

Current and emerging treatments for amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a relatively rare neurodegenerative disorder of both upper and lower motoneurons. Currently, the management of ALS is essentially symptoms-based, and riluzole, an ant glutamatergic agent, is the only drug for the treatment of ALS approved by the food and drug administration.

Objective: We reviewed current literature concerning emerging treatments for amyotrophic lateral sclerosis.

Methods: A Medline literature search was performed to identify all studies on ALS treatment published from January 1st, 1986 through August 31st, 2009. We selected papers concerning only disease-modifying therapy.

Results: Forty-eight compounds were identified and reviewed in this study.

Conclusions: Riluzole is the only compound that demonstrated a beneficial effect on ALS patients, but with only modest increase in survival. Although several drugs showed effective results in the animal models for ALS, none of them significantly prolonged survival or improved quality of life of ALS patients. Several factors have been implicated in explaining the predominantly negative results of numerous randomized clinical trials in ALS, including methodological problems in the use of animal-drug screening, the lack of assessment of pharmacokinetic profile of the drugs, and methodological pitfalls of clinical trials in ALS patients.

Keywords: amyotrophic lateral sclerosis, therapy, drug, survival

Introduction

Amyotrophic lateral sclerosis (ALS) is a relatively rare neurodegenerative disorder characterized by progressive loss of both upper and lower motor neurons in the brain, brainstem, and spinal cord. The progression of the disease is usually rapid, leading to death on average within 3–5 years.¹

The underlying cause of ALS remains unclear, but an interplay between endogenous (genetic, metabolic) and exogenous factors (environmental, lifestyle) is believed to be involved in the development of the disease.^{2,3}

Although ALS usually develops sporadically, 5%–10% of cases are familial and hereditary. Twenty percent of familial ALS are caused by the mutation in Cu/Zn superoxide dismutase-1 (SOD1) gene.¹

The development of animal models of ALS has provided progress in understanding the underlying mechanisms of the disease because the sporadic and the familiar forms of ALS share similar clinical and pathological features.^{3,4}

Several animal models have been extensively used in ALS through the years, including different transgenic mouse models, wobbler mouse and one canine model.^{3,4}

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The most clinically relevant animal model of ALS is the SOD1 transgenic rodent (mainly mice) model, that is genetically engineered to express a mutant form of the human SOD1 gene. The most commonly used SOD1 mouse harbors the glycine to alanine mutation at position 93 (G93A). This mutation results in a toxic gain of function of Cu/Zn SOD1 that enhances the generation of damaging oxygen radicals.³

A wide range of mechanisms are thought to be implicated in the pathogenesis of the disease: these include mitochondrial dysfunction, excitotoxicity, oxidative stress, protein misfolding, proteosomal dysfunction, aberrant growth factor signaling, microinflammatory process and glial activation.²⁻⁵

Riluzole, an antiglutamatergic agent that inhibits the presynaptic release of glutamate, is the only drug for the treatment of ALS approved by the US Food and Drug Administration (FDA).⁶ However, it is known to have limited therapeutic benefits and only modest effects on survival of ALS patients.⁶ Therefore, to date there is no effective cure for ALS and the management of ALS in clinical practice remains essentially supportive and symptoms-based.^{6,7}

In recent years, great efforts have been made in the search for effective treatments of ALS; a large number of neuroprotective agents have been proposed candidates for the treatment of ALS and several clinical trials have been planned and conducted.⁸ The purpose of this review is to summarize the current and emerging treatments for amyotrophic lateral sclerosis.

Methods

A Medline literature search was performed to identify all studies on neuroprotective treatment of ALS published from January 1st, 1986 through August 31st, 2009, using the MeSH terms “motor neuron disease”, “motor neurons”, “amyotrophic lateral sclerosis”, “treatment”, “therapy”, “clinical trials”, “experimental studies”, and “drugs”. Articles and abstracts were included only when published in English. Additional references were taken from article citations. For the purpose of this review we considered only disease-modifying therapy.

Results

Following data extraction, we identified a group of 48 potential therapeutic agents. These compounds were grouped and reviewed based on their hypothetical mechanisms of action (Table 1). A list of undergoing clinical trials for ALS is also reported (Table 2).

Antiglutamate agents

Riluzole

Riluzole is an antiglutamatergic agent thought to inhibit the presynaptic release of glutamate.⁹ In a mouse model of ALS, treatment with riluzole significantly delayed the onset of the disease and slowed the decline in motor function.^{8,9} Recently, a systematic review of ALS treatment with riluzole has been performed by the Cochrane Neuromuscular Diseases group.⁶ The review included four clinical trials (overall number of treated patients: 1,477).⁶ Based on this meta-analysis, riluzole treatment with 100 mg daily was considered safe, well tolerated and was associated with a statistically significant improvement in tracheostomy-free survival. The effect size was however small, as the median increase in survival is about two to three months.⁶

Results from population-based studies indicated that riluzole therapy increased survival rates at 12 months by approximately 10% and prolonged survival by 4–6 months.^{10,11} One study observed also a stronger beneficial effect amongst bulbar-onset ALS and patients aged >70 years (increase in median survival time: eight months).¹¹ The favorable effect of the drug was transient and lost in prolonged follow-up (after 18 months).^{10,11} A study on transgenic rats demonstrated that the deficit in glutamate uptake becomes more severe by end-stage of the disease and is probably the cause for the loss of efficacy of the drug in advanced ALS.¹² More studies are therefore needed, especially to clarify the effects of riluzole in older patients, in bulbar-ALS, and in patients with more advanced disease.

Memantine

Memantine is a low-affinity, noncompetitive antagonist of both open-channel N-methyl-D-aspartate (NMDA) and α -calcium-permeable-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors.^{13,14} It permits the blockade of excessive NMDA receptors activity, without disrupting normal synaptic transmission.¹³ Various *in vitro* and *in vivo* models of excitotoxicity showed that memantine has neuroprotective properties¹⁴ and the drug has been used clinically with excellent safety in various neurodegenerative disorders, including Alzheimer's disease.¹⁵ Two recent animal studies on SOD1 transgenic mice found that the drug is effective in slowing progression and increasing survival of transgenic mice.^{16,17} In one study, the administration of memantine had therapeutic effects, even when given at symptoms onset.¹⁷ Data on ALS patients are lacking, although one phase II clinical trial in US and combined phase II–III clinical trials are ongoing.¹⁸

Table I List of drugs tested in ALS, with hypothetical mechanisms of action and currently available results

