Archives Italiennes de Biologie, 149: 151-167, 2011.

Strategies for clinical approach to neurodegeneration in amyotrophic lateral sclerosis

C. CARLESI[1], L. PASQUALI[1], S. PIAZZA[1], A. LO GERFO[1], E. CALDARAZZO IENCO[1], R. ALESSI[1], F. FORNAI[2,3], G. SICILIANO[1]

[1] Department of Neuroscience, Clinical Neurology, University of Pisa, Italy;
[2] Department of Human Morphology and Applied Biology, University of Pisa, Italy;
[3] Neurobiology of Movement Disorder Unit INM IRCCS Neuromed, Pozzilli (IS), Italy

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and ultimately fatal neurodegenerative disorder of unknown aetiology that involves the loss of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord. Significant progress in understanding the cellular mechanisms of motor neuron degeneration in ALS has not been matched with the development of therapeutic strategies to prevent disease progression, and riluzole remains the only available therapy, with only marginal effects on disease survival. More recently alterations of mRNA processing in genetically defined forms of ALS, as those related to TDP-43 and FUS-TLS gene mutations have provided important insights into the molecular networks implicated in the disease pathogenesis. Here we review some of the recent progress in promoting therapeutic strategies for neurodegeneration.

Key words
Amyotrophic lateral sclerosis • Clinical trial • Therapeutic strategies

Introduction

Neurodegeneration is a term used for diseases characterized by progressive loss of structure and/or function of neurons, including death. Many neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS), Parkinson, Alzheimer and Huntington occur as a result of neurodegenerative processes in selective areas. As research progresses, many similarities appear which relate these diseases to one another on a sub-cellular level. Several molecular studies are designed both in animal models and in humans to discover the physiopathology of the disease in order to eventually develop new approaches for neurodegeneration (Bossy-Wetzel et al., 2004).

ALS is a neurological disease of unknown origin characterized by a selective degeneration and death of upper and lower motor neurons, initiating in mid adult life and almost invariably progressing to paralysis and death over a 1-5 year time course (Cleveland, 1999). The clinical manifestations reflect the involvement of both upper and lower motor neurons. ALS diagnosis is based on the El Escorial criteria revised mainly on clinical and electrophysiological findings in four body regions (Brooks et al., 2000). About 95% of ALS patients are sporadic, whereas 5% are familial (Mackenzie et al.,

Corresponding Author: Gabriele Siciliano, MD, PhD, Department of Neuroscience, Clinical Neurology, University of Pisa, via Roma 67, 56126 Pisa, Italy - Tel.: +39 050 993046 - Fax: +39 050 554808 - Email: g.siciliano@med.unipi.it

2010). In this group, approximately 15% are caused by mutations in the SOD1 gene (on chromosome 21) (Rosen, 1993) that codes for the Cu,Zn-superoxide dismutase 1 (SOD1), an enzyme that catalyzes the dismutation of superoxide to molecular oxygen and hydrogen peroxide (Klug et al., 1972), and a further 3%-4% of cases are due to pathogenic variants in either the TDP-43 or FUS gene (Mackenzie et al., 2010). The aetiology of sporadic ALS cases remains unknown. The symptoms and pathology of familial ALS patients with SOD1 mutations closely resemble those of patients with sporadic ALS, suggesting common mechanisms of neurodegeneration in both forms of the disease (Manfredi and Xu, 2005).

Several potential mechanisms of motor neuron degeneration in ALS have been proposed. These include the involvement of environmental factors (Wicklund, 2005), genetic factors (Simpson and Al-Chalabi, 2006), autoimmune phenomena (Pagani et al., 2006), increased oxidative stress (Beckman et al., 2001), glutamate toxicity (Van Den Bosch et al., 2006), viral infections (Portegies and Cohen, 2002), protein aggregation (Wood et al., 2003), mitochondrial dysfunction (Xu, 2004), cytoskeletal abnormalities (Cairns et al., 2004), impairment of axonal transport (Lariviere and Julien, 2004) and pro-apoptotic alterations (Przedborski, 2004). However the cause of ALS is still unclear.

In recent years the idea that in ALS not only motor neurons, but also surrounding glial cells are affected, and the findings of altered interactions between these cell types, has emerged as an additional mechanism in the disease pathogenesis (Rothstein, 1996; Appel and Simpson, 2001).

Each mechanism involved in the pathogenesis of ALS may represent a possible therapeutic approach to counteract neurodegeneration.

Over the years several studies have been performed with different types of therapeutic agents, acting on the mechanisms involved in motor neuron degeneration. Traynor and collaborators (2006) identified over a hundred therapeutic agents as potentially beneficial in patients with ALS and in order to maintain transparency of the process, expand discussion, and further scientific rigor, circulated the list of these drugs within the ALS research community. Among these drugs only twenty-four were judged to be the most promising agents (Traynor et al., 2006). The present review focuses on some therapeutic clinical trials actually recruiting ALS patients or recently concluded.

Pharmacological strategies in ALS

Excitotoxicity in ALS and therapeutic strategies

Glutamate, the primary excitatory neurotransmitter in the Central Nervous System (CNS), acts at both ionotropic and metabotropic receptors, the primary ionotropic receptor classes being N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate (AMPA)/kainate. Extracellular glutamate levels are regulated by transporters; there are distinct classes of transporters on neurons and on astrocytes but the bulk of glutamate uptake appears to be mediated by astrocytes (Anderson and Swanson, 2000). Excessive glutamate exposure is toxic to neurons, probably resulting in large part from glutamate-triggered Ca^{2+} entry (Choi, 1988).

Inhibitors of glutamate uptake cause selective motor neuron damage in organotypic slice (Rothstein et al., 1993) and in dissociated spinal cord culture models (Carriedo et al., 1996), suggesting that the increased extracellular glutamate concentration could contribute to motor neuron damage in ALS. Observations of deficient glutamate transport capacity in affected regions of spinal cord and motor cortex provide a likely basis for these rises in extracellular glutamate concentration (Rothstein et al., 1992). Abnormalities in glutamate metabolism have been reported in ALS patients (Plaitakis and Caroscio, 1987), with elevated glutamate levels in the cerebrospinal fluid (Shaw et al., 1995a). Indeed, the only drug proven to slow the course of human ALS is the anti-excitotoxic compound, Riluzole (Bensimon et al., 1994). Riluzole inhibits the release of glutamate by inactivating voltage-dependent Na^+ channels on glutamatergic nerve terminals, as well as activating a G-protein dependent signal transduction process, thus slowing down disease progression and significantly increasing patients' survival by a few months (Doble, 1996). Moreover, riluzole can also block some of the postsynaptic effects of glutamate by non-competitive inhibition at NMDA and AMPA receptors (Albo et al., 2004). A noncompetitive modulator of AMPA glutamate receptors, primarily under development as an antiepileptic agent, *Talampanel* (8-methyl-7H-1,3-dioxolo(2,3)benzodiazepine, IVAX Corporation) has been used in a clinical trial in ALS patients. A pilot study showed a slower decline in Amyotrophic Lateral Sclerosis

Functional Rating Scale-revised (ALS-FRS-r) score rate during 9-months treatment. Subsequently a "Multinational, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Assess the Efficacy, Tolerability and Safety of Talampanel in Subjects With Amyotrophic Lateral Sclerosis (ALS-TAL-201)", was performed enrolling 550 ALS patients (NCT00696332), starting on September 2008. On May 2010 global pharmaceutical company Teva announced that the results of this study were conclusively negative. The ALS-FRS-r score showed no difference in the progression rate of ALS patients treated with placebo or either with 2 doses of Talampanel. Side effects were more common in participants treated with Talampanel, while the dropout rates were very similar in all groups (www.asla.org 2/7/2010).

Several studies underlined that also the clearance of glutamate from neuromuscular synapses is diminished in patients with ALS because of the loss of the astroglial glutamate transporter EAAT2 (excitatory amino acid transporter 2), that is of major importance for synaptic glutamate reuptake (Rothstein et al., 1995). A loss of high-affinity glutamate transport was identified in specific brain regions and spinal cord of patients with ALS. These results suggested that the defect in glutamate transport could be responsible for sustained elevations in extracellular glutamate, which might be injurious to neurons (Rothstein et al., 1992). These findings have supported the use of *cephalosporins* in ALS because of their antiexcitatory proprieties (Rothstein et al., 2005), by increasing EAAT2 (also known as glutamate transporter GLT1) promoter activity (Rothstein et al., 1993). The increases in GLT1 expression and function were documented in SOD1G93A mice, a mouse model for ALS, treated with ceftriaxone at symptom onset (Rothstein et al., 2005). For human studies third generation ceftriaxone was selected because of its superior CNS penetration and long half-life (Nau et al., 1993). Several studies have been performed with cephalosporins showed controversial results (Robberecht, 1992; Carelli et al., 1994; Carod Artal et al., 1994; Couratier et al., 1994; Gil Llano and Casado Naranjo, 1994; Norris, 1994). Case reports with cephalosporins indicated a clinical improvement (Harvey and Martz et al., 2007; Siciliano et al., 2010). We performed an open label clinical trial with bimonthly drug cycles of Ceftriaxone (2 g/day, for 14 days every two months) in a one year study in ALS patients. We observed a significant improvement of antioxidant oxidative stress status, without clinical improvement, in ALS patients after treatment (Siciliano et al., 2010).

