as well as limb weakness. Bulbar upper motor neuron defect causes spastic dysarthria and dysphagia. The sporadic form (SALS) is the most frequent, making up approximately 90% of all cases, whereas the familial variant (FALS) affects about 10% of patients. The incidence is around two to three cases per 100,000 general population annually, and the prevalence is around four to six per 100,000 [3, 4]. The first identified FALS causative gene is the copper–zinc superoxide dismutase-1 (*SOD1*) gene on chromosome 21q that causes approximately 20% of FALS [5]. One of the most important recent advances in the ALS field has been the identification of new genes including those for TAR DNA binding protein (TDP)43, fused in sarcoma (FUS), optineurin, and spatacsin [6–10]. These findings reinforce the idea that multiple pathogenic mechanisms can lead to a common motor neuron phenotype, with obvious implications in therapy design.

Although the majority of human ALS is sporadic and without known genetic causes, another motor neuron disease, spinal muscular atrophy (SMA), has a completely

defined genetic defect in the survival motor neuron 1 (*SMN1*) gene [11]. In 2010, Kaspar et al. [12–15] and three other groups described the use of a viral vector expressing human SMN that was administered into a mouse model of SMA. They reported the successful expression of SMN in the mouse spinal cord, and, of greater importance, a nearly complete rescue of the phenotype. These studies are promising as a model for gene therapy in diseases of known genetic mutation and protein deficiency, and human clinical trials are now planned. In the context of these remarkable pre-clinical gene therapy successes in other motor neuron diseases, the crucial questions are how to translate them in the ALS field and if we really need to wait to identify new molecular targets for developing a gene therapy for ALS.

Thus, the focus of our review is ALS research reports on gene therapy approaches, discussing molecular strategies that seem promising in terms of developing clinically translatable therapies. We analyzed: (1) RNA interference (RNAi) and antisense approaches involving both (a) specific FALS causative genes like *SOD1* and (b) other molecular pathogenetic targets; and (2) gene therapy approaches delivering neuroprotective substances. Breakthroughs in terms of efficacy achieved with the use of viral vectors such as adeno-associated virus (AAV) vectors are discussed.

RNAi and antisense therapies

RNAi therapy

RNAi is a process by which noncoding micro RNA (miRNA) inhibits and regulates gene expression by binding mRNA [16]. This endogenous gene-silencing mechanism is now being used to study potential therapeutic applications in autosomal-dominant (heterozygous) diseases that might be cured by effective silencing of the dominant mutant allele.

Specific genes are experimentally targeted for silencing through the administration of small interfering (si)RNA produced in vitro or through the transfection of interfering short hairpin RNA (shRNA) or miRNA using viral vectors. Several hereditary neurodegenerative diseases are being targeted with RNAi therapy, including ALS [17].

Oligonucleotide therapy

Today, antisense oligonucleotides (AONs) are in clinical trials for several applications. They are relatively small ($\sim$10 kDa), have favorable biodistribution properties, and can be generated on a large scale under good manufacturing practice conditions. Short, synthetic oligonucleotides (15–25 nucleotides) bind with the target mRNA in a sequence-specific manner. Once bound, the antisense agent either disables or induces the degradation of the target RNA or can alter splicing. This method of reducing the expression of a target gene is both precise and sequence-specific. Effective delivery into the brain and spinal cord is crucial for using AONs. The antisense strategy has application as a tool in gene function and target validation studies and is emerging as a therapeutic technology [18]. AONs have therapeutic potential and are currently in various phases of translational investigation [19–23].