Compound	Mechanism of action	Principal results
Riluzole	Antiglutamatergic	Preclinical studies with positive results ^{8,9} In three clinical trials was associated with increase in survival of about two months ⁶ Results from population-based studies indicated an increase in survival rates at 12 months by 10% and in median survival by 4–6 months ^{10,11} A trend for stronger beneficence amongst bulbar-onset ALS and patients aged >70 years was also observed ^{10,11} The favorable effect was transient, as it was lost in prolonged follow-up (after 18 months) ^{10,11}
Memantine	Antiglutamatergic	Neuroprotective in <i>in vitro</i> and <i>in vivo</i> studies ¹⁴ Positive preclinical studies on SOD1 transgenic mice ^{16,17} No data on humans
L-Arginine	Antiglutamatergic	Positive preclinical studies on SOD1 transgenic mice ¹⁹ Lower plasma L-arginine levels in ALS patients ²⁰ No clinical trials on ALS patients
Ceftriaxone	Antiglutamatergic	Positive preclinical studies on SOD1 transgenic mice ^{21,22} Limited data on humans
Cobalamin	Antiglutamatergic	Two trials on SOD1 transgenic mice gave positive results ^{26,27} A small double-blind trial found that short term (four weeks) high-dosage (0.5 mg/day) was effective in improving compound motor action potential, used as indicator of lower motoneuron number ²⁸ Patients with a good response to treatment presented slower disease progression (disease duration: 23.1 vs 18.8 months; $P = 0.02$) ²⁸ The clinical benefit was however transient, as it was followed by deterioration after 1–3 months ²⁸
Talampanel	Antiglutamatergic	Prolongs survival in SOD1 transgenic mice ⁸ In a phase II study on 60 patients with ALS showed safe and well tolerated results ^{8,23} No data on efficacy
N-acetylated alpha-linked acidic dipeptidase	Antiglutamatergic	Positive results from <i>in vivo</i> studies on motor neurons ³¹ and <i>in vivo</i> studies on SOD1 transgenic mice ³⁰ No data on safety and efficacy in ALS patients
Topiramate	Antiglutamatergic	Preclinical studies with conflicting results: effective <i>in vitro</i> , but not <i>in vivo</i> ³² A randomized clinical trial showed no benefits. Patients receiving the drug had more pulmonary emboli, deep vein thrombosis, and renal calculi ³³
Gabapentin	Antiglutamatergic	Preclinical results with gabapentin suggested that this agent may prolong motor neuron survival ³⁴ A phase II randomized trial found a slowing of the rate of strength decline ³⁵ A phase III clinical trial found no benefit on survival ³⁶
Lamotrigine	Antiglutamatergic	Animal model with axotomy gave positive results ³⁸ Two small sample randomized clinical trials found no beneficial effects on survival and markers of motor performances ^{39,40}
r-IGF-I	Neuroprotective	<i>In vitro</i> and <i>in vivo</i> studies gave positive results ⁴¹ A first clinical trial found beneficial effect, ⁴² while two further clinical trials gave negative results ^{43,44}
Mechano-growth factor	Neuroprotective	A variant of IGF-I was more effective on survival of SOD1 transgenic mice, compared to IGF-I ⁴⁷ No data on humans
Ciliary neurotrophic factor	Neuroprotective	Preclinical study with positive results ⁴⁸ Two clinical trials revealed it was ineffective in ALS patients ^{50,51} A trend for higher serious adverse events was noted ^{50,51}
EPO	Neuroprotective Anti-inflammatory Antiapoptotic	Neuroprotective effects in <i>in vivo</i> and <i>in vitro</i> studies ^{53,54} Safe and well tolerated in a phase II clinical trial ⁵⁵ No data on efficacy in humans

(Continued)

Table I (Continued)

Compound	Mechanism of action	Principal results
VEGF	Neuroprotective (against excitotoxicity)	VEGF polymorphisms have been associated with an increased risk for ALS ²³ Preclinical studies found efficacy on SOD1 transgenic mice ^{56,57} No data regarding safety, tolerability or efficacy in humans. The compound requires intrathecal delivery ²³ A phase II clinical trial is underway ²⁴
rh-GSF	Neuroprotective Antiapoptotic	Preclinical studies on ALS animal models gave positive results ⁶⁰ Safe and well tolerated in two small sample open-label pilot studies ^{6,62} One study found a trend of slowing disease progression ⁶²
rh-HGF	Neuroprotective Antiapoptotic Antiglutamatergic	Prolonged survival in different studies on SOD1 animal models ^{63–65} Safety or efficacy data in patients with ALS are lacking and the compound requires intrathecal delivery
BDNF	Neuroprotective	Positive results in preclinical studies on ALS animal models ^{68,69} A trend for prolonged survival after the subcutaneous infusion of BDNF was found in a phase I/II study ⁷⁰ A large phase III placebo-controlled clinical trial found no beneficial effect from subcutaneous administration of BDNF ⁷¹ Intrathecal infusion was safe and well tolerated in 25 ALS patients ⁶⁶
GDNF	Neuroprotective	Treatment with GDNF mediated by either an adeno-associated virus vector ^{72–74} or by mesenchymal stem cells ^{75,76} was effective in preclinical <i>in vitro</i> and <i>in vivo</i> studies No data on ALS patients
Xaliproden	Neuroprotective	Modest effects on vital capacity but not on survival in phase II and III clinical trials ^{79,80}
Coenzyme Q	Antioxidant Mitochondrial cofactor	Prolongs survival in SOD1 transgenic mice ⁸¹ Safe and well tolerated in a recent phase II clinical trial ⁸² Not effective in a phase II fertility trial ⁸³
Creatine	Antioxidant Mitochondrial cofactor	Preclinical studies with positive results ^{23,84} Several phase II clinical trials on ALS patients using doses up to 10 g/day gave negative results ^{85–87} Treatment with 20 g/day increases maximal isometric power in ALS patients ⁸⁸ Clinical trials using high dosage or a combination with celecoxib are ongoing ²⁴
Vitamin E	Antioxidant	Effective in ALS animal models ⁹ High intake of vitamin E was associated with a 50%–60% decreased risk of developing ALS, in a recent retrospective case-control study ⁹³ Safe, well tolerated, but not effective in two double blind, placebo-controlled, clinical trials as add-on to riluzole ^{91,92}
Edavarore	Antioxidant (Free-radicals scavenger)	Preclinical studies with positive results ⁹⁴ Was safe and well tolerated and there was a suggestion of slowed disease progression in an open-label phase II study ⁹⁵
R(+) Pramipexole	Antioxidant Antiapoptotic	Prolonged survival in an animal study on SOD1 transgenic mice ⁹⁶ A nonsignificant reduction in disease progression was observed in a recent phase II clinical study ⁹⁷
AEOL-10150	Antioxidant	Effective in prolonging survival in animal study on SOD1 transgenic mice ^{99–101} In a recent open-label study on patients with ALS was safe and well tolerate ¹⁰²
Ammonium tetrathiomolybdate	Antioxidant (copper-chelating drug)	Positive preclinical studies on SOD1 transgenic mice ¹⁰⁴ No data on humans
N-acetylcysteine	Antioxidant	Prolonged survival and delayed onset of motor impairment in ALS animal study ¹⁰⁵ Not effective in a double-blind placebo-controlled clinical trial ¹⁰⁶
TRO19622	Antioxidant	Positive results from <i>in vitro</i> and <i>in vivo</i> studies ¹⁰⁷ No data on ALS patients
Tamoxifen	Antioxidant	A phase II clinical trial indicated a trend for survival benefit with administration of tamoxifen at the dose of ≥ 20 mg/day ¹⁰⁸

(Continued)

Table I (Continued)