A wide double blind placebo controlled clinical trial on about 600 patients is currently recruiting participants in the United States of America (NCT00349622) [www.clinicaltrial.gov] since July 2006. Final data collection for the primary outcome measure (survival and rate of change in ALSFRSr - stage 3) will be available in June 2012.

Another pharmacological approach to protect against motor neuron degeneration in ALS may be represented by the use of *Memantine*, a low-affinity, noncompetitive NMDA receptor antagonist, since NMDA-mediated neurotoxicity and oxidative stress have been implicated in the pathogenesis of the disease (Chen et al., 1992; Chen and Lipton, 2006). Joo and colleagues (2007) conducted a study on G93A SOD1 mice treated with memantine supplied daily with drinking water beginning at 75 days of age with a low-dose (30 mg/kg/day) and high-dose (90 mg/kg/day). Mice treated with memantine had an improvement in motor performances and a significantly prolonged survival compared to controls. Recently de Carvalho and coworkers (2010) described the results of a phase II/III, 12-months, double-blinded, single-centre, randomized clinical trial performed to evaluate the efficacy and safety of memantine (20 mg/day) in 63 ALS patients. Primary (12-months ALSFRS decline) and secondary (forced vital capacity, manual muscle testing, visual analogue scale, quality of life, motor unit number estimation and neurophysiological index) outcomes were not significantly different between the two groups. The results showed that memantine is well tolerated and safe in ALS patients. Actually is ongoing and currently recruiting participants in Canada a "Randomized, Double-blind, Dose Ranging Study to Determine the Effect of Memantine on Functional Outcomes and Motor Neuron Degeneration in Patients With ALS" (NCT00409721). The estimated study completion date is December 2010.

Oxidative stress in ALS and therapeutic strategies

The involvement of oxidative stress in ALS is suggested by the observation that mutations in the SOD1 gene are responsible for approximately 15%

of familial ALS, mainly inherited with an autosomal dominant trait (Rosen, 1993). Whether oxidative stress is a primary cause of pathogenesis in ALS, or is merely a consequence of the disease, has long been debated. However, extensive evidence shows increased oxidative damage in spinal cord and motor cortex motorneurons (Shaw et al., 1995b; Ferrante et al., 1997) in both sporadic and SOD1 familial ALS post mortem tissue (Abe et al., 1995; 1997; Beal et al., 1997). Moreover oxidative damage to DNA measured by levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), and lipid oxidation occurs (Fitzmaurice et al., 1996; Shibata et al., 2001). Imbalance of oxidative stress has been reported also in cerebrospinal fluid (CSF) of ALS patients (Smith et al., 1998; Ihara et al., 2005). Siciliano and collaborators (2007) found a significantly decrease of the total antioxidant capacity and an increased content of oxidation protein products both in CSF and in plasma in ALS patients compared to controls. Based on these findings a large variety of antioxidants compounds have been tested in patients with ALS.

Mitochondria are implicated in production of oxidative stress, as the major source of reactive oxygen species (ROS), and are also targets of ROS, with decreased mitochondrial efficiency caused by oxidative damage (Beal, 2002; Lenaz et al., 2002; Genova et al., 2004). Although preclinical studies demonstrated a prolonged survival following *coenzyme Q10* treatment (Matthews et al., 1998), an antioxidant, essential mitochondrial cofactor facilitating electron transfer in the respiratory chain, the phase II study in ALS (NCT00243932) showed insufficient promise to warrant Phase III testing (Kaufmann et al., 2009). Several studies with manganoporphyrin, *AEOL 10150*, an antioxidant molecule that catalytically neutralizes superoxide, hydrogen peroxide and peroxynitrite, and inhibits lipid peroxidation (Patel and Day, 1999) have been performed on ALS patients after positive results obtained in animal models (Crow et al., 2005). A pilot study showed that single doses of AEOL 10150 ranging from 3 mg to 30 mg were tolerated without serious adverse events [www.rideforlife.com].

In keeping with maintaining mitochondrial function in ALS, a two-part Phase II randomized, double-blind trial (NCT00647296) with *KNS-760704* [(6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride, RPPX] including 102 ALS patients, recently concluded. Preliminary results showed that KNS-760704 is safe and well-tolerated and that 300 mg/daily may slow the rate of motor function loss, assessed with the ALSFRS-r scale (Bozik et al., 2009).

Edaravone is a free-radical scavenger is (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186), approved by the Ministry of Health, Labor, and Welfare of Japan in 2001, now widely used for the treatment of acute cerebral infarction (Watanabe et al., 1994; The Edaravone Acute Infarction Study Group, 2003) for its neuroprotective effect against oxidative damage (Shichinohe et al., 2004; Uno et al., 2005). Ito and colleagues (2008) performed a randomized blind trial in G93A SOD1 mice with edaravone. The drug administered at clinical onset determined a slowing in symptom progression and motor neuron degeneration in the ALS mice model. Yoshino and Kimura (2006) investigated the efficacy and safety of edaravone in ALS patients. Within an open trial design, 20 subjects with ALS received either 30 mg (5 subjects) or 60 mg (15 subjects) of edaravone intravenously once per day. Two weeks of administration were followed by a two-week observation period. This four-week cycle was repeated six times. The primary endpoint was the change in the revised ALSFRS-r score, while the secondary endpoint was 3-nitrotyrosine (3NT) level in CSF. Efficacy was evaluated in the 60 mg group. During the six-month treatment period, the decline in the ALSFRS-R score was significantly less than that in the six months prior to edaravone administration. In almost all patients, CSF 3NT, a marker for oxidative stress, was markedly reduced to almost undetectable levels at the end of the six-month treatment period. This study suggested that edaravone is safe and may delay the progression of functional motor disturbances by reducing oxidative stress in ALS patients. However, the evidence about the effects of edaravone on human ALS patients awaits the publication of the results of a phase III clinical trial of ALS, currently ongoing in Japan (Expanded Controlled Study of MCI-186 for Treatment of Amyotrophic Lateral Sclerosis in Double-Blind, Parallel-Group, Placebo-Controlled Manner - NCT00424463).

Apoptosis in ALS and therapeutic strategies

Apoptosis mediates the precise and programmed natural death of neurons and is a physiologically important process in neurogenesis during maturation

of the CNS. However, premature apoptosis and/or an aberration in apoptosis regulation is implicated in the pathogenesis of neurodegeneration, a multifaceted process involved in several neurodegenerative disease, such as Alzheimer's, Parkinson's, Huntington's diseases and ALS (Okouchi et al., 2007).

Transcriptional dysfunction has been implicated in the pathogenesis of many neurodegenerative diseases including ALS (Kanai et al., 2004; Rouaux et al., 2007; Cudkowicz et al., 2008). Therefore, histone deacetylase (HDAC) inhibitors may be promising candidates. *Valproic acid* (VPA) is a HDAC-inhibiting drug that promotes gene transcription (Kernochan et al., 2005) and inhibits neuronal cell death by counterbalancing apoptosis, oxidative stress and glutamate toxicity (Hassel et al., 2001; Morland et al., 2004). Based on the finding that VPA and other HDAC inhibitors increased survival in G93A SOD1mice (Sugai et al., 2004; Leng et al., 2008), 163 ALS patients received VPA 1.500 mg or placebo daily (A Randomized, Double-Blind, Placebo-Controlled Sequential Clinical Trial of Sodium Valproate in ALS - NCT00136110). VPA, at a dose used in epilepsy, did not show a beneficial effect on survival or disease progression in patients with ALS. *TCH346 (Dibenzo(b,f)oxepin-10-ylmethyl-methyl-prop-2-ynylamine)* is an antiapoptotic compound that exerts its effects by binding to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and blocking the apoptotic pathway in which GAPDH is involved. In the "Long-Term Extension Study of TCH346 and Placebo Administered Once Daily in Patients With Amyotrophic Lateral Sclerosis (ALS)" (NCT00230074) conducted on 591 ALS patients enrolled from 42 sites in Europe and North America, patients received either placebo or one of four doses of TCH346 (1.0, 2.5, 7.5, or 15 mg/day) administered orally once daily for at least 24 weeks. The trial design included a 16-week lead-in phase to determine each patient's rate of disease progression. At the end of the study there were no significant differences between placebo and active treatment groups in the mean rate of decline of the ALSFRS-R or in the secondary outcome measures (survival, pulmonary function, and MMT).