Silencing of specific FALS causative genes

RNAi SOD1

SOD1 FALS arises through toxic gain-of-function mutations rather than enzyme deficiency and is a good target for RNAi therapy. Several studies have demonstrated effective SOD1 silencing and improved motor outcomes in mice. Some of the early studies demonstrating RNA silencing in ALS included muscle injections of a lentiviral vector with shRNA, which reduced SOD1 expression, improved motor neuron survival, and delayed the onset of ALS symptoms [24]. Intraspinal injections of a viral-mediated RNAi also slowed the onset and progression of disease [25], and the development of a transgenic SOD1 mutant mouse with anti-SOD1 siRNA inhibited mutant protein production and prevented the development of clinical disease [26]. This protective effect was solely attributed to gene silencing in the central nervous system (CNS), as muscle-restricted SOD1 knockdown yielded no benefits [27]. Further studies have confirmed the ability to suppress mutant SOD1 production, and in most cases, to delay clinical disease progression in rodent models of RNAi [28–31]. Two studies in 2009 highlighted the promise of RNAi therapy for ALS in the SOD1 model [32, 33]. Rizvanov et al. [32] showed that siRNA applied to the proximal stump of a severed sciatic nerve in the SOD1 mutant mouse decreased SOD1 mRNA levels in the lumbar spinal cord. Therefore, retrograde transport of siRNA in motor nerves could deliver RNAi therapy. Using a viral vector, Wu et al. [33] delivered RNAi with both nerve and muscle injections, silencing mutant SOD1. However, only nerve injections had the double effect of slowing disease progression and prolonging survival.

In 2011, Kubodera et al. [34] refined the in vivo application of an RNA strategy for the selective silencing of the mutant SOD1 allele. The authors previously highlighted an approach for selective suppression of mutant alleles, applying this method to spinocerebellar ataxia type 6; both mutant and wild-type alleles are silenced by the most effective siRNA, and wild-type protein is re-expressed with

the use of mRNA mutated to be resistant to the siRNA [35]. Since the expression of the endogenous wild-type *SOD1* gene was reduced by more than 80% in the previously reported anti-SOD1 shRNA transgenic mice [35], in this work [34] the authors aimed to restore wild-type SOD1 protein expression in the same mice. Therefore, they generated a transgenic mouse resistant to SOD1 shRNA and crossed it with anti-SOD1 shRNA mice [34]. siRNA-resistant SOD1 sequence was generated to encode the same amino acid sequence as that of native SOD1, but contained mutations in the nucleotide sequence to avoid the shRNA matching [34].

Concern has arisen about the applicability of this new technology in people and for ALS. The translation of RNAi methods to patients without a known genetic mutation is untried. It might be considered for blocking production of the TDP43 protein. In general, with RNAi therapies, safety and efficacy must be demonstrated in comparing modes of RNAi administration.

Direct siRNA administration is easily controlled because it is local and can be interrupted in case of side-effects, but, on the other hand, the treatment is short-acting and difficult to deliver to the CNS. Conversely, viral vectors seem to be more efficient in delivering RNAi, but there are concerns about the long-term and off-target effects of RNAi binding [36].

In 2011, Towne and colleagues examined recombinant AAV (rAAV) vectors expressing shRNA for silencing mouse (m)SOD1 [37]. AAV vectors constitute a promising system for in vivo clinical applications because of their neuronal tropism, stable transgene expression in quiescent cells, low pathogenicity, and poor rate of integration into the host genome [38, 39]. Towne et al. examined the ability of rAAV serotype 6, a vector capable of axonal retrograde transport, to silence mSOD1 in motor neuron pools from the periphery. Mouse neonate muscle injections of AAV6-shSOD1 vector reduced mSOD1 levels in skeletal muscle and motor neurons, conferring neuroprotection and complete protection against muscle wasting [37]. However, this approach did not therapeutically modify disease progression in the ALS mouse model. These results underlie the complexity of gene delivery for mSOD1 silencing and the difficulty of translating local neuroprotective effects into functional improvements. As reported by Towne in 2011, reduction of mutated SOD1 protein in the studies reported above, although was achieved, is not followed by a modification of disease phenotype (Table 1). This is likely due to a limited efficacy of the intramuscular route of AAV injection to direct global motor neuron transduction. Only the work of Ralph et al. has demonstrated a remarkable extension in survival through intramuscular injection. This result is probably due to the efficiency of the specific lentiviral vector used