Compound	Mechanism of action	Principal results
Minocycline	Antiapoptotic Anti-inflammatory	Extends survival in mouse models of some neurological conditions ¹⁰⁹⁻¹¹¹ Safe and well tolerated in phase-II clinical trials ¹¹² A recent multicenter, randomized placebo-controlled phase III trial found that minocycline in escalating doses of up to 400 mg/day for nine months has a harmful effect on patients with ALS ¹¹³
TCH-346	Antiapoptotic	A small sample double-blind placebo controlled clinical trial conducted find no beneficial effects ¹¹⁴
zVAD-fmk	Antiapoptotic	Significantly delayed disease onset and prolonged survival in SOD1 transgenic mice ¹¹⁶ Data on ALS patients are still not available
Pentoxifylline	Antiapoptotic	A large phase II randomized clinical trial found that the drug is not effective in ALS and should be avoided in patients treated with riluzole ¹¹⁷
Celecoxib	Anti-inflammatory (cyclo-oxygenase-2 inhibitor)	Positive preclinical studies on SOD1 transgenic mice ^{119,120} A double-blind, placebo-controlled, clinical trial demonstrated that celecoxib (800 mg/day) was safe but did not have a beneficial effect on patients with ALS ¹²¹ A clinical trial evaluating the association with creatine at high dose is underway ²⁴
Glatiramer acetate	Anti-inflammatory Antiglutamatergic growth factor-stimulating effects	Conflicting results from preclinical studies: some studies found that it prolonged survival in SOD1 mutant mice, ¹²⁴ while others did not ¹²⁵ Was safe and well tolerated in a phase II trial ¹²⁶ A double-blind, randomized, placebo-controlled, multicenter trial confirmed safety and tolerability but did not show any beneficial effect ¹²⁷
AM-1241	Anti-inflammatory (via cannabinoid receptors)	Delayed the disease progression in animal studies ^{128,129} No data on humans are available
Celastrol	Anti-inflammatory Antioxidant Interfere with protein aggregation (by heat-shock protein induction)	Significantly improved weight loss, motor performance and delayed the onset of ALS in SOD1 transgenic mice ¹³⁰ No data on humans are available
Thalidomide	Anti-inflammatory	Enhanced motor performance, decreased motor neuron cell death, and significantly increased the life span in animal studies ¹³¹ A small open-label study find no improvement in progression of the disease and a high risk for side effects ¹³²
Nordihydroguaiaretic acid	Anti-inflammatory Antiglutamatergic	Extends survival and slows disease progression in animal studies ¹³⁵ No data are available on humans
Pioglitazone	Anti-inflammatory	Significantly improved muscle strength and body weight, delayed disease onset, and prolonged survival in SOD1 transgenic mice ¹³⁶⁻¹³⁸ No data are available on humans
RO-28-2653	Anti-inflammatory (inhibits matrix metalloproteases)	Prolongs survival in ALS SOD1 transgenic mice ¹³⁹ No data on humans are available
ONO-2506	Anti-inflammatory Restores astrocytes Antiglutamatergic	Limited data on preclinical studies and on humans ¹⁴⁰ Trials have been however recently completed
Lithium	Inductor of autophagy Antioxidant	Effective in preclinical studies on SOD1 transgenic mice ¹⁴¹ In a small sample open label study delayed disease progression in 44 patients affected by ALS ¹⁴¹ Two large clinical trials are ongoing ²⁴
Sodium phenylbutyrate	Inhibit protein aggregation (histone deacetylase inhibitor)	Promotes cell survival, alone or in combination with riluzole in animal studies ^{144,145} Safe and well tolerated in a recent open-label study ¹⁴⁶
Valproate	Inhibit protein aggregation (histone deacetylase inhibitor)	Preclinical studies on SOD1 mutant mice gave discordant results, ¹⁴⁹⁻¹⁵² some studies found that it prolongs survival, ^{149,150} while others did not ^{151,152} Safe but not effective in a recent sequential clinical trial ¹⁵³

(Continued)

Table 1 (Continued)

Compound	Mechanism of action	Principal results
Scriptaid	Inhibit protein aggregation (histone deacetylase inhibitor)	Preliminary positive results in cultural cells transfected with SOD1 ¹⁵⁴ No data on animal models and humans are available
Ariclomol	Inhibit protein aggregation (by heat-shock protein induction)	Effective in preclinical studies ^{155,156} Safe and well tolerated in a phase II clinical trial ¹⁵⁷ No data on efficacy on ALS patients

Abbreviations: ALS, amyotrophic lateral sclerosis; r-IGF-I, recombinant insulin-like growth factor; EPO, recombinant human erythropoietin; VEGF, vascular endothelial growth factor; rh-GSF, recombinant human granulocyte-macrophage colony-stimulating factor; rh-HGF, recombinant human hepatocyte growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; SOD1, superoxide dismutase-1.

L-Arginine

L-Arginine is a semiessential amino acid that serves as sole substrate for enzymes involved in diverse cell processes. Preclinical studies have found that L-arginine protects cultured motor neurons from glutamate excitotoxic injury.¹⁹ The mechanism underlying these favorable effects is still not known but may be related to the synthesis of neuroprotective polyamines, essential for neuronal survival and regeneration.¹⁹ L-Arginine supplementation in SOD1 transgenic ALS mice, administered both prior to and after the onset of motor neuron degeneration, significantly slowed the progression of neuropathology in lumbar spinal cord, delayed onset of motor dysfunction, and prolonged life span.¹⁹ Moreover, lower plasma L-arginine concentrations have been reported in ALS patients, probably due to malnutrition associated with advanced ALS.²⁰ Although L-arginine has potent *in vitro* and *in vivo* neuroprotective properties and may be a candidate for therapeutic trials in ALS, data on humans are lacking.^{8,18}

Ceftriaxone

Ceftriaxone, a beta-lactam antibiotic, modulates the expression of glutamate transporter GLT1 via gene activation and may also act as metal chelator.²¹ Preclinical studies demonstrated that it prolongs survival in different animal models of ALS.^{21,22} This compound has been used extensively in humans and is safe.²³ However, intravenous administration is required and there is limited safety experience in ALS patients.²³ A combined long-term clinical trial of intravenous treatment with ceftriaxone has been started. The study consists of three stages. The first two stages will evaluate brain penetration, safety and side effects. The third stage will determine whether the study drug prolongs survival and slows decline in function due to ALS.^{18,24}

Cobalamin

Vitamin B12 (cobalamin) has multiple protective effects that can be potentially relevant in ALS. Accumulating

evidence indicates that B-vitamin inhibits the cytotoxicity induced by NMDA and protects cultured neurons against glutamate excitotoxicity.²⁵ Cobalamin also has antioxidant and antiapoptotic properties.²⁶ In two controlled trials on G93A SOD1 transgenic mice, multivitamin therapy with cobalamin, folic acid and pyridoxine significantly prolonged average lifespan improved motor performance and delayed disease onset of treated mice, compared to controls.^{26,27} Furthermore, cobalamin administered pre-symptomatically significantly delayed the onset of motor neuron disease in one of the studies.²⁶ In a small sample double blind clinical trial conducted on 24 Japanese ALS

Table 2 List of compounds undergoing clinical trials for ALS¹⁶³

Compound	Phase	Mechanism of action
Ariclomol	II/III	Heat-shock protein induction
Ceftriaxone	II/III	Antiglutamatergic
Cobalamin	II/III	Antiglutamatergic
Creatine	II/III	Antioxidant
Celecoxib		Anti-inflammatory
Edavarore	II/III	Free-radicals scavenger Reduces mutant SOD1 deposition
Lithium	a) II/III b) II/III	Inductor of autophagy
Memantine	II/III	Antiglutamatergic
Pioglitazone	II	Anti-inflammatory
R(+)-pramipexole	II	Antioxidant Antiapoptotic
Talampanel	II	Antiglutamatergic
Thalidomide	a) I b) II	Anti-inflammatory
Valproate	II/III	Inhibits protein aggregation by acting as a histone deacetylase inhibitor
VEGF	II	Protects motor neurons against excitotoxicity

Abbreviations: ALS, amyotrophic lateral sclerosis; SOD1, superoxide dismutase-1; VEGF, vascular endothelial growth factor.

patients short term (four weeks) high-dosage (0.5 mg/day) administration of methyl-cobalamin was effective in improving compound motor action potential, used as indicator of lower motoneuron number.²⁸ Patients with a good response to treatment presented slower disease progression (disease duration: 23.1 vs 18.8 months; $P = 0.02$) and predominant lower motor neuron involvement, compared to nonresponders.²⁸ The clinical benefit however was transient, as it was followed by deterioration after 1–3 months.²⁸ A large-scale long-term clinical trial is ongoing in Japan to evaluate the long-term efficacy and the safety of ultra-high-dose methylcobalamin for ALS.²⁹

Talampanel

Talampanel is a noncompetitive modulator of glutamate AMPA glutamate receptors primarily developed as an antiepileptic agent. Talampanel significantly prolonged survival in SOD1 ALS transgenic mice.⁸ In a phase II study on 60 patients with ALS, talampanel was safe and well tolerated.^{8,23} A trend for slower decline in ALS Functional Rating Scale (ALS-FRS) score was also observed in the treated subgroup, although the study was not powered to detect efficacy.^{8,23} Therefore, there are still no data on its efficacy on patients with ALS.