The administration *Minocycline* to ALS patients in a multicentre, randomized placebo-controlled phase III trial (NCT00047723) showed negative results with ALSFRS-r score deterioration that was faster in the minocycline group than in the placebo group (Gordon et al., 2007) even if it prolongs survival by 10-22% in transgenic mouse models of ALS (Kriz et al., 2002; Van Den Bosch et al., 2002).

A small Phase II trial (NCT00877604) in Italy involving 20 people with MND is now underway to assess whether the addition of *Tauroursodeoxycholic Acid (TUDCA)* to riluzole can slow the progression of the disease. The preclinical studies showed antioxidant, antiapoptotic and neuroprotective properties of TUDCA in the central nervous system, in both *in vitro* and *in vivo* models (Castro et al., 2004). The trial will run for 12 months and results are expected in early 2011.

Neuroinflammation in ALS and therapeutic strategies

Inflammation in ALS may be a secondary response to neuronal injury by genetic, biochemical, or environmental insults. Inflammatory cells surround degenerating neurons (Hirano, 1991), leading to the accumulation of proinflammatory cytokines and free radicals that likely contribute to neurodegeneration (Shaw et al., 1995b). Therefore, modulation of inflammation may reduce cell death. Pre-clinical studies on SOD1G93A mice suggested that anti-inflammatory agents may be effective in treating this disease (McGeer and McGeer, 2002).

Several reports have demonstrated that pro-inflammatory cytokines have a toxic role in the pathogenesis of ALS (Kiaei et al., 2006; Wu et al., 2006). Inflammatory cascades contribute to motor neuron death in the spinal cord in the G93A SOD1 transgenic mice, as well as in human ALS patients (Kiaei et al., 2006). Proinflammatory cytokines, such as tumor necrosis factor (TNF), are upregulated in ALS. *Thalidomide* shows potent anti-inflammatory properties through the modulation of inflammatory cytokines such as TNF-α and appreciably penetrates the CSF (Franks et al., 2004). Although a pilot study with thalidomide was interrupted for safety concerns, a non-randomized, open label phase II study was further performed (NCT00140452) and showed that thalidomide can cause adverse effects without effectively modulating disease progression (Stommel et al., 2009).

Celastol a potent anti-inflammatory and antioxidant triterpene that suppresses TNF-α, IL-1β, and inducible nitric oxide production (Allison et al., 2001) that induces a heat shock protein response (Westerheide

et al., 2004), showed improved motor performance and survival of SOD1G93A mice (Kipiani et al., 2004). However, data concerning toxicity and safety of Celastol in ALS patients and optimum dose remain to be collected.

Neurotropic factors in ALS and therapeutic strategies

Neurotrophic factors are involved in the regulation of neuronal survival and differentiation and in maintaining neuronal structural integrity. In SOD1 G93 mice treatment with insulin-like growth factor (IGF)-1 or glial-cell-line-derived neurotrophic factor (GDNF) have beneficial effects on survival and motorneurons morphology, also decreasing gliosis (Kaspar et al., 2003; Dobrowolny et al., 2005).

Insulin-like growth factor 1 (IGF-1) is a neurotrophic factor that been tested in a North American and in a European phase III trial, enrolling 266 and 183 ALS patients respectively. The two trials showed contrasting results, since IGF-1 either slowed the rate of functional decline by 26% (Lai et al., 1997), or did not produce positive effects (Borasio et al., 1998). Subsequently another phase III trial using IGF-1 treatment for two years has been performed on 330 ALS patients, showing that IGF-1 did not produce beneficial effects on manual muscle testing, ALSFRS-r or in delaying tracheostomy (Sorenson et al., 2008).

Vascular endothelial growth factor (*VEGF)* was originally discovered for its role in affecting vascular permeability and angiogenesis (Senger et al., 1983), but more recent studies have demonstrated that this endogenous protein is able to promote neurogenesis, neuronal survival and neurite outgrowth (Sondell et al., 2000; Jin et al., 2000; 2002). G93A SOD1 mice lacking VEGF show a more severe motor impairment than controls (Lambrechts et al., 2003) and overexpression or administration of VEGF protects motor neurons from degeneration and prolongs mice survival (Azzouz et al., 2004; Zheng et al., 2004; Wang et al., 2007). In ALS patients VEGF expression is decreased in the spinal cord (Brockington et al., 2006). Based on these findings, a double-blind, randomised, parallel group study to evaluate safety and tolerability with VEGF165 administered intracerebroventricularly (NCT00800501) is currently recruiting participants [www.clinicaltrial.gov].

Heat shock proteins inducer

Heat shock proteins have been reported to be involved in the pathogenesis of ALS. In a very recent study Crippa et al. (2010) showed that the small heat shock protein HspB8 is overexpressed in SOD1G93A mice motorneurons, where it removes the accumulated SOD1, by inducing autophagy.

Arimoclomol is a molecule able to enhance the heat shock response, which determines an increased production of the heat shock proteins, this protecting cells form protein aggregation and death (Kieran et al., 2004). In SOD1G93A mice, arimoclomol treatment determined a prolonged survival either if treatment was started in pre-symptomatic stage (Kieran et al., 2004) or at the onset of symptoms (Kalmar et al., 2008). After a Phase IIa arimoclomol trial showed that the drug is safe and well tolerated (Cudkowicz et al., 2008), a phase II/III adaptive, randomized, placebo-controlled clinical trial is still recruiting inherited or familial ALS patients (NCT00706147) [www.clinicaltrial.gov].

Autophagy inducers

Several targets and signaling pathways regulate autophagy, including the mammalian target of rapamycin, mTOR, as a negative regulator. By inhibiting mTOR, rapamycin is currently used to upregulate autophagy, and has been shown to be beneficial in models of neurodegenerative diseases (Ravikumar et al., 2004; Berger et al., 2006; Rubinsztein et al., 2007). On the contrary, the activation of activating mTOR by the inhibition of GSK-3beta downregulates autophagy (Sarkar et al., 2008). Lithium is another pharmacological agent that regulates autophagy. Lithium targets different molecules, and acts on different pathways, the final effect critically depending on the dose. At therapeutically relevant concentrations, lithium is a powerful inhibitor of the intracellular enzyme inositol monophosphatase (IMP)ase (Ki = 0.8 mmol/L) and inositolopolyphosphatase (IPP)ase 1 (Ki = 0.3 mmol/L) (Berridge et al., 1989), while at higher concentrations (Ki 2 mM) it inhibits the activity of glycogen synthase kinase (GSK-3beta), (Stambolic et al., 1996). Lithium induces autophagy by inhibiting the IMPase (Sarkar et al., 2005). The inhibition of GSK-3beta determines instead a negative regulation of autophagy via mTOR (Sarbassov et al., 2005; Yang and Guan, 2007). Lithium-induced autophagy counteracts neu-

rodegeneration (Madeo et al., 2009; Pasquali et al., 2009; Crews et al., 2010) and appears to be neuroprotective for motor neurons both in organotypic slice cultures of spinal cord (Calderò et al., 2010) and in G93A SOD1 mice, where it improves motor function and slows disease progression (Shin et al., 2007; Feng et al., 2008; Fornai et al., 2008; Ferrucci et al., 2010). Defective autophagy has been found in diseased motorneurons (Venkatachalam et al., 2008) and when authopagy is blocked in G93A SOD1 mice, SOD 1 accumulates and neuroprotection does not occur (Crippa et al., 2010). Moreover, a 15-month pilot clinical trial in randomized ALS patients showed that lithium and riluzole cotreatment markedly reduced mortality when compared with matched control patients treated with riluzole alone (Fornai et al., 2008). However, inconsistent results have also been reported. In a sibling-matched, gender-balanced, investigator-blinded trial, using a standard mouse model of familial ALS chronic lithium treatment showed no benefit (Gill et al., 2009). Another study also found no therapeutic or neuroprotective effects of lithium in female ALS mice (Pizzasegola et al., 2009). A randomised, double-blind, placebo-controlled trial involving 84 ALS patients, was conducted by the Northeast ALS (NEALS) and Canadian ALS (CALS) Consortia. The study showed that lithium did not produce safety concerns and that it was interrupted for futility at the first interim analysis (24 weeks), when a 43% improvement did not occur in ALS patients treated with lithium (Aggarwal et al., 2010).