Table 1 Summary of SOD1 silencing with RNAi

Reference	Injection site	Dose	Vector	% of transduction	SOD1 protein reduction	Extension in survival
Ralph et al. [24]	Intramuscular (hindlimb, facial, tongue, diaphragm and intercostals)	120 µl (7×10^8–1×10^9 transducing units/ml)	Lentiviral vectors based on the equine infectious anemia virus	95% in vitro	70% in vitro	227 days
Raoule et al. [25]	Bilaterally in the lumbar L3–L4 region	3 µl (60,000 ng of p24 antigen/ml)	Self-inactivating lentiviral vectors	20% in vivo	70% in vitro 52% in vivo	–
Miller et al. [28]	Intra-muscular (right lower hind limb)	25 µl (1×10^{12} particles/ml)	AAV2-siRNA-GFP	–	–	–
Miller et al. [27]	Intra-muscular (hindlimbs)	1×10^{11} AAV2	AAV2-siRNA	–	60% in vivo	–
		1×10^7 lentiviral particles	Lentiviral siRNA		60% in vivo	
Towne et al. [30]	Intramuscular (gastrocnemius)	30 µl (2×10^9 vg)	AAV6 shSOD1	3.9% in vivo	59% in vivo	No effect
	intravenous	200 µl (2×10^{11} vg)		–	–	–
Rizvanov et al. [32]	Lumbar spinal cord (sciatic nerve)	–	No vectors	–	–	–
Wu et al. [33]	Intraspinal cord (sciatic nerve)	7 µl (1.25×10^9 viral particles)	RAd-shR–SOD1-EGFP	–	–	10 days
		7 µl (5×10^8 viral particles)	AAV2-U6–shR-SOD1-EGFP			No effect
Towne et al. [37]	Intramuscular (hindlimb, forelimb, intercostal, facial muscle, thoracic cavity)	2×10^7 transducing units	AAV6-shSOD	–	83% in vivo	No effect

(70% protein reduction in vitro, 95% transduction efficiency in vitro) [24]. Considering the studies described above, lentiviral vectors have been shown to be more efficient in improving disease phenotype than AAV after intramuscular injection.

Antisense

SOD1 can be silenced also by modified AONs that are complementary to a target sequence in its mRNA. In 2006, Smith and colleagues demonstrated that modified AONs, continuously infused intraventricularly, reach different CNS areas in both rodents and primates, including the area involved in the major neurodegenerative diseases [40]. Using this route of administration, this group found that AONs to SOD1 diminished both SOD1 protein and mRNA levels in the brain and spinal cord. The authors tested several SOD1-AONs, and all were phosphorothioate-modified chimeric oligonucleotides composed of five 2′-O-(2-methoxy)ethyl modifications on both the 5′ and 3′ ends and ten oligodeoxynucleotides in the center to support, but had different nucleotide sequence. Infusion of SODr/h333611, the lead compound, did not affect disease onset and slowed disease progression of 37%, however, the treatment did not significantly extended survival in SODG93A rats (only for 10 days). These results support the hypothesis that direct administration of AONs could be an efficacious, dose-regulatable strategy for treating neurodegenerative diseases, including ALS, in which target proteins are identified [40], even if its therapeutic effect has yet to be clearly demonstrated.

Moreover, this experimental strategy using antisense is being prepared for a clinical trial in patients with FALS linked to SOD1. The timing of treatment for the animal model was near symptom onset, reflecting the scenario for patients who often have definite and even advanced signs of motor neuron loss by the time of ALS diagnosis. Thus, this therapeutic approach may produce a similar therapeutic benefit in SOD1 patients. In 2010, Isis Pharmaceuticals, Inc., started a phase 1 study of AONs (ISIS-SOD1Rx) designed to reduce production of mutant SOD1. The trial is a placebo-controlled, dose-escalation study designed to assess the safety, tolerability, and pharmacokinetic profile of ISIS-SOD1Rx. The molecule used for patients is ISIS 333611 (http://www.clinicaltrials.gov/ct2/show/NCT01041222) that is described in the paper by Smith et al. [40]. The drug will be injected intrathecally (through an external pump and a temporary catheter to administer the molecule into the spinal fluid) during a single, 12-h infusion. The proposed trial design includes four dosage cohorts, each with eight participants: six participants will be randomly assigned to receive SOD1Rx and two participants will receive placebo.