N-acetylated alpha-linked acidic dipeptidase (NAALADase)

N-acetylated alpha-linked acidic dipeptidase (NAALADase) is an inhibitor of glutamate carboxypeptidase II, which converts the neuropeptide N-acetylaspartylglutamate to glutamate.³⁰ Glutamate carboxypeptidase II inhibitors may provide neuroprotection by simultaneously decreasing glutamate production and inhibiting glutamate release.³⁰ Preclinical *in vitro* studies in SOD1 transgenic mice found that treatment with selective inhibitors of glutamate carboxypeptidase II significantly delays the onset of clinical symptoms and prolongs life.³⁰ Glutamate carboxypeptidase II inhibitors were protective against histological abnormalities induced by mutant SOD1 in *in vitro* studies on motor neurons cultures.³¹ In phase I single dose and repeat dose trials treatment with NAALADase was safe and well tolerated by both healthy volunteers and diabetic patients.³⁰ There are however still no data on safety and efficacy in ALS patients.

Topiramate

Topiramate is an anticonvulsant with antiglutamatergic properties. It reduces glutamate release from neurons and blocks AMPA receptors. *In vitro* studies found that topiramate protects motor neurons in an organotypic spinal cord

culture system in which glutamate transport is inhibited by pharmacological blockade.³² Conversely, the drug did not increase survival in G93A SOD1 transgenic mice.³²

A randomized placebo controlled clinical trial has been recently conducted in 296 ALS patients from the US. Patients were randomized (2:1) to receive topiramate (maximum tolerated dose up to 800 mg/day) or placebo for 12 months.³³ At the dosages studied, topiramate did not have a beneficial effect for patients with ALS. Moreover, high-dose topiramate treatment was associated with a faster rate of decline in muscle strength and with an increased risk for several adverse events, such as pulmonary emboli, deep vein thrombosis, and renal calculi.³³

Gabapentin

Gabapentin is another antiepileptic drug with antiglutamatergic properties. Gabapentin may reduce the pool of releasable glutamate and thus decrease glutamate excitotoxicity.³⁴ Preclinical studies with gabapentin suggested that this agent may prolong motor neuron survival.³⁵ A six-month phase II randomized trial in 150 patients with ALS found a nonstatistically significant trend towards slowing of the rate of strength decline in patients taking gabapentin (up to 2,400 mg day), compared with those taking placebo (mean difference 24%, median 37%; $P = 0.057$).³⁵ In a phase III randomized placebo controlled clinical trial 204 ALS patients received oral gabapentin 3,600 mg or placebo daily for nine months. The mean rate of decline of the arm muscle strength was not significantly different between the groups.³⁶ Moreover, there was no beneficial effect on the rate of decline of other secondary measures, as vital capacity, survival and ALS-FRS score.³⁶ Confirming these findings, a recent small proton magnetic resonance spectroscopy study on 18 ALS patients showed that treatment with gabapentin was not associated with improvement in spectroscopic markers of neuronal integrity in motor and nonmotor cerebral regions.³⁷

Lamotrigine

Lamotrigine is an antiepileptic drug that inhibits glutamate release.³⁸ Treatment with lamotrigine was associated with a reduction in motor neuron loss in an animal model using axotomy.³⁸ Two small sample, randomized phase I clinical trials found no beneficial effects on survival and markers of motor performances on total 97 ALS patients.^{39,40}

Neurotrophic factor

Recombinant insulin-like growth factor (rIGF-1)

Recombinant insulin-like growth factor (r-IGF-1) is a potent neurotrophic factor that has neuroprotective properties in the central and peripheral nervous systems.⁴¹ Due to the efficacy

of IGF-I in the treatment of other diseases and its ability to promote neuronal survival in both *in vitro* and *in vivo* studies, IGF-I has been extensively studied in ALS.^{23,41}

The efficacy and safety of r-IGF-1 in ALS has been tested in three clinical trials.^{42–44} With the exception of an increased risk of injection site reactions with r-IGF-I, the drug showed otherwise safe and well tolerated results. However, the benefit on survival was inconsistent across the studies. One study showed a slowing in functional decline,⁴² whilst no benefit was observed in the second.⁴³ The combined analysis from both trials performed by the Cochrane Group showed a trend towards for beneficial effect favoring the treated group.⁴⁵ A third placebo-controlled trial has been recently completed.⁴⁴ There was no difference between treatment groups in the primary and secondary outcome measures after a two-year follow-up period.⁴⁴ In conclusion, r-IGF-I is well tolerated but, although so far is the only agent other than riluzole to show on any ALS markers of disease progression, can not be considered beneficial for patients with ALS. Recently, an adeno-associated virus has been engineered to contain the gene for IGF-1 (IGF-1/AAV).²³ Theoretically, after the intramuscular injection, this vector could allow to deliver IGF-1 to motor neurons.²³ Preclinical studies revealed that IGF-1/AAV can prolong survival in SOD1 ALS transgenic mice.⁴⁶ However, there are no data on safety, tolerability or pharmacokinetics of IGF-1/AAV in humans with ALS.

Mechano-growth factor (MGF)

The mechano-growth factor (MGF), an IGF-I splice variant, has been shown to have greater neuroprotective effects than IGF-I in a number of models of neurodegeneration.²³ In an animal study on SOD1 transgenic mice the intramuscular administration of a mammalian expression plasmid containing MGF or, for comparison, the IGF-I DNA sequence resulted in a significant improvement in hind-limb muscle strength, and an increase in motor unit and motor neuron survival.⁴⁷ Significantly more motor neurons survived in MGF treated mice.⁴⁷ There are still no data on safety and efficacy in humans.

Ciliary neurotrophic factor (CNTF)

Ciliary neurotrophic factor (CNTF) is a neuroactive cytokine found in Schwann cells, which seems to be released in response to nerve injury.²³ CNTF maintains survival of adult motor neurons and mice lacking the CNTF gene develop mild, progressive motor neuron loss.⁴⁸ In a recent study, serum level of CNTF was significantly higher in ALS patients than in controls.⁴⁹ There was no difference between familial and sporadic ALS, and a trend for higher levels was observed in patients with spinal-onset ALS, compared to patients with a bulbar onset

of the disease.⁴⁹ ALS patients in two trials (n = 1,300) were treated with subcutaneous CNTF.^{50,51} No significant difference in either primary or secondary outcomes was observed between CNTF and placebo groups.^{50–52} However, a significant increase of the incidence of several adverse events was noted in groups treated with higher doses of CNTF.⁵² Therefore CNTF can not be considered beneficial for patients with ALS.

Recombinant human erythropoietin (EPO)

Recombinant human erythropoietin (EPO) is used to stimulate red blood cell production in patients with anemia. Preclinical studies in different models of peripheral and central nervous system diseases revealed that EPO has also anti-inflammatory and antiapoptotic properties.^{53,54} A recent phase II double-blind, randomized, placebo-controlled study on 23 patients showed that treatment with subcutaneous EPO was safe and well tolerated.⁵⁵ However, larger studies are warranted to confirm safety and to investigate different dose schedule and efficacy.

Vascular endothelial growth factor (VEGF)

VEGF polymorphisms have been associated with an increased risk for ALS in some, but not all populations.²³ Therefore VEGF deficiency may play a role in the pathogenesis of ALS. The most important limitation as for other growth factors, is that requires invasive administration (intraventricular or via viral vectors).²³ Preclinical studies on different ALS animal models found that intracerebral or intraspinal treatment with VEGF prolongs survival and reduces disease progression, particularly when given before the onset of symptoms.^{56,57} *In vitro* studies showed that VEGF protects motor neurons against excitotoxicity.⁵⁸ Finally, intratecal transplantation of neural stem cells overexpressing VEGF was effective in several animal studies.⁵⁹ There are, however, no data regarding safety, tolerability or efficacy in humans, although a phase II clinical trial is ongoing.²⁴

Recombinant human granulocyte-stimulating factor (rh-GSF)

Recombinant human granulocyte-stimulating factor (rh-GSF), used to stimulate white blood cell production in patients with leucopenia, has been proposed for ALS because the GSF receptor is expressed by motor neurons, has neurotropic effects, and protects cultured motor neuronal cells from apoptosis.⁶⁰ In a recent animal study, continuous subcutaneous delivery of GSF, given at the stage of the disease where muscle denervation is already evident, significantly improved motor performance, delayed the onset of severe motor impairment and prolonged overall survival

of SOD1 transgenic mice model.⁶⁰ In two small sample open-label pilot studies on 39 ALS patients overall, rh-GSF was safe and well tolerated.^{61,62} One study found a trend of slowing disease progression following rh-GSF treatment, as shown by lower decline of quality of life and ALS-FRS score.⁶² Larger studies are needed.