Non-pharmacological strategies in ALS

Physical exercise

Current medical treatment of ALS is limited to medication and supportive care. The question of whether regular exercise is beneficial or not in ALS patients is still controversial (Francis et al., 1999). Moderate regular exercise is helpful in the management of many neuromuscular diseases (Milner-Brawn and Miller, 1988; Aitkens et al., 1993), allowing weak muscles to increase their mitochondrial content and to enhance muscle blood flow and strength (Holloszy et al., 1977). Frequently ALS patients do not perform any type of physical activity in order to preserve their muscle strength and minimize overwork muscle damage (Longstreth et al., 1991). Some epidemiological studies suggest that vigorous physical activity in the form of heavy labor or competitive athletics increases ALS risk (for an extensive review see Chiò et al., 2005). On the contrary other studies report earlier disease onset among individuals with a greater amounts of leisure time and reduced physical activity (Veldink et al., 2005) or that physical activity is not a risk factor for developing ALS (Qureshi et al., 2006).

Pre clinical studies in the transgenic mice model of ALS have demonstrated beneficial effects of physical activity on motor function. Carreras and colleagues (2010) investigated the effects of high and moderate levels of exercise in G93ASOD1 mice. Their study showed preservation of motor performance that correlates with higher motor neuron density in the ventral horn of the lumbar spinal cord in G93A mice undergoing moderate exercise. Clinical trials on ALS patients have suggested that regular physical exercise may be neuroprotective, ameliorating symptoms and improving functionality (Drory et al., 2001; Bello-Haas et al., 2007).

Drory and colleagues (2001) conducted an observational study to determine the effect of moderate regular exercise under professional guidance on motor deficit, disability, fatigue, musculoskeletal pain and perceived quality-of-life. They randomized 25 ALS patients to perform either a specific moderate daily exercise program or just their daily physical activity. At 3 months, patients who performed regular exercise showed less deterioration on ALS-FRS and Ashworth spasticity scales; at 6 months, there was no significant difference between groups, although a trend towards less deterioration in the treated group on most scales was observed. At 9 and 12 months there were too few patients in each group for statistical evaluation. Their results showed that a regular moderate physical exercise program has a short-lived positive effect on disability in ALS patients.

Bello-Haas and colleagues (2010) performed a study in which they investigated the effects of resistance exercise on function, fatigue, and quality of life in 27 ALS patients using ALS-FRS, the Fatigue Severity Scale, Short Form-36, FVC and maximum voluntary isometric contraction at baseline and monthly for 6 months. Patients in mild stage of disease were randomly assigned to a resistance exercise group (daily stretching and resistance exer-

cises three times weekly) or to a usual care group (only the daily stretching exercises). Eight of the thirteen enrolled resistance exercise patients and 10 of the 14 enrolled usual care patients completed the trial. At 6 months, the resistance exercise group had significantly higher ALSFRS and Short Form -36 physical function subscale scores and less decline in leg strength, as measured by maximum voluntary isometric contraction.

Physical exercise exerts positive effects on healthy motorneurons, by enhancing dendritic restructuring, protein synthesis, axonal transport and neuromuscular communication (Gardiner et al., 2006). In ALS several studies indicated that alterations of motorneurons neuromuscular junctions and axonal transport, the latter including also specific gene mutations responsible for familial ALS, play an important role in the pathogenesis of the disease. Furthermore non neuronal cell types besides astrocytes and endothelial cells are involved in ALS. Therefore physical exercise, by acting at these specific levels, may represent a neuroprotective strategy which needs to be further investigated. However, investigation of some oxidative stress markers during submaximal incremental exercise at 40% pnPOmax (a power level corresponding to the anaerobic lactate threshold in patients), showed an increased production of lactate and lipoperoxides (Siciliano et al., 2002), suggesting that regular aerobic physical activity, rather than anaerobic exercise, may be useful in ALS. Given this evidence, we performed a study to investigate some peripheral blood oxidative stress markers during exercise, before and after an antioxidant treatment based on a cysteine donor food integrator called Prother (Dietetic Metabolic Food, Milan, Italy) in ALS patients (Siciliano et al., 2010). Cysteine is a critical factor in glutathione synthesis and glutathione plays an important role in protecting cells from free radical attacks. The 16 enrolled patients performed an incremental muscle test with a hand-grip dynamometer (Digital Multi-Myometer, MIE Medical Research, UK) for the assessment of advanced oxidation protein products (AOPP) in the resting state, at the end of the exercise and after 15 minutes recovery, before and after cysteine donor treatment. Total glutathione was assessed only at resting conditions, before and after treatment. At the start of each experiment the patients performed 3 brief maximal efforts on the hand grip dynamometer. The highest tension recorded was taken as the maximal voluntary contraction (MVC). 15 minutes later the test begun with a first bout at 10% of MVC, then continued trough successive 10% increments, up to 70% of MVC. Each bout consisted of 1 minute intermittent contractions on the hand grip dynamometer followed by a 2 minutes rest.

In vivo, plasma levels of AOPP closely correlate with levels of dityrosine, a hallmark of oxidized protein, and with pentosidine, a marker of enzymatic protein glycation tightly related to oxidative stress.

At rest conditions, AOPP levels were significantly increased and total GSH levels significantly decreased in ALS compared to controls. After 45 days of therapy ALS patients showed, during incremental exercise, a significant decrease, compared to pre-treatment condition, of peak exercise blood AOPP levels and a not significant increase of total GSH. The evidence of a significant total GSH reduction in ALS patients strongly suggests that GSH depletion could be an important factor in the disease pathogenesis. The present study underlines the beneficial effects of antioxidant cysteine-donor therapy on biochemical markers of protein oxidation, also during exercise, in ALS.

Repetitive transcranial magnetic stimulation

Another approach to modulate glutamatergic circuits of human motor cortex is represented by *repetitive transcranial magnetic stimulation* (rTMS) (Paulus et al., 2008). This technique has potential therapeutic effects in several neurological diseases including ALS (Ridding and Rothwell, 2007; Lefaucheur, 2008). Repetitive stimulation of the motor cortex was performed for five consecutive days every month for six consecutive months in 20 patients with definite ALS participating to a double-blind, placebo-controlled trial (Di Lazzaro et al., 2006). Results of the study showed that both active and sham patients deteriorated during treatment, with active patients having a modest but significant slowing of the deterioration rate. Based on these findings, Di Lazzaro and colleagues (2010) performed a double blind, placebo-controlled trial on 20 ALS patients randomly allocated to blinded real or placebo stimulation, to test the hypothesis that Repetitive Transcranial Magnetic Stimulation (rTMS) given as continuous theta burst stimulation (cTBS), repeated monthly for one year, would affect

disease progression. cTBS of the motor cortex was performed for five consecutive days every month for one year. Primary outcome was the rate of decline as evaluated with the ALSFRS-r. Treatment was well tolerated but there was no significant difference in the ALSFRS-r score between patients treated with real or placebo stimulation. The author concluded that although cTBS is a safe procedure, a larger randomized trial would not be justified in ALS patients, at least in advanced stage of the disease (Di Lazzaro et al, 2010).

Gene therapy in ALS

Accumulation of altered, misfolded and aggregated proteins in both fALS and sALS seems to be involved in the pathogenesis of the disease. Decreasing levels of these proteins, by downregulating the genes responsible for such accumulations, may represent a therapeutic strategy aimed to ameliorate the disease state. Modified antisense oligonucleotides, widely distribute throughout the CNS in animals model of neurodegenerative diseases. Antisense oligonucleotides to SOD1 administered near onset in SOD1 G93A rats reduced SOD1 in brain and in spinal cord and significantly slowed disease progression (Smith et al., 2006). Based on these findings, a clinical trial using antisense oligonucleotides to SOD1 will start in familial ALS patients with SOD1 mutations [www.alsa.org].

Stem cell therapy in ALS

Stem-cell transplantation is an attractive strategy for neurological diseases. Previous studies conducted in neurodegnerative disease animal models generated optimism about restoring function or delaying degeneration in human beings. The restricted potential of adult stem cells has been challenged over the past 5 years by reports on their ability to acquire new unexpected fates beyond their embryonic lineage (transdifferentiation). Given that evidence, autologous or allogeneic stem cells, undifferentiated or transdifferentiated and manipulated epigenetically or genetically, could represent a new therapeutic strategy in ALS (Silani et al., 2004).

Although scientifically based clinical trials using stem cells to treat neurodegenerative diseases have already been carried out, none of them has reached a real benefit. Nonetheless, such still no proven treatments are somewhere offered around the world, in ALS as in other neurodegenerative diseases, but with no support of strong rational and scientific basis and with poor clinical management, often underestimating the potential risks of this procedure (Lau et al., 2008).

For a successful development of stem cell-based therapy in neurodegenerative diseases is necessary to establish strict laboratory guidelines, well established pre-clinical studies, rigorous clinical protocols and follow-ups, in addition to a comprehensive dealing with ethical, social and economic implications.