Silencing of other molecular pathogenetic targets in ALS

The RNAi strategy can be applied also to other molecular pathogenetic targets. In 2003, Turner et al. demonstrated that the death-signaling p75 neurotrophin receptor (p75NTR) can be a promising antisense target in the treatment of ALS. Intraperitoneal injection into pre-symptomatic SOD1G93A mice of antisense peptide nucleic acid (PNA) targeting p75NTR significantly delayed locomotor injury and prolonged survival [41]. Using the same strategy, Rembach et al. [42] silenced the Ca^{2+} permeable glutamate receptor subunit 3 (GluR3), which has been implicated in several neurologic conditions regulating intracellular calcium ion (Ca^{2+}) levels in SOD1G93A mice. Mice treated with intraperitoneal injections of the antisense PNA at postnatal day 50 had an approximate 10% increase in lifespan compared to that of controls. Western-blot analysis, however, did not reveal a significant reduction in GluR3 protein levels in whole extracts of the lumbar spinal cord. Indeed the entry of antisense PNA into the spinal cord and motor neurons needs to be clearly demonstrated [42].

However, RNAi-mediated knockdown of the Fas receptor has produced the most significant rescue [43], probably because of direct administration in the CNS. In that study, Locatelli et al. investigated the role of Fas-linked motor neuron death in the pathogenesis of ALS through in vitro and in vivo silencing of the Fas receptor on motor neurons carrying the SOD1-G93A mutation. After in vitro treatment, motor neurons demonstrated increased survival and a reduction in cytochrome C release from the mitochondria, whereas in vivo intrathecal administration of Fas siRNA improved motor function and survival in SOD1-G93A mice. This study demonstrated that Fas silencing can interfere with motor neuron degeneration in SOD1-G93A mice, providing new insights into ALS pathogenesis and suggesting possible strategies of molecular therapy for SALS and FALS [43].

Gene Therapy with AAV

Viral vector-based gene delivery: latest-generation viral vectors

A gene therapy strategy often relies on viral vectors that promise efficacy for delivery of genes to the CNS and to enhance motor neuron survival. Vectors based on AAV and lentiviruses are proving to be particularly effective for the treatment of neurological disorders [44]. These vectors are attractive for their simplicity and their high transduction efficiency for neurons [45]. Given the recent experimental success with AAVs, here we describe only these vectors.

AAVs

AAV is a non-pathogenic, single-stranded DNA virus. Vectors derived from AAV can transduce both dividing and non-dividing cells, confer long-term stable gene expression without associated toxicity, and be produced at high clinical-grade titers [46–48]. The number of defined AAV serotypes expands each year, and over 100 AAV variants have been isolated from different animal species [49]. The first ten serotypes are the best characterized, and AAV2 is the most widely studied and employed. The absence of destructive effects on tissue and the presence of long-term gene expression have laid the groundwork for the therapeutic use of AAVs in the nervous system. Consequently, the vast majority of ongoing and planned clinical trials for gene therapy application in neurodegenerative diseases are based on AAV vectors [50]. AAV1, 2, and 5 have all been approved by the U.S. Food and Drug Administration for use in clinical trials [51]. The viral coat profoundly affects tropism; AAV serotypes differ in their capsid protein amino acid structure, but the mechanism triggering distinct or selective tropisms remains unknown. However, differences in the capsid proteins can alter the in vivo tropism of the AAV vectors [52]. In general, most of the novel serotypes exhibit a predominant neuronal tropism and biodistribution in the rodent CNS, particularly in the brain. AAV2 has a strong neuronal tropism but limited spread when injected intraparenchymally because of its propensity to bind heparin sulfate proteoglycan present in the extracellular matrix [53, 54]. Unlike AAV2, AAV4 preferentially transduces astrocytes and ependymal cells [55], while AAV5 also transduces neurons [56, 57]. AAV3 failed to transduce any astrocytes and demonstrated a low transduction efficacy for all CNS cell types [58]. AAV1 and AAV6 transduce neurons with greater efficiency and distribution compared with AAV2 [58]. Finally, AAV8 and AAV9 can be more efficient than AAV1 and 2 after intravascular and/or intraventricular delivery in neonate or adult mice [59–61], and AAV8 has a potential oligodendrocyte tropism, which is demonstrated with two recent studies [58, 62].