Recombinant human hepatocyte growth factor (rh-HGF)

Recombinant human hepatocyte growth factor (rh-HGF) has, in addition to its neurotropic effects, antiapoptotic and antiglutamatergic properties.^{63,64} Intrathecal administration and gene therapy significantly prolonged survival in different studies on SOD1 animal models, even if delivered at symptom onset.⁶³⁻⁶⁵ A recent immunohistochemical study on both familial (SOD1) and sporadic ALS found that HGF is expressed on the anterior horn cells of the spinal cord, supporting the hypothesis that disruption of HGF system thereby contributes to the acceleration of neuronal degeneration in ALS patients.⁶⁶

However, safety or efficacy data in patients with ALS are lacking and the compound requires intrathecal administration.

Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that supports the survival and growth of developing motor neurons.⁶⁷ Preclinical studies in several animal models found that BDNF treatment significantly prolongs survival and slows the loss of motor neurons.^{8,68,69} In phase I/II study, the subcutaneous infusion of BDNF increased survival and retard loss of pulmonary function in ALS patients,⁷⁰ but a large phase III placebo-controlled clinical trial of subcutaneous administration of 25 or 100 µg/kg n 1.135 ALS patients failed to demonstrate a statistically significant effect of BDNF on survival.⁷¹ Post hoc analyses revealed a statistically significant benefit in ALS patients with an early respiratory impairment. Higher subcutaneous dosage or an intrathecal delivery have been proposed to emphasize the possible beneficial effects of the drug. Recently, in a phase I/II trial intrathecal infusion of recombinant methionyl human BDNF in doses of up to 150 µg/day showed safe and well tolerated results in 25 ALS patients, although reversible mild sensory symptoms were reported in the higher-dosage subgroup.⁶⁷ Studies on the efficacy of intrathecal BDNF are therefore required.

Glial cell-derived neurotrophic factor (GDNF)

Glial cell-derived neurotrophic factor (GDNF) has a potent trophic effect on motor neurons.⁷¹ Several preclinical

in vitro and *in vivo* studies found that treatment with GDNF mediated by either an adeno-associated virus vector⁷²⁻⁷⁴ or by mesenchymal stem cells^{75,76} is effective in prolonging motor neurons survival. Conversely, studies from patients with sporadic ALS gave conflicting results.^{77,78} Increased cerebrospinal fluid levels of GDNF in patients with ALS compared to controls⁷⁷ and upregulation of GDNF gene in both spinal cord and muscle of sporadic ALS have been indeed observed.^{77,78} These findings indicate that the capacity to synthesize GDNF is enhanced in ALS. Clinical trials of GDNF in ALS patients are however lacking.

Xaliproden

Xaliproden is a nonpeptidic compound with growth factor activities.⁸ A double-blind, placebo-controlled phase II study conducted in 54 ALS patients treated for up to 32 weeks showed a significantly slower rate of deterioration in vital capacity (VC) (43%; $P = 0.046$) in xaliproden-treated patients.⁷⁹ Two randomized phase III clinical trials have been conducted: one with xaliproden and riluzole and the other with xaliproden alone. Two primary endpoints were defined: time to death, tracheostomy, or permanent assisted ventilation and time to VC of less than 50%.⁸⁰ The drug demonstrated in both studies modest benefits for VC but not for the other endpoints.⁸⁰ Therefore the drug is not significantly effective in ALS.

Antioxidant

Coenzyme Q 10

Coenzyme Q 10 has multiple potential mechanisms that can be relevant in ALS. It acts as an antioxidant and an essential mitochondrial cofactor that facilitates electron transfer in the respiratory chain.²³ Animal studies revealed that coenzyme Q 10 can prolong survival in SOD1 transgenic mice.⁸¹ In an open-label, dose-escalation study, doses up to 3,000 mg per day administered orally over eight months was safe and well tolerated in 31 patients with ALS.⁸² Conversely, results of a phase II futility trial on 185 patients showed no benefit on survival of 2,700 mg daily oral treatment with coenzyme Q 10.⁸³ Long term safety and efficacy in humans are limited, but several randomized studies in patients with ALS recently terminated recruitment.²⁴

Creatine

Creatine has multiple potential effects that might be relevant in ALS, including its antioxidant properties, stabilization of the mitochondrial transition pore and facilitation of mitochondrial ATP synthesis. Important advantages of creatine are also its oral administration, elevate brain penetration and the excellent

safety profile.^{8,23} Preclinical studies on SOD1 transgenic mice revealed that creatine significantly increases survival, when given before the onset of the disease.^{23,84} Three double blind, placebo-controlled clinical trials on creatine monohydrate use have been recently conducted.^{85–87} In one clinical trial creatine was administered at doses of 10 mg/day over a 16-month follow-up period,⁸⁵ while the other two studies used a dosage of 5 mg/day over a six- and nine-month period of observation.^{86,87} All these studies gave negative results as creatine failed to show a benefit on survival or multiple markers of disease progression. A possible explanation of these negative results may be that these trials did not use doses that optimize brain phosphocreatine levels, as preliminary results demonstrated that treatment with 20 g/day increases maximal isometric power in ALS patients.⁸⁸ Alternatively, the combination of higher doses of creatine with other drugs may be used to maximize its benefit, as indicated by results from recent animal studies.⁸⁹ Confirming these observations, an innovative phase II “selection” trial, in which creatine at 20 g/day was used in combination with either minocycline or celecoxib, found that the mean decline in ALS Functional Rating Scale (ALS-FRS) score was lower in the celecoxib-creatine group compared to the minocycline-creatine group and an historical cohort.⁹⁰ The celecoxib-creatine may be therefore a preferable combination for further evaluation. Two clinical trials with high dose creatine and with celecoxib-creatine association are underway.²⁴

Vitamin E

Vitamin E (alpha-tocopherol) is the most important lipid-soluble antioxidant and protects cell membranes from oxidation by reacting with lipid radicals.²³ Preclinical studies showed that treatment with vitamin E slows down the onset and progression of the paralysis in SOD 1 transgenic mice.⁹ Two double blind, placebo-controlled, clinical trials on ALS patients from Germany and France evaluated the safety and efficacy of high-dose vitamin E (5000 mg per day) when given added to riluzole, over a follow up period of 18 and 12 months, respectively.^{91,92} No significant difference between placebo and treatment group could be detected either in the primary or the secondary outcome measures, although the French trial observed that patients receiving alpha-tocopherol were less likely to progress from the milder state to the more severe state, according to the ALS Health State scale.⁹¹ In a recent retrospective case-control study, a high intake of vitamin E was associated with a 50%–60% decreased risk of developing ALS.⁹³ Further clinical trials with longer follow-up or larger sample sizes are needed.