Summary

Several years of biochemical and biological experimentations in cells and in transgenic SOD1 animal models have yielded a bunch of information on ALS pathological mechanisms, suggesting new molecular targets and possible therapeutic approaches. However, most pre-clinical pharmacological studies failed to translate to ALS patients. The reasons underlying this lack of effect might be that animals used in the studies carry the same mutation expressed in a homogeneous genetic background and are of the same age, while ALS patients are heterogeneous in terms of disease onset, progression and severity and only a small percentage have a familial form of ALS. Furthermore animal studies are conducted in different types of animal models, with different experimental designs and conditions, such as timing and method of drug delivery, motor function tests. Genetic studies confirm how different molecular pathways can lead to motor neuron degeneration (Shatunov et al., 2010). Moreover some drugs have been shown to be effective only if given before onset, while in ALS patients the therapeutic time window varies, depending on time of diagnosis and severity of progression.

Although studies in animal models shed lights on the pathological mechanisms of the disease, the causes of ALS remain obscure. In the last few years the concept that ALS is a multifactorial and multisystemic disease, where several cell types are affected, has emerged. In fact in ALS it has been demonstrated the occurrence of interactions between motorneurons and glia and between motoneurons and muscle (Bruijn et al., 2004). Therefore it is conceivable that

a combination of therapies addressing the different cell types involved as well as intercellular and intracellular mechanisms in ALS could be successful in slowing disease progression with an additive or synergistic mechanism. Although such combination therapy has been successful in oncology, it has to be taken into account that multiple drugs administration increases the incidence of side effects. It is therefore necessary to rationally select pharmacological and non-pharmacological approaches for clinical testing in patients with ALS in order to evaluate new therapies as they emerge.

Acknowledgements

The financial supports of Health Ministry (Project RF-INP-2007-627227) and Fondazione Monte dei Paschi di Siena (Project 39167) to the Department of Neuroscience, Clinical Neurology, University of Pisa, are gratefully acknowledged.

References

Abe K., Pan L.H., Watanabe M., Kato T., Itoyama Y. Induction of nitrotyrosine-like immunoreactivity in the lower motor neuron of amyotrophic lateral sclerosis. *Neurosci. Lett.*, **199**: 152-154, 1995.

Abe K., Pan L.H., Watanabe M., Konno H., Kato T., Itoyama Y. Upregulation of protein-tyrosine nitration in the anterior horn cells of amyotrophic lateral sclerosis. *Neurol. Res.*, **19**: 124-128, 1997.

Aggarwal S.P., Zinman L., Simpson E., McKinley J., Jackson K.E., Pinto H., Kaufman P., Conwit R.A., Schoenfeld D., Shefner J., Cudkowicz M. Northeast and Canadian Amyotrophic Lateral Sclerosis consortia. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.*, **9**: 481-488, 2010.

Aitkens S.G., McCrory M.A., Kilmer D.D., Bernauer E.M. Moderate resistance exercise program: its effect in slowly progressive neuromuscular disease. *Arch. Phys. Med. Rehabil.*, **74**: 711-715, 1993.

Albo F., Pieri M., Zona C. Modulation of AMPA receptors in spinal motor neurons by the neuroprotective agent riluzole. *J. Neurosci. Res.*, **78**: 200-207, 2004.

Allison A.C., Cacabelos R., Lombardi V.R., Alvarez X.A., Vigo C. Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **25**: 1341-1357, 2001.

Anderson C.M. and Swanson R.A. Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia*, **32**: 1-14, 2000.

Appel S.H. and Simpson E.P. Activated microglia: the silent executioner in neurodegenerative disease? *Curr. Neurol. Neurosci. Rep.*, **1**: 303-305, 2001.

Azzouz M., Ralph G.S., Storkebaum E., Walmsley L.E., Mitrophanous K.A., Kingsman S.M., Carmeliet P., Mazarakis N.D. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature*, **429**: 413-417, 2004.

Beal M.F. Oxidatively modified proteins in aging and disease. *Free Radical Biol. Med.*, **32**: 797-803, 2002.

Beal M.F., Ferrante R.J., Browne S.E., Matthews R.T., Kowall N.W., Brown Jr. R.H. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Ann. Neurol.*, **42**: 644-654, 1997.

Beckman J.S., Estevez A.G., Crow J.P., Barbeito L. Superoxide dismutase and the death of motoneurons in ALS. *Trends Neurosci.*, **24**: S15-S20, 2001.

Bello-Haas, V.D., Florence, J.M., Kloos, A.D., Scheirbecker, J., Lopate, G., Hayes, S.M., Pioro, E.P., Mitsumoto, H. A randomized controlled trial of resistance exercise in individuals with ALS. *Neurology*, **68**: 2003-2007, 2007.

Bensimon G., Lacomblez L., Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *N. Engl. J. Med.*, **330**: 585-591, 1994.

Berger Z., Ravikumar B., Menzies F.M., Oroz L.G., Underwood B.R., Pangalos M.N., Schmitt I., Wullner U., Evert B.O., O'Kane C.J., Rubinsztein D.C. Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum. Mol. Genet.*, **15**: 433-442, 2006.

Berridge M.J., Downes C.P., Hanley M.R. Neural and developmental actions of lithium: A unifying hypothesis. *Cell*, **59**: 411-419, 1989.

Borasio G.D., Robberecht W., Leigh P.N., Emile J., Guiloff R.J., Jerusalem F., Silani V., Vos P.E., Wokke J.H., Dobbins T. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group. *Neurology*, **51**: 583-586, 1998.

Bossy-Wetzel E., Schwarzenbacher R., Lipton S.A. Molecular pathways to neurodegeneration. *Nat. Med.*, **10** Suppl: S2-9, 2004.

Bozik M., Ingersoll E., Volles L., Mather J., Amburgey C., Moritz J., Archibald D., Sullivan M., Gribkoff V., Miller R., Mitsumoto H., Moore D., Schoenfeld D., Shefner J., Cudkowicz M. KNS-760704-CL201, Part 1: A 12-Week Phase 2 Study of the Safety, Tolerability, and Clinical Effects of KNS 760704 in ALS Subjects. In: 20th International Symposium on ALS/MND, Berlin, Germany, 8-10 December; Clinical Trials and Trial Design, 2009.

Brockington A., Wharton S.B., Fernando M., Gelsthorpe C.H., Baxter L., Ince P.G., Lewis C.E., Shaw P.J. Expression of vascular endothelial growth factor and its receptors in the central nervous system in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.*, **65**: 26-36, 2006.

Brooks B.R., Miller R.G., Swash M., Munsat T.L. World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.*, **1**: 293-299, 2000.

Bruijn L.I., Miller T.M., Cleveland D.W. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu. Rev. Neurosci.*, **27**: 723-749, 2004.

Cairns N.J., Lee V.M., Trojanowski J.Q. The cytoskeleton in neurodegenerative diseases. *J. Pathol.*, **204**: 438-449, 2004.

Calderó J., Brunet N., Tarabal O., Piedrafita L., Hereu M., Ayala V., Esquerda J.E. Lithium prevents excitotoxic cell death of motoneurons in organotypic slice cultures of spinal cord. *Neuroscience*, **165**: 1353-1369, 2010.

Carelli V., Liguori R., Cordivari C., Bianchedi G., Montagna P. Ceftriaxone is ineffective in ALS. *Ital. J. Neurol. Sci.*, **15**: 66, 1994.

Carod Artal F.J., Perez Lopez-Fraile I., Gracia Naya M., Giron Mombiela J.A. Failure of empirical treatment with ceftriaxone in motor neuron disease. *Neurologia*, **9**: 29-31, 1994.

Carreras I., Yuruker S., Aytan N., Hossain L., Choi J.K., Jenkins B.G., Kowall N.W., Dedeoglu A. Moderate exercise delays the motor performance decline in a transgenic model of ALS. *Brain Res.*, **1313**: 192-201, 2010.

Carriedo S.G, Yin H.Z., Weiss J.H. Motor neurons are selectively vulnerable to AMPA/kainate receptor-mediated injury in vitro. *J. Neurosci.*, **16**: 4069-4079, 1996.

Castro R.E., Solá S., Ramalho R.M., Steer C.J., Rodrigues C.M. The bile acid tauroursodeoxycholic acid modulates phosphorylation and translocation of bad via phosphatidylinositol 3-kinase in glutamate-induced apoptosis of rat cortical neurons. *J. Pharmacol. Exp. Ther.*, **311**: 845-852, 2004.

Chen H.S, Pellegrini J.W., Aggarwal S.K., Lei S.Z., Warach S., Jensen F.E., Lipton S.A. Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity. *J. Neurosci.*, **12**: 4427-4436, 1992.

Chen H.S. and Lipton S.A. The chemical biology of clinically tolerated NMDA receptor antagonists. *J. Neurochem.*, **97**: 1611-1626, 2006.

Chiò A., Benzi G., Dossena M., Mutani R., Mora, G. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. *Brain*, **128**: 472-476, 2005.

Choi D.W. Glutamate neurotoxicity and diseases of the nervous system. *Neuron*, **1**: 623-634, 1988.