The binding properties of AAV can also be altered by mixing the capsid proteins from different serotypes to generate novel AAV vectors with unique tropisms [63]. Most recently, the generation of mosaic or chimeric AAV vectors through AAV display [64] or DNA shuffling [65] has yielded targeted vectors with selective/enhanced features. In these strategies, random capsid recombinations are screened for specific binding or transport properties either in vitro or in vivo. Finally, gene expression can be restricted to neuronal subtypes or glia with the use of neuronal- or glial-specific promoters, as described for lentiviral and adenoviral vectors [66]. Two groups recently demonstrated the efficacy of AAVs, reporting efficient and widespread transduction of sensory and motor neurons in the spinal cord after intravascular delivery of self-complementary (sc)AAV9 to the facial vein of neonate mice [59, 60]; indeed, scAAV9 crossed the blood–brain barrier. Moreover, excellent results have been achieved by four groups with AAV carrying SMN gene in a SMA mouse model, showing the efficacy of AAV as therapeutic strategy [12–15]. However, Duque and collaborators [59] have advanced a step further by demonstrating the efficacy of this approach in adult mice and successful translation in a large animal (i.e., adult cats) without pharmacological disruption of the blood–brain barrier [59]. Of interest, in these studies, scAAV vectors performed better than single-stranded vectors.

Delivery of factors for neuroprotection

In diseases with identified genetic defects, ongoing research focuses on targeted gene therapy using viral vectors to improve abnormal protein synthesis. However, because the precise molecular mechanisms underlying SALS are still not understood, gene therapy strategies have targeted general mechanisms including neuroprotection through trophic factors, anti-apoptotic proteins, and anti-oxidants and anti-excitotoxicity genes. Federici and Boulis [67] have provided a detailed description of these experiments up to 2006. Here, we have reviewed papers from 2006 to 2010, but all known available papers are listed in Tables 2 and 3.

Neurotrophic factors hold much promise for therapy in ALS [68]. Angiogenin (ANG) is a potent inducer of neovascularization [69–71], and its expression has been detected in motor neurons [72–74]. In 2008, Kieran's team examined the biological role of ANG in controlling motor neuron survival. They showed that ANG has neuroprotective activities on motor neurons in vitro and in vivo. In particular, in vitro expression of ANG protected against cell death produced by endoplasmic reticulum stress and trophic factor withdrawal and against excitotoxic injury. Moreover, SOD1(G93A) mice treated systemically with human recombinant ANG protein from either 50 days (before symptom onset) or 90 days (after symptom onset) showed an increase in lifespan and in motor neuron survival. These data suggest that ANG is a critical determinant of motor neuron survival and demonstrate an important role for ANG in the pathophysiology of ALS [75].

Another promising neurotrophic factor for ALS is insulin-like growth factor-I (IGF-I). Delivery of IGF-I protein reduces motor neuron loss [76] and prevents motor neuron death [77, 78]. In 2007, Lepore and colleagues injected an AAV2-based vector encoding human IGF-1 into the ventral gray matter in the lumbar L4–L5 region of