Edaravone

Edaravone (MCI-186) is an agent widely used for cerebral ischemia in Japan that acts as a free-radical scavenger.⁹⁴ In a randomized blind trial, intraperitoneally administration of multiple doses of edaravone in an ALS mice model significantly slowed the motor decline and motor neuron degeneration of the transgenic mice, even when administered after the onset of the disease.⁹⁴ Furthermore, high-dose edaravone treatment was associated with a significant decrease in the area of mutant SOD1 deposition in the spinal cord.⁹⁴ The favorable effects of the drug might be attributable to its primary antioxidant properties or alternatively to the reduction of mutant SOD1 accumulation.⁹⁴

In an open-label phase II study of 20 patients with ALS, the intravenous administration of edaravone was safe and well tolerated and there was a suggestion of slowed disease progression, measured by the ALS-FRS scale during the six-month treatment period, compared with the six months before the administration of edaravone.⁹⁵ Treatment with edaravone also resulted in a marked reduction of 3-nitrotyrosine, a marker of oxidative stress.⁹⁵ A phase III clinical trial is undergoing in Japan.²⁴

R(+) pramipexole

R(+) pramipexole is the enantiomeric homolog of the dopamine agonist used in Parkinson's disease and can reduce oxidative stress in patients with ALS.⁹⁶ *In vitro* and *in vivo* studies revealed that it is concentrated into the brain and mitochondria and efficiently scavenges reactive oxygen and nitrogen species,^{96,97} and blocks caspase activation.⁹⁸ As it has less affinity for dopamine receptors than pramipexole, it should have fewer side effects.⁹⁶ In SOD1 ALS transgenic mice, treatment with R(+) pramipexole prolongs survival.⁹⁸ A small open-label dose-escalation study on 30 ALS patients revealed a nonsignificant 17% reduction in the rate of decline of ALS-FRS in the group of patients receiving the highest dosage (60 mg/day).^{8,97} A study on safety and tolerability has just terminated the recruitment.²³ Further studies are however warranted.

AEOL-10150

The manganese porphyrin AEOL-10150, is a small-molecule antioxidant analogous to the catalytic site of superoxide dismutase, that scavenges peroxynitrite and other deleterious oxidants.^{8,23} It has been indicated as a potential subcutaneous treatment for ALS.⁹⁸ The administration of AEOL-10150 at symptom onset markedly prolonged survival in SOD1 transgenic mice.^{99–101} Recently, the single dose subcutaneous

treatment with AEOL-10150 was safe and well tolerated in 25 patients with ALS.¹⁰² A multiple dose phase II safety study is underway.^{24,102} Although there are limited data in humans with ALS, a recent meta-analysis of preclinical trials conducted on SOD1 transgenic mice found that AEOL-10150 can be considered the most promising compound for evaluation in a treatment trial.¹⁰³

Ammonium tetrathiomolybdate (TTM)

Ammonium tetrathiomolybdate (TTM) is a copper-chelating drug that is capable of removing a copper ion from copper-thiolate clusters, such as SOD1.¹⁰⁴ A recent preclinical study on SOD1 transgenic mice found that treatment with TTM significantly delayed disease onset, slowed disease progression, and prolonged survival by approximately 20%, 42%, and 25%, respectively.¹⁰⁴ TTM was also effective in depressing the spinal copper ion level and inhibiting the lipid peroxidation, with a significant suppression of SOD1 enzymatic activity in SOD1.¹⁰⁴ There are still no data on humans.

N-acetylcysteine

N-acetyl-L-cysteine is an antioxidant agent that reduces free radical damage.⁸ Preclinical studies in transgenic mice with SOD1 mutation showed that N-acetyl-L-cysteine significantly extends survival and delayed onset of motor impairment.¹⁰⁵ However, in a double-blind placebo-controlled clinical trial on 110 ALS patients, acetylcysteine 50 mg/kg daily subcutaneous infusion did not result in a major increase in 12-month survival or a reduction in disease progression.¹⁰⁶ Therefore, the beneficial effects of cysteine in ALS seem questionable.

TRO19622

TRO19622 is a cholest-4-en-3-one steroidal oxime identified via through-put screening.¹⁰⁷ TRO19622 may increase mitochondrial stability by directly bounding to two components of the mitochondrial permeability transition pore: the voltage-dependent anion channel and the translocator protein.¹⁰⁷ *In vitro* studies found that TRO19622 promotes motor neuron survival in a dose-dependent manner.¹⁰⁷ *In vivo*, TRO19622 rescued motor neurons from axotomy-induced cell death promoted nerve regeneration.¹⁰⁷ Finally, treatment with TRO19622 significantly improved motor performances, delayed the onset of the disease and extended survival in SOD1 transgenic mice.¹⁰⁷ There are still no data on safety and efficacy on humans.

Tamoxifen

Tamoxifen is a selective estrogen receptor modulator that belongs, as TRO19622, to the family of steroidal oximes.⁸

Along with the well known antineoplastic activity, tamoxifen may inhibit the action of protein kinase C and may bind the mitochondrial permeability transition pore.⁸ Preliminary results of a 24-month phase II clinical trial indicated a trend for survival benefit with administration of tamoxifen at the dose of ≥ 20 mg/day.¹⁰⁸

Antiapoptotic

Minocycline

Minocycline is a tetracycline antibiotic that has antiapoptotic and anti-inflammatory effects *in vitro* (prevents microglial activation and caspase activation). Minocycline extends survival in mouse models of some neurological conditions, as ALS.^{109–111} Two double-blind, randomized, placebo-controlled phase II clinical trials demonstrated that the drug is safe and well tolerated in 42 ALS patients;^{23,112} however these studies were not powered for efficacy.²³ A recent multicenter, randomized placebo-controlled phase III trial on 412 patients found that minocycline in escalating doses of up to 400 mg/day for nine months has a harmful effect on patients with ALS. A faster ALS-FRS score deterioration and greater mortality (hazard ratio, 1.32; 95% confidence interval [CI]: 0.83–2.10; $P = 0.23$) was observed in the minocycline group than in the placebo group.¹¹³ These results indicate that minocycline is not effective in ALS patients.

TCH346

TCH346 is an antiapoptotic agent that binds to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and blocks the apoptotic pathway in which GAPDH is involved. TCH346 treatment delayed disease onset and slowed the clinical course of the disease in the ALS mouse model.¹¹⁴ A small sample double-blind placebo-controlled clinical trial conducted on 591 patients from Europe and North America failed to find any beneficial effect of TCH346 given at several dosages on disease progression in patients with ALS.¹¹⁵

N-benzyloxycarbonyl-Val-Asp-fluoromethylketone (zVAD-fmk)

N-benzyloxycarbonyl-Val-Asp-fluoromethylketone (zVAD-fmk) is a broad enzymatic caspase inhibitor.¹¹⁶ Intraventricular administration of zVAD-fmk in the late presymptomatic stage significantly delayed disease onset and prolonged survival in SOD1 transgenic mice.¹¹⁶ Data on ALS patients are still not available.

Pentoxifylline

Pentoxifylline is a phosphodiesterase inhibitor that increases cellular cyclic AMP and GMP and demonstrates

antiapoptotic properties.¹¹⁷ A randomized clinical trial conducted on 400 European ALS patients found that treatment with pentoxifylline as add-on to riluzole was not associated with appreciable effect on functional measures.¹¹⁷ Moreover, pentoxifylline had a negative effect on survival. At the end of follow-up period, 51.7% of patients were alive in the pentoxifylline group compared to 59.7% in the placebo group (unadjusted risk 1.28, $P = 0.107$; adjusted risk 1.43, $P = 0.02$).¹¹⁷

Anti-inflammatory

Cyclooxygenase (COX-2) inhibitors

The enzyme cyclooxygenase-2 (COX-2) has been proposed as an attractive therapeutic target in ALS because its increase in the spinal cord stimulates astrocytic glutamate release.¹¹⁸ Elevated levels of COX-2 and prostaglandin E₂ have been observed in the spinal cord of SOD1 mutant mice and ALS patients.¹¹⁸ Celecoxib, a COX-2 inhibitor has been shown to be beneficial in preclinical testing, prolonging survival of SOD1 mice.^{119,120} A 12-month double-blind placebo-controlled clinical trial was conducted on 300 patients with ALS. Subjects were randomized (with a 2:1 ratio) to receive celecoxib (800 mg/day) or placebo for 12 months.¹²¹ Treatment with celecoxib showed safe results but did not have a beneficial effect on the decline in muscle strength, vital capacity, motor unit number estimates, ALS-FRS score, or survival in patients with ALS.¹²¹

Nimesulide has been indicated as the preferential COX-2 inhibitor because of has additional antioxidant properties and can be administered via multiple routes, including orally.²³ Preclinical observations revealed that nimesulide administration decreases prostaglandin E₂ levels in the spinal cord of SOD1G93A mice and preserves motor skill integrity.¹²² However, its putative mechanism of action is the same as celecoxib and safety concerns surrounding long-term administration of this medication class may limit the use of COX-2 inhibitors in patients with ALS.²³ Their combination with other compounds such as creatine is under evaluation.²⁴