Cleveland D.W. From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. *Neuron*, **24**: 515-520, 1999.

Couratier P., Vallat J.M., Merle L., Preux P.M., Hugon J. Report of six sporadic cases of ALS patients receiving ceftriaxone. *Therapie*, **49**: 146, 1994.

Crews L., Spencer B., Desplats P., Patrick C., Paulino A., Rockenstein E., Hansen L., Adame A., Galasko D., Masliah E. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy. *PLoS One*, **5**: e9313, 2010.

Crippa V., Sau D., Rusmini P., Boncoraglio A., Onesto E., Bolzoni E., Galbiati M., Fontana E., Marino M., Carra S., Bendotti C., De Biasi S., Poletti A. The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum. Mol. Genet.*, **19**: 3440-3456, 2010.

Crow J.P., Calingasan N.Y., Chen J., Hill J.L., Beal M.F. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. *Ann. Neurol.*, **58**: 258-265, 2005.

Cudkowicz M.E., Shefner J.M., Simpson E., Grasso D., Yu H., Zhang H., Shui A., Schoenfeld D., Brown R.H., Wieland S., Barber J.R., Northeast ALS Consortium. Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. *Muscle Nerve*, **38**: 837-844, 2008.

de Carvalho M., Pinto S., Costa J., Evangelista T., Ohana B., Pinto A. A randomized, placebo-con-

trolled trial of memantine for functional disability in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler*., **11**: 456-460, 2010

Di Lazzaro V., Dileone M., Pilato F., Profice P., Cioni B., Meglio M., Papacci F., Sabatelli M., Musumeci G., Ranieri F., Tonali P.A. Long-term motor cortex stimulation for amyotrophic lateral sclerosis. *Brain Stimul*., **3**: 22-27, 2010.

Di Lazzaro V., Dileone M., Pilato F., Profice P., Ranieri F., Musumeci G., Angelucci F., Sabatelli M., Tonali P.A. Repetitive transcranial magnetic stimulation for ALS. A preliminary controlled study. *Neurosci. Lett.*, **408**: 135-140, 2006.

Doble A. The pharmacology and mechanism of action of riluzole. *Neurology*, **47**: S233-241, 1996.

Dobrowolny G., Giacinti C., Pelosi L., Nicoletti C., Winn N., Barberi L., Molinaro M., Rosenthal N., Musarò A. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *J. Cell Biol*., **168**: 193-199, 2005.

Drory V.E., Goltsman E., Reznik J.G., Mosek A., Korczyn A.D. The value of muscle exercise in patients with amyotrophic lateral sclerosis. *J. Neurol. Sci*., **191**: 133-137, 2001.

Edaravone Acute Infarction Study Group. Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. *Cerebrovasc. Dis*., **15**: 222-229, 2003.

Feng H.L., Leng Y., Ma C.H., Zhang J., Ren M., Chuang D.M. Combined lithium and valproate treatment delays disease onset, reduces neurological deficits and prolongs survival in an amyotrophic lateral sclerosis mouse model. *Neuroscience*, **155**: 567-572, 2008.

Ferrante R.J., Browne S.E., Shinobu L.A., Bowling A.C., Baik M.J, MacGarvey U., Kowall N.W., Brown Jr. R.H., Beal M.F. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J. Neurochem.*, **69**: 2064-2074, 1997.

Ferrucci M., Spalloni A., Bartalucci A., Cantafora E., Fulceri F., Nutini M., Longone P., Paparelli A., Fornai F. A systematic study of brainstem motor nuclei in a mouse model of ALS, the effects of lithium. *Neurobiol. Dis*., **37**: 370-383, 2010.

Fitzmaurice P.S., Shaw I.C., Kleiner H.E., Miller R.T., Monks T.J., Lau S.S., Mitchell J.D., Lynch P.G. Evidence for DNA damage in amyotrophic lateral sclerosis. *Muscle Nerve*, **19**: 797-798, 1996.

Fornai F., Longone P., Cafaro L., Kastsiuchenka O., Ferrucci M., Manca M.L., Lazzeri G., Spalloni A., Bellio N., Lenzi P., Modugno N., Siciliano G., Isidoro C., Murri L., Ruggieri S., Paparelli A. Lithium delays progression of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U.S.A*., **105**: 2052-2057, 2008.

Francis K., Bach J.R., DeLisa J.A. Evaluation and rehabilitation of patients with adult motor neuron disease. *Arch. Phys. Med. Rehabil*., **80**: 951-963, 1999.

Franks M.E., Macpherson G.R., Figg W.D. Thalidomide. *Lancet*, **363**, 1802-1811, 2004.

Gardiner P., Dai Y., Heckman C.J. Effects of exercise training on alpha-motoneurons. *J. Appl. Physio*l., **101**: 1228-1236, 2006.

Genova M.L., Pich M.M., Bernacchia A., Bianchi C., Biondi A., Bovina C., Falasca A.I., Formiggini G., Castelli G.P., Lenaz G. The mitochondrial production of reactive oxygen species in relation to aging and pathology. *Ann. N.Y. Acad. Sci.*, **1011**: 86-100, 2004.

Gil Llano J.R. and Casado Naranjo I. Lack of improvement of motoneuron disease with ceftriaxone. *Neurologia*, **9**: 205-206, 1994.

Gill A., Kidd J., Vieira F., Thompson K., Perrin S. No benefit from chronic lithium dosing in a sibling-matched, gender balanced, investigator-blinded trial using a standard mouse model of familial ALS. *PLoS One*, **4**: e6489, 2009.

Gordon P.H., Moore D.H., Miller R.G., Florence J.M., Verheijde J.L., Doorish C., Hilton J.F., Spitalny G.M., MacArthur R.B., Mitsumoto H., Neville H.E., Boylan K., Mozaffar T., Belsh J.M., Ravits J., Bedlack R.S., Graves M.C., McCluskey L.F., Barohn R.J., Tandan R., Western ALS Study Group. Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. *Lancet Neurol*., **6**: 1045-1053, 2007.

Harvey W.T. and Martz D. Motor neuron disease recovery associated with IV ceftriaxone and antibabesia therapy. *Acta Neurol. Scand.*, **115**: 129-131, 2007.

Hassel B., Iversen E.G., Gjerstad L., Tauboll E. Up-regulation of hippocampal glutamate transport during chronic treatment with sodium valproate. *J. Neurochem*., **77**: 1285-1292, 2001.

Hirano A. Cytopathology in amyotrophic lateral sclerosis. *Adv. Neurol*., **56**: 91-101, 1991.

Holloszy J.O., Rennie M.J., Hickson R.C., Conlee R.K., Hagberg J.M. Physiological consequences of the biochemical adaptation to endurance exercise. *Ann. N.Y. Acad. Sci*., **301**: 440-450, 1977.

Ihara Y., Nobukuni K., Takata H., Hayabara T. Oxidative stress and metal content in blood and cerebrospinal fluid of amyotrophic lateral sclerosis patients with and without a Cu,Zn-superoxide dismutase mutation. *Neurol. Res.*, **27**: 105-108, 2005.

Ito H., Wate R., Zhang J., Ohnishi S., Kaneko S., Ito H., Nakano S., Kusaka H. Treatment with edaravone, initiated at symptom onset, slows motor decline and decreases SOD1 deposition in ALS mice. *Exp. Neurol.*, **213**: 448-455, 2008.

Jin K.L., Mao X.O., Greenberg D.A. Vascular endothelial growth factor: direct neuroprotective effect in in vitro ischemia. *Proc. Natl. Acad. Sci. U.S.A.*, **97**: 10242-10247, 2000.

Jin K., Zhu Y., Sun Y., Mao X.O., Xie L., Greenberg D.A. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.*, **99**: 11946-11950, 2002.

Joo I.S., Hwang D.H., Seok J.I., Shin S.K., Kim S.U. Oral administration of memantine prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J. Clin. Neurol.*, **3**: 181-186, 2007.

Kalmar B., Novoselov S., Gray A., Cheetham M.E., Margulis B., Greensmith L. Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. *J. Neurochem.*, **107**: 339-350, 2008.

Kanai H., Sawa A., Chen R.W., Leeds P., Chuang D.M. Valproic acid inhibits histone deacetylase activity and suppresses excitotoxicity-induced GAPDH nuclear accumulation and apoptotic death in neurons. *Pharmacogenomics J.*, **4**: 336 -344, 2004.

Kaspar B.K., Lladó J., Sherkat N., Rothstein J.D., Gage F.H. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science*, **301**: 839-842, 2003.