Table 2 Summary of causative genes silencing in familial ALS

Silencing agent	Animal model	Dose	Administration	Start (days)	Onset change (%)	Survival change (%)	Author
EIAV-shRNA SOD1	SOD1G93A mouse	$\sim 1 \times 10^8$ units	Intramuscular	7	↑115	↑76	Ralph et al. [24]
LV-shRNA SOD1	SOD1G93A mouse	90 ng p24 antigen	Intraspinal	40	↑20	nd	Raoul et al. [25]
Anti-SOD1 shRNA	Anti-SOD1 siRNA Tg mouse	nd	Electroporation in ES cells	Over the entire 300-day duration of the experiment, no differences were observed with respect to wild-type			Saito et al. [26]
AAV2-siRNA-SOD1	SOD1G93A mouse	25×10^9 particles	Intramuscular	49	nd	nd	Miller et al. [28]
AAV-shRNA	SOD1G93A mouse	1 × 107 units	Intramuscular	40	ns	nd	Miller et al. [27]
Oligonucleotide targeting SOD1	SOD1G93A rat	100 μg/d	Infusion with an osmotic pump in lateral ventricle	65	0	↑8.2	Smith et al. [40]
Anti-SOD1G93A shRNA	B3neo/Gur or B3neo/G93A mouse	nd	Pronuclear injection of the fertilized eggs	nd	↑40 in B3neo/G93A	↑50 in B3neo/G93A ↑30 in B3neo/Gur	Xia et al. [29]
siRNA Fas	SOD1-G93A	1 mg/kg/day	Infusion with osmotic minipump intrathecally	90	↑18.75	↑12.7	Locatelli et al. [43]
AAV6-RNAi-SOD1	SOD1-G93A mouse	2×10^{11} vg	Intravenously	42	0	0	Towne et al. [30]
siRNA SOD1	B6SJL-TG(SOD1-G93A) dl1Gur/J	100 nM, total volume 7 _m	Injection into sciatic nerve	252	nd	nd	Rizvanov et al. [32]
AAV2 shRNA SOD1	SOD1G93A mouse	1.25×10^9 particles.	Into sciatic nerve	94	Treatment starts at disease onset	↑7.5	Wu et al. [33]
rAAV-2/8-shRNA or -shRNA/resistant SOD1	SOD1G93A mouse	1×10^{12} vector genomes	Intravenously	nd	nd	nd	Kubodera et al. [34]

Table 3 Summary of factors delivery with gene therapy

Agent	Model	Administration	Dose	Start (days)	Onset change (%)	Survival change (%)	Reference
GDNF plasmid	SOD1G93A	Intramuscular	50 mg plasmid fortnightly	63	↑	ns	
AV-CT-1	SOD1G93A	Intramuscular	1×10^8 units	5–6	↑21	↑8	
AV-GDNF	SOD1G93A	Intramuscular	5×10^9 units	5–7	↑7	↑12	
AAV-Bcl-'2	SOD1G93A	Intramuscular	$\sim 1 \times 10^8$ units	35	↑8	Not significant	
AAV-GDNF	SOD1G93A	Intramuscular	1×10^{11} units	63	↑13	↑14	
AAV-GDNF	SOD1G93A	Intramuscular	1×10^{10} units	60	↑18	↑9	
AAV-GDNF	SOD1G93A	Intramuscular	1×10^{10} units	90	nd	↑6	
AAV-Follistatin	SOD1G93A	Intramuscular	1×10^{11} units	40	nd	Not significant	Miller et al. [27]
AAV-IGF-1	SOD1G93A	Intramuscular	1×10^{10} units	60	↑34	↑30	
AAV-IGF-1	SOD1G93A	Intramuscular	1×10^{10} units	90	nd	↑18	
AAV-IGF-1	SOD1G93A	Intraspinal	5×10^{10} units	60	↑20	↑10	Lepore et al. [80]
EIAV-VEGF	SOD1G93A	Intramuscular	1×10^8 units	21	↑30	↑30	
EIAV-VEGF	SOD1G93A	Intramuscular	1×10^8 units	90	nd	↑18	
PNA GluR3	SOD1G93A	Intraperitoneal	2.5 mg/kg, 3 times a week	50	↑9	↑9	Rembach et al. [42]
PNA $p75^{NTR}$	SOD1G93A	Intraperitoneal	2.5 mg/kg, 3 times a week	60	↑10	↑8	Turner et al. [41]
siRNA Fas	SOD1G93A	Intraspinal	1 mg/kg days	90	↑	↑14	Locatelli et al. [43]