Glatiramer acetate

Glatiramer acetate, a combination of four amino acids, is the analogous of myelin basic protein and it is used to reduce the frequency of relapses in patients with multiple sclerosis.²⁰ It requires subcutaneous administration and is believed to act by enhancing regulatory T-cell immunity.^{23,123} In addition, it may also have antiglutamatergic and growth factor-stimulating effects.¹²³ Results of preclinical studies are limited and conflicting, some studies found that it prolongs

survival in SOD1 mutant mice,¹²⁴ while others did not.¹²⁵ In a phase II trial conducted on 20 ALS patients the drug showed safe, well tolerated results and affected the immune system at the dosage studied.¹²⁶ A recent large-scale double-blind, randomized placebo-controlled multicenter trial on 366 ALS patients confirmed safety and tolerability of glatiramer acetate at a dose of 40 mg/day but did not show any beneficial effect of the drug on rate of deterioration of the ALS-FRS scale, or time to death, tracheostomy or permanent assisted ventilation.¹²⁷ Further studies are required.

AM-1241

Cannabinoids produce anti-inflammatory actions via cannabinoid receptor 1 and 2 (CB2) and delay the progression of neuroinflammation.¹²⁸ AM-1241 is a selective agonist at the CB2 cannabinoid receptors, that are dramatically up-regulated in inflamed neural tissues associated with CNS disorders.¹²⁸ Animal studies on SOD1 mutant mice reported that the injections at symptom onset can dramatically prolong survival.^{128,129} However, there is no experience with this compound on humans and administration is likely to be parenteral.²³

Celastrol

Celastrol, a natural product from southern China, has multiple effects that can be relevant to ALS. It exerts potent anti-inflammatory and antioxidative effects, by suppression of tumor necrosis factor- α (TNF- α), interleukin-1B, and nitric oxide.²³ It also acts potently to increase expression of heat shock proteins.¹³⁰ The oral administration before the onset of symptoms significantly improved weight loss, motor performance and delayed the onset of ALS in SOD1 transgenic mice.¹³⁰ However, there is a lack of safety and pharmacokinetic data in humans with ALS.²³

Thalidomide

Thalidomide, is a historical sedative and now is used again in the treatment of leprosy, myeloma and cachexia. It has a number of interesting mechanisms of action for neurodegenerative disorders such as ALS, including suppression of TNF- α .²³ When administered orally to SOD1 mutant mice, it enhanced motor performance, decreased motor neuron cell death, and significantly prolonged life span.¹³¹ However a small open-label study found no improvement in progression of the disease. In addition, treatment with thalidomide was associated with several side effects.¹³² Further clinical trials are however underway.²⁴ Because of thalidomide's side effects, lenalidomide may offer a safer alternative.^{131,133}

Nordihydroguaiaretic acid

Nordihydroguaiaretic acid is a lipoxygenase inhibitor that inhibits TNF- α activation of microglia¹³⁴ and enhances glutamate uptake in motor neuronal cells.¹³⁵ A recent animal study on SOD1 transgenic mice found that nordihydroguaiaretic acid extends survival and slowed motor dysfunction. These favorable effects were observed even when administration was begun relatively late in life.¹³⁴ There are still no data on ALS patients.

Pioglitazone

Pioglitazone is a peroxisome proliferator-activated receptor- γ (PPAR- γ) agonist. It is used as an oral antidiabetic, but may also act as potent anti-inflammatory drug.¹³⁶ Three recent animal studies on SOD1 transgenic mice found that the oral administration of pioglitazone significantly improves muscle strength and body weight, delayed the disease onset and prolonged survival.^{136–138} To date, no information on safety and efficacy on ALS patients are available; however, a phase II clinical trial is ongoing.²⁴

RO-28-2653

RO-28-2653 acts as an anti-inflammatory agent by specifically inhibiting the activation of matrix metalloprotease enzymes that digest the extracellular matrix. An increased expression of matrix metalloproteinases and the degradation of the extracellular matrix in postmortem spinal cord tissue have been observed in ALS.¹³⁹ RO-28-2653 prolonged survival in familial ALS mice if given before the onset of symptoms;¹³⁹ however, the administration of the drug at disease onset did not significantly improve survival time.¹³⁹ Despite the unique mechanism of action among ALS relevant therapies, there is a lack of safety or efficacy data for this agent in ALS patients.²⁴

ONO-2506

ONO-2506 is an enantiomeric homolog of valproic acid, which has multiple potential mechanisms for ALS, as anti-inflammatory COX-2 inhibitor properties and antiglutamate functions.¹⁴⁰ ONO-2506 also restores normal astrocytes functions after brain damage and prevents reactive astrocytosis.¹⁴⁰ European phase I and II studies of 1,200 mg per day oral formulation have been conducted in humans with ALS, but results are not yet available.^{23,24} A phase III study has recently been initiated in Europe.¹⁴⁰

Autophagy inducer

Lithium

Both *in vitro* and *in vivo* studies revealed that the autophagy pathway is involved during motor neuron death with a protective role.¹⁴¹ Lithium is a compound used as a mood stabilizer,

which is neuroprotective in a variety of disease models.¹⁴¹ At low doses is a well-known autophagy inducer that clears misfolded proteins and altered mitochondria from motor neurons. In addition, lithium preserves mitochondria and sustains their genesis.¹⁴¹ Finally, lithium has been reported to decrease glial proliferation in the ALS spinal cord and induces sprouting in cortico-spinal fibers.¹⁴² Preclinical study on SOD1 transgenic mice found that lithium delayed disease onset and duration and augmented the life span.¹⁴³ These effects were associated with the activation of autophagy, an increase in the number of the mitochondria in motor neurons and suppression of reactive astrogliosis. In a small sample open label study, daily doses of lithium, leading to plasma levels ranging from 0.4 to 0.8 mEq/liter, delayed disease progression in on 44 patients affected by ALS.¹⁴³ Larger clinical trials are ongoing.²⁴

Protein aggregation: Histone deacetylase inhibitors and heat-shock protein gene inducers

Sodium phenylbutyrate

Sodium phenylbutyrate improves transcription and post-transcriptional pathways, by inhibiting histone deacetylase enzyme. Transcription dysregulation and consequent abnormal protein aggregation play a role in the pathogenesis of ALS.³ Ubiquitin cytosolic inclusions indeed represent one of the pathologic hallmark of ALS.⁸ In the mouse model of ALS sodium phenylbutyrate promoted cell survival, alone or in combination with riluzole.^{144,145} A recent 20-week open-label study found that the oral administration of sodium phenylbutyrate to 26 ALS patients was safe and tolerable.¹⁴⁶ Blood histone acetylation levels were significantly increased after sodium phenylbutyrate administration, even at the lowest dosage (9 g/day).¹⁴⁶ Further animal studies and clinical trials on long-term safety and efficacy are required.

Valproic acid

Valproic acid is a well-known antiepileptic drug that may modulate transcriptional dysregulation by acting as a histone deacetylase inhibitor.^{147,148} It also may upregulate the antiapoptotic protein Bcl-2.^{147,148} Preclinical studies on SOD1 mutant mice gave discordant results;^{149–152} some studies found that it prolongs survival when given before or at symptoms onset,^{149,150} while others did not.^{151,152} Furthermore, a recent sequential clinical trial found that treatment with valproic acid, at a dose used in epilepsy, is safe but does not show a beneficial effect on survival or disease progression in 163 patients with ALS.¹⁵³ Other clinical trials are underway.²⁴

Scriptaid

Scriptaid is a small molecule that acts as a histone deacetylase inhibitor. *In vitro* studies found that treatment with scriptaid disrupts aggresome formation in cultured cells transfected with mutant SOD1.¹⁵⁴ Trials on safety and efficacy of this compound both in animal models and ALS patients are still unavailable.