Kaufmann P., Thompson J.L., Levy G., Buchsbaum R., Shefner J., Krivickas L.S., Katz J., Rollins Y., Barohn R.J., Jackson C.E., Tiryaki E., Lomen-Hoerth C., Armon C., Tandan R., Rudnicki S.A., Rezania K., Sufit R., Pestronk A., Novella S.P., Heiman-Patterson T., Kasarskis E.J., Pioro E.P., Montes J., Arbing R., Vecchio D., Barsdorf A., Mitsumoto H., Levin B., QALS Study Group. Phase II trial of CoQ10 for ALS finds insufficient evidence to justify phase III. *Ann. Neurol.*, **66**: 235-244, 2009.

Kernochan L.E., Russo M.L., Woodling N.S., Huynh T.N., Avila A.M., Fischbeck K.H., Sumner C.J. The role of histone acetylation in SMN gene expression. *Hum. Mol. Genet.*, **14**: 1171-1182, 2005.

Kiaei M., Petri S., Kipiani K., Gardian G., Choi D.K., Chen J., Calingasan N.Y., Schafer P., Muller G.W., Stewart C., Hensley K., Beal M.F. Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J. Neurosci.*, **26**, 2467-2473, 2006.

Kieran D., Kalmar B., Dick J.R., Riddoch-Contreras J., Burnstock G., Greensmith L. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nat. Med.*, **10**: 402-405, 2004.

Kipiani K., Kiaei M., Chen J., Calingasan N.Y., Beal M.F. Celastrol blocks motor neuron cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. *J. Neurochem.*, **90** (Suppl 1): 92, 2004.

Klug D., Rabani J., Fridovich I. A direct demonstration of the catalytic action of superoxide dismutase through the use of pulse radiolysis. *J. Biol. Chem.*, **247**: 4839-4842, 1972.

Kriz J., Nguyen M., Julien J. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol. Dis.*, **10**: 268-278, 2002.

Lai E.C., Felice K.J., Festoff B.W., Gawel M.J., Gelinas D.F., Kratz R., Murphy M.F., Natter H.M., Norris F.H., Rudnicki S.A. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology*, **49**: 1621-1630, 1997.

Lambrechts D., Storkebaum E., Morimoto M., Del-Favero J., Desmet F., Marklund S.L., Wyns S., Thijs V., Andersson J., van Marion I., Al-Chalabi A., Bornes S., Musson R., Hansen V., Beckman L., Adolfsson R., Pall H.S., Prats H., Vermeire S., Rutgeerts P., Katayama S., Awata T., Leigh N., Lang-Lazdunski L., Dewerchin M., Shaw C., Moons L., Vlietinck R., Morrison K.E., Robberecht W., Van Broeckhoven C., Collen D., Andersen P.M., Carmeliet P.VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nat. Genet.*, **34**: 383-394, 2003.

Lariviere R.C. and Julien J.P. Functions of intermediate filaments in neuronal development and disease. *J. Neurobiol.*, **58**: 131-148, 2004.

Lau D., Ogbogu U., Taylor B., Stafinski T., Menon D., Caulfield T. Stem cell clinics online: the direct-to-consumer portrayal of stem cell medicine. *Cell Stem Cell*, **3**: 591-594, 2008.

Lefaucheur J.P. Principles of therapeutic use of transcranial and epidural cortical stimulation. *Clin. Neurophysiol.*, **119**: 2179-2184, 2008

Lenaz G., Bovina C., D'Aurelio M., Fato R., Formiggini G., Genova M.L., Giuliano G., Merlo Pich M., Paolucci U., Parenti Castelli G., Ventura B. Role of mitochondria in oxidative stress and ageing. *Ann. N.Y. Acad. Sci.*, **959**: 199-213, 2002.

Leng Y., Liang M.H., Ren M., Marinova Z., Leeds P., Chuang D.M. Synergistic neuroprotective effects of lithium and valproic acid or other histone deacetylase inhibitors in neurons: roles of glycogen synthase kinase-3 inhibition. *J. Neurosci.*, **28**: 2576-2588, 2008.

Longstreth W.T., Nelson L.M., Koepsell T.D., van Belle G. Hypotheses to explain the association between vigorous physical activity and amyotrophic lateral sclerosis. *Med. Hypotheses*, **34**: 144-148, 1991.

Mackenzie I.R., Rademakers R., Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol.*, **9**: 995-1007, 2010.

Madeo F., Eisenberg T., Kroemer G. Autophagy for the avoidance of neurodegeneration. *Genes. Dev.*, **23**: 2253-2259, 2009.

Manfredi G. and Xu Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. *Mitochondrion*, **5**: 77-87, 2005.

Matthews R.T., Yang L., Browne S., Baik M., Beal M.F. Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc. Natl. Acad. Sci. U.S.A.*, **95**: 8892-8897, 1998.

McGeer P.L. and McGeer E.G. Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve*, **26**: 459-470, 2002.

Milner-Brown H.S. and Miller R.G. Muscle strengthening through highresistance weight training in patients with neuromuscular disorders. *Arch. Phys. Med. Rehabil.*, **69**: 14-19, 1988.

Morland C., Boldingh K.A., Iversen E.G., Hassel B. Valproate is neuroprotective against malonate toxicity in rat striatum: an association with augmentation of high-affinity glutamate uptake. *J. Cereb. Blood Flow Metab.*, **24**: 1226-1234, 2004.

Nau R., Prange H.W., Muth P., Mahr G., Menck S., Kolenda H., Sörgel F. Passage of cefotaxime and ceftriaxone into cerebrospinal fluid of patients with uninflamed meninges. *Antimicrob. Agents Chemother.*, **37**: 1518-1524: 1993.

Norris F.H. Ceftriaxone in amyotrophic lateral sclerosis. *Arch. Neurol.*, **51**: 447, 1994.

Okouchi M., Ekshyyan O., Maracine M., Aw T.Y. Neuronal apoptosis in neurodegeneration. *Antioxid. Redox Signal.*, **9**: 1059-1096, 2007.

Pagani M.R., Reisin R.C., Uchitel O.D. Calcium signaling pathways mediating synaptic potentiation triggered by amyotrophic lateral sclerosis IgG in motor nerve terminals. *J. Neurosci.*, **26**: 2661-2672, 2006.

Pasquali L., Longone P., Isidoro C., Ruggieri S., Paparelli A., Fornai F. Autophagy, lithium, and amyotrophic lateral sclerosis. *Muscle Nerve*, **40**: 173-194, 2009.

Patel M. and Day B.J. Metalloporphyrin class of therapeutic catalytic antioxidants. *Trends Pharmacol. Sci.*, **20**: 359-364, 1999.

Paulus W., Classen J., Cohen L.G., Large C.H., Lazzaro V.D., Nitsche M., Pascual-Leone A., Rosenow F., Rothwell J.C., Ziemann U. State of the art: pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. *Brain Stimulation*, **1**: 151-163, 2008.

Pizzasegola C., Caron I., Daleno C., Ronchi A., Minoia C., Carri M.T., Bendotti C. Treatment with lithium carbonate does not improve disease progression in two different strains of SOD1 mutant mice. *Amyotroph. Lateral. Scler.*, **10**: 221-228, 2009.

Plaitakis A. and Caroscio J.T. Abnormal glutamate metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.*, **22**: 575-579, 1987.

Portegies P. and Cohen E.S. Possible etiological role retroviruses and enteroviruses in the development of amyotrophic lateral sclerosis. *Ned Tijdschr Geneeskd*, **146**: 1398-1400, 2002.

Przedborski S. Programmed cell death in amyotrophic lateral sclerosis: a mechanism of pathogenic and therapeutic importance. *Neurologist*, **10**: 1-7, 2004.

Qureshi M.M., Hayden D., Urbinelli L., Ferrante K., Newhall K., Myers D., Hilgenberg S., Smart R., Brown R.H., Cudkowicz M.E. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (ALS). *Amyotroph. Lateral Scler.*, **7**: 173-182, 2006.

Ravikumar B., Vacher C., Berger Z., Davies J.E., Luo S., Oroz L.G., Scaravilli F., Easton D.F., Duden R., O'Kane C.J., Rubinsztein D.C. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat. Genet.*, **36**: 585-595, 2004.

Ridding M.C. and Rothwell J.C. Is there a future for therapeutic use of transcranial magnetic stimulation? *Nat. Rev.*, **8**: 559-567, 2007

Robberecht W. Lack of improvement with ceftriaxone in motoneuron disease. *Lancet*, **340**: 1096-1097, 1992.

Rosen D.R. Mutations in Cu/Zn superoxide dismutase gene are associated with amyotrophic lateral sclerosis. *Nature*, **362**: 59-62, 1993.

Rothstein J.D., Martin L.J., Kuncl R.W. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N. Engl. J. Med.*, **326**: 1464-1468, 1992.

Rothstein J.D., Jin L., Dykes-Hoberg M., Kuncl R.W. Chronic inhibition of glutamate uptake produces a model of slow neurotoxicity. *Proc. Natl. Acad. Sci. U.S.A.*, **90**: 6591-6595, 1993.