60-day-old SOD1G93A mice. Animals treated with AAV2 IGF-1 showed a significantly greater number of motor neurons compared to control, demonstrating that IGF-1 delivery could partially rescue motor neuron loss. Disease onset was slowed, and survival significantly increased [79]. The following year, Dodge et al. delivered an IGF-1–expressing viral vector to the CNS through bilateral injection into the deep cerebellar nuclei (A–P: −5.75; M–L: −1.8; D–V: −2.6 from bregma and dura) of 88- to 90-day-old SOD1G93A mice. A benefit of this delivery approach is that it facilitates access from a single injection site to several areas involved in neurodegeneration in ALS, without administration of the vector directly into the spinal cord where neurodegeneration is occurring. This approach reduced ALS neuropathology, ameliorated weakness, and significantly improved lifespan in ALS mice. Moreover, through an in vitro model of ALS, this group supported their in vivo findings, demonstrating that IGF-1 reduces the pathological activity of non-neuronal cells that are important for disease progression [80]. In 2009, Franz et al. [81] confirmed these results, injecting AAV2 with IGF-1 in presymptomatic (80 days of age) hSOD1G93A rat spinal cord.

Recently, Dodge et al. repeated experiments with IGF-1 and evaluated the effect of AAV4-mediated delivery of another promising trophic factor, vascular endothelial growth factor (VEGF) [82]. Injection into the cellular components of the ventricular system and spinal cord central canal in SOD1(G93A) transgenic mice resulted in delayed motor decline and a significant extension of survival. On the other hand, the combination of IGF-1- and VEGF-165-expressing AAV4 vectors showed no additional efficacy, suggesting that these trophic factors act on similar signaling pathways [82].

Petruska et al. [83] engineered AAV serotypes AAV1, AAV2, and AAV5 with the neurotrophin-3 (*NT-3*) gene, a neurotrophic factor that stimulates sensory axon regeneration. After virus injection into the medial gastrocnemius muscle of adult rats, motor neuron intracellular recording revealed complex electrophysiological changes. They found novel correlations between modified NT-3 expression and single-cell electrophysiological parameters, indicating that intramuscular administration of AAV(NT-3) can exert long-lasting effects on synaptic transmission to motor neurons [83].

Granulocyte-colony stimulating factor (G-CSF) is a cytokine first described as a hematopoietic growth factor that stimulates proliferation and differentiation of myeloid precursors [84]. Furthermore, G-CSF protects motor neurons, ameliorates functional outcomes, and improves lifespan of SOD-1(G93A) mice when administered subcutaneously [85]. In 2010, Henriques et al. [86] used AAV to directly target G-CSF expression to the spinal cord, showing that intraspinal delivery improved motor function, delayed disease progression, and increased survival by 10%.

Conclusions

Up to now, no effective therapies have been developed for ALS, the pathogenesis of which remains largely unknown.

Only a few genes have been identified as being differentially expressed and specifically linked with ALS pathogenesis, including SOD1 [5], and more recently, TDP43 and FUS [6]. Different methods targeting known deregulated genes in ALS have been proposed. Gene suppression can be achieved with oligonucleotide approaches, such as RNAi and AONs. These methods have been tested only with two genes, SOD1 and Fas, and important results have been reported in animal models [40]. However, downregulation of SOD1 causes delayed disease progression only with injection into the CNS, and clinical applicability is not yet defined [32]. The only trial in patients with FALS resulting from a SOD1 mutation was started in 2010 by Isis Pharmaceuticals, involving injection of SOD1 inhibitor directly into cerebrospinal fluid. The main problem with these new drug strategies is delivery of gene regulators to the target cells—the motor neurons—because of the necessity of crossing the blood–brain barrier and of obtaining compounds with long-lasting effects. To overcome these obstacles, different strategies have been tested, such as direct injection into the CNS with a pump or catheter or the use of AAVs. Viral vectors seem to be particularly promising for gene therapy. AVV vectors have the greatest potential, especially AAV9, which can pass the blood–brain barrier and exhibit neuronal tropism, while AAV2 is particularly specific for neuron tissue but must be administered directly into the CNS. These vectors have been employed to deliver factors identified as crucial for neuronal survival such as IGF-1, G-CSF, ANG, or NT-3. These methods have not only been tested in small animal models, particularly in mice, but also in bigger animals such as cats and monkeys [87]. All of these new approaches have yielded quite promising results, although much experimental work remains to be done to achieve effective therapy for ALS patients.

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