Arimoclomol

Arimoclomol amplifies heat shock protein gene expression and induces heat-shock protein during cell stress.¹⁵⁵ This drug may interfere with protein aggregation and apoptosis, mechanisms likely to be involved in ALS pathogenesis. It significantly prolonged survival in SOD1 mice, when administered either before the onset or at the symptoms onset.^{155,156} In a recent early-stage clinical trial it was administered orally at three different dosages to 84 patients with ALS over 12 weeks.¹⁵⁷ The drug showed safe and well tolerated results at doses up to 300 mg/day.¹⁵⁷ An efficacy study in ALS patients has been planned but is not yet open for recruitment, because the drug has been placed on hold by the FDA until results of preclinical toxicology studies become available.¹⁵⁸

Discussion

ALS remains a devastating disease that dramatically reduces quality of life and survival of patients, despite in recent years advances in understanding the mechanisms of ALS have been provided by the development of animal models of ALS and a large number of drugs have been tested.

The management of ALS patients is still supportive and symptoms-based and, actually, riluzole is the only compound that demonstrated a beneficial effect on ALS patients, but with only modest increase in survival. Although several drugs (such as creatine, celecoxib, IGF-1, CNF, gabapentin, topiramate, lamotrigine, minocycline, thalidomide, valproate, vitamin E) gave positive results in preclinical animal studies, none of these compounds, when tested in humans, significantly prolonged survival or improved quality of life of ALS patients.

Several factors have been implicated in the explaining the predominantly negative results of numerous randomized clinical trials in ALS, including methodological problems in the use of animal-drug screening, the lack of assessment of pharmacokinetic profile of the drugs and methodological pitfalls of clinical trials.

Use of animal-drug screening

The therapeutic successes obtained in the SOD1 ALS rodent model has not translated into effective therapy for

ALS patients. Riluzole, the only effective drug in ALS, was developed without the use of the SOD1 transgenic mice model.¹⁵⁸ Based on these observations, the utility of animal models in the preclinical phase for identifying therapeutic agents in ALS has been doubted.^{23,103}

Several possible explanations are conceivable for the discrepancy between successful animal studies and ineffective clinical trials in humans. First, most of the available therapeutic trials for ALS conducted on mice model present several methodological pitfalls, as pointed out by recent meta-analyses.^{103,159} First, the lack of control in most of the studies for critical biological confounding variables, including sex, that should be ruled out when designing and interpreting results from efficacy studies.^{103,159} A second explanation could be that treatment has been started before the onset of symptoms in more than 80% of the studies.¹⁰³ Although this approach may be more effective in showing a delay in the onset or slowing in the progression of the disease, it cannot be used in patients with sporadic ALS, as to date subjects who are at high risk for developing ALS cannot be identified. Third, only the minority of studies was randomized and investigators were blinded in an even smaller number.¹⁰³

Furthermore, the intra-species differences in pharmacokinetics, difficulties in establishing dose equivalence to obtain in humans a biologic activity similar to that observed in mice, the difference between laboratories in the design of the animal study, may also concur to explain the contrast between results of preclinical studies and ALS clinical trials.^{8,23,103}

Established consensus guidelines have been therefore advocated to ensure that ALS animal drug studies are conducted in a uniform manner.²³ With this objective, a recent study established several parameters for optimal study design in the SOD1 transgenic mouse model.¹⁵⁹ Using these new study design criteria several compounds (minocycline, creatine, celecoxib, sodium phenylbutyrate, ceftriaxone, thalidomide, and riluzole) were retested and no benefit on survival was found for any compounds, including riluzole.¹⁵⁹

Finally, another possible explanation for the contrast between results of preclinical studies and ALS clinical trials may be that the current mouse model of familial ALS is not able to evaluate the drug effect in patients with sporadic ALS. Animal drug-screening studies in ALS almost exclusively utilized the mutant SOD1 (G93A) mouse, but it remains to be firmly demonstrated that the SOD1 transgenic mouse models are an accurate and useful model for sporadic ALS.^{103,159} The role of biochemically altered SOD1 in sporadic ALS remains speculative and some pathogenetic mechanisms are different between familial and sporadic ALS.³ Alternative models that better

represent pathological features observed in sporadic ALS should be therefore obtained.²³ However, until a model of sporadic ALS will be developed, a possible strategy will be to require multiple preclinical information both from *in vitro* and *in vivo* studies before the start of clinical trials on ALS patients.

Correct assessment of pharmacokinetic profile

There has been a tendency for potentially beneficial candidates to move rapidly to large ALS clinical trials, before an adequate assessment of parameters as the pharmacokinetic profile, the safety/toxicity properties. Dose-ranging studies are a prerequisite to phase III studies to determine the most effective and safe dosage.²³ This is particularly relevant if we consider that the tolerability of a dose in healthy patients may not be taken as indication that the same dose will be safe in patients with ALS. In the clinical trial of topiramate in ALS, the frequency of adverse events was higher in patients with ALS compared to that observed in patients with epilepsy,³⁴ probably relating to the dehydration and malnutrition in patients with ALS. Finally, the lack of ability of a drug to cross the human blood–brain barrier may not represent an important issue for the efficacy of newly developed drugs in ALS. Recent studies indeed found that blood–brain barrier is compromised in the areas of motor neuron degeneration of ALS mouse models and that tight junction proteins are down-regulated in ALS patients.^{160,161}

Methodological pitfalls of ALS clinical trials

Several methodological pitfalls have been underlined in the design of most of ALS clinical trials, including the small sample size, the inclusion of heterogeneous populations, the short follow-up, and the use of inadequate efficacy measures. The small sample size is believed to prevent the assessment of mild/moderate drug effects, as we may expect in ALS.¹⁶²

The inclusion of patients with variable disease duration, site of onset (bulbar vs spinal), values of forced vital capacity may represent a remarkable source of bias.¹⁶² The enrolment of ALS of newly diagnosed cases from population-based cohorts has been proposed to test the efficacy of new pharmaceutical compounds, because an early start of treatment is an important issue in evaluating efficacy for devastating disease, such as ALS.¹⁶² Population-based cohorts may offer the advantages of a greater potential response to a given treatment, when compared to prevalent cohorts with long-lasting disease, as observed by studies conducted on riluzole.^{10,11} Moreover, a rigorous control of confounding

factors is necessary in ALS clinical trials, given the presence of prognostic indicators that may significantly affect the primary end-points of the study.

The study end-points are an important issue for the choice of the study design. A wide range of end-points have been included, from death or tracheostomy, gastrostomy, mechanical ventilation, and a number of disability measures, like ALS-FRS.²³ Another crucial point is the short duration (6–12 months) of the large majority of clinical trials; this is an important issue to evaluate the efficacy at the late stage of the disease.^{10,11}

Comments

We believe that the development of more potent riluzole analogs should be an important issue in the near future, as riluzole is the only therapy to date that slows disease progression in patients with ALS.⁶

Until this is confirmed, the use of a single medication that targets more than one pathogenic pathway or combining agents with different mechanisms of action could represent a therapeutic approach to the disease. Although multiple drug interactions might hypothetically increase the incidence of side effects, such combination therapy may be successful, as observed in oncology.²³

Clinical trials including “cocktail therapies” should also be designed using new drugs as add-on therapies to riluzole.²³ Pre-clinical studies in SOD1 transgenic mice indicated that therapy combinations are more effective than individual agents.^{119,144} This approach has recently been considered in a phase II clinical trial and it appeared feasible, efficient, and has been demonstrated some beneficial effect on ALS patients.⁸⁹ Furthermore, important news should be provided in the next years by research focused on drug delivery via viral vectors or compounds interfering with transcriptional dysregulation, protein aggregation, and disease-causing mutations. Thus, results from ongoing trials of phenylbutyrate (which may reverse transcriptional dysregulation) and arimoclomol (which may mediate protein aggregation) will provide important information for everyday clinical practice.

Disclosures

The authors report no conflicts of interest in this work.

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