Rothstein J.D., Van Kammen M., Levey A.I., Martin L.J., Kuncl R.W. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann. Neurol.*, **38**: 73-84, 1995.

Rothstein J.D. Excitotoxicity hypothesis. *Neurology*, **47** (Suppl. 2): S19-S25, 1996.

Rothstein J.D., Patel S., Regan M.R., Haenggeli C., Huang Y.H., Bergles D.E., Jin L., Dykes Hoberg M., Vidensky S., Chung D.S., Toan S.V., Bruijn L.I., Su Z.Z., Gupta P., Fisher P.B. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*, **433**: 73-77, 2005.

Rouaux C., Panteleeva I., Rene F., Gonzalez de Aguilar J.L., Echaniz-Laguna A., Dupuis L., Menger Y., Boutillier A.L., Loeffler J.P. Sodium valproate exerts neuroprotective effects in vivo through CREB-binding proteindependent mechanisms but does not improve survival in an amyotrophic lateral sclerosis mouse model. *J. Neurosci.*, **27**: 5535-5545, 2007.

Rubinsztein D.C., Gestwicki J.E., Murphy L.O., Klionsky D.J. Potential therapeutic applications of autophagy. *Nat. Rev. Drug. Discov.*, **6**: 304-312, 2007.

Sarbassov D.D., Ali S.M., Sabatini D.M. Growing roles for the mTOR pathway. *Curr. Opin. Cell. Biol.*, **17**: 596-603, 2005.

Sarkar S., Floto R.A., Berger Z., Imarisio S., Cordenier A., Pasco M., Cook LJ, Rubinsztein, D.C. Lithium induces autophagy by inhibiting inositol monophosphatase. *J. Cell. Biol.*, **170**: 1101-1111, 2005.

Sarkar S., Krishna G., Imarisio S., Saiki S., O'Kane C.J., Rubinsztein D.C. A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. *Hum. Mol. Genet.*, **17**: 170-178, 2008.

Senger D.R., Galli S.J., Dvorak A.M., Perruzzi C.A., Harvey V.S., Dvorak H.F. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*, **219**: 983-985, 1983.

Shatunov A., Mok K., Newhouse S., Weale M.E., Smith B., Vance C., Johnson L., Veldink J.H., van Es M.A., van den Berg L.H., Robberecht W., Van Damme P., Hardiman O., Farmer A.E., Lewis C.M., Butler A.W., Abel O., Andersen P.M., Fogh I., Silani V., Chiò A., Traynor B.J., Melki J., Meininger V., Landers J.E., McGuffin P., Glass J.D., Pall H., Leigh P.N., Hardy J., Brown R.H. Jr, Powell J.F., Orrell R.W., Morrison K.E., Shaw P.J., Shaw C.E., Al-Chalabi A. Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. *Lancet Neurol.*, 2010 *in press*.

Shaw P.J., Forrest V., Ince P.G., Richardson J.P., Wastell H.J. SF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration*, **4**: 209-216, 1995a.

Shaw P., Ince P., Falkous G., Mantel D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. *Ann. Neurol.*, **38**: 691-695, 1995b.

Shibata N., Nagai R., Uchida K., Horiuchi S., Yamada S., Hirano A., Kawaguchi M., Yamamoto T., Sasaki S., Kobayashi M. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. *Brain Res.*, **917**: 97-104, 2001.

Shichinohe H., Kuroda S., Yasuda H., Ishikawa T., Iwai M., Horiuchi M., Iwasaki Y. Neuroprotective effects of the free radical scavenger Edaravone (MCI-186) in micepermanent focal brain ischemia. *Brain Res.*, **1029**: 200-206, 2004.

Shin J.H., Cho S.I., Lim H.R., Lee J.K., Lee Y.A., Noh J.S., Joo I.S., Kim K.W., Gwag B.J. Concurrent administration of Neu2000 and lithium produces marked improvement of motor neuron survival, motor function, and mortality in a mouse model of amyotrophic lateral sclerosis. *Mol. Pharmacol.*, **71**: 965-975, 2007.

Siciliano G., D'Avino C., Del Corona A., Barsacchi R., Kusmic C., Rocchi A., Pastorini E., Murri L. Impaired oxidative metabolism and lipid peroxidation in exercising muscle from ALS patients.

Amyotroph. Lateral Scler. Other Motor Neuron Disord., **3**: 57-62, 2002.

Siciliano G., Piazza S., Carlesi C., Del Corona A., Franzini M., Pompella A., Malvaldi G., Mancuso M., Paolicchi A., Murri L. Antioxidant capacity and protein oxidation in cerebrospinal fluid of amyotrophic lateral sclerosis. *J. Neurol.*, **254**: 575-580, 2007.

Siciliano G., Carlesi C., Pasquali L., Piazza S., Pietracupa S., Fornai F., Ruggieri S., Murri L. Clinical trials for neuroprotection in ALS. *CNS Neurol. Disord. Drug Targets*, **9**: 305-313, 2010.

Silani V., Cova L., Corbo M., Ciammola A., Polli E. Stem-cell therapy for amyotrophic lateral sclerosis. *Lancet*, **364**: 200-202, 2004.

Simpson C.L. and Al-Chalabi A. Amyotrophic lateral sclerosis as a complex genetic disease. *Biochim. Biophys. Acta*, **1762**: 973-985, 2006.

Smith R.G., Henry Y.K., Mattson M.P., Appel S.H. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann. Neurol.*, **44**: 696-699, 1998.

Smith R.A., Miller T.M., Yamanaka K., Monia B.P., Condon T.P., Hung G., Lobsiger C.S., Ward C.M., McAlonis-Downes M., Wei H., Wancewicz E.V., Bennett C.F., Cleveland D.W. Antisense oligonucleotide therapy for neurodegenerative disease. *J. Clin. Invest.*, **116**: 2290-2296, 2006.

Sondell M., Sundler F., Kanje M. Vascular endothelial growth factor is a neurotrophic factor which stimulates axonal outgrowth through the flk-1 receptor. *Eur. J. Neurosci.*, **12**: 4243-4254, 2000.

Sorenson E.J., Windbank A.J., Mandrekar J.N., Bamlet W.R., Appel S.H., Armon C., Barkhaus P.E., Bosch P., Boylan K., David W.S., Feldman E., Glass J., Gutmann L., Katz J., King W., Luciano C.A., McCluskey L.F., Nash S., Newman D.S., Pascuzzi R.M., Pioro E., Sams L.J., Scelsa S., Simpson E.P., Subramony S.H., Tiryaki E., Thornton C.A. Subcutaneous IGF-1 is not beneficial in 2-year ALS trial. *Neurology*, **71**: 1770-1775, 2008.

Stambolic V., Ruel L., Woodgett J.R. Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signaling in intact cells. *Curr. Biol.*, **6**: 1664-1668, 1996.

Stommel E.W., Cohen J.A., Fadul C.E., Cogbill C.H., Graber D.J., Kingman L., Mackenzie T., Channon Smith J.Y., Harris B.T. Efficacy of thalidomide for the treatment of amyotrophic lateral sclerosis: A phase II open label clinical trial. *Amyotroph. Lateral Scler.*, **10**: 393-404, 2009.

Sugai F., Yamamoto Y., Miyaguchi K., Zhou Z., Sumi H., Hamasaki T., Goto M., Sakoda S. Benefit of valproic acid in suppressing disease progression of ALS model mice. *Eur. J. Neurosci.*, **20**: 3179-3183, 2004.

Traynor B.J., Bruijn L., Conwit R., Beal F., O'Neill G., Fagan S.C., Cudkowicz M.E. Neuroprotective agents for clinical trials in ALS: a systematic assessment. *Neurology*, **67**: 20-27, 2006.

Uno M., Kitazato K.T., Suzue A., Matsuzaki K., Harada M., Itabe H., Nagahiro S. Inhibition of brain damage by edaravone, a free radical scavenger, can be monitored by plasma biomarkers that detect oxidative and astrocyte damage in patients with acute cerebral infarction. *Free Radic. Biol. Med.*, **39**: 1109-1116, 2005.

Van Den Bosch L., Van Damme P., Bogaert E., Robberecht W. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochim. Biophys. Acta*, **1762**: 1068-1082, 2006.

Van Den Bosch L., Tillkin P., Lemmens G., Robberecht W. Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport*, **13**: 1067-1070, 2002.

Veldink J.H., Kalmijn S., Groeneveld G.J., Titulaer M.J., Wokke J.H., van den Berg L.H. Physical activity and the association with sporadic ALS. *Neurology*, **64**: 241-245, 2005.

Venkatachalam K., Long A.A., Elsaesser R., Nikolaeva D., Broadie K., Montell C. Motor deficit in a Drosophila model of mucolipidosis type IV due to defective clearance of apoptotic cells. *Cell*, **135**: 838-851, 2008.

Wang Y., Mao X.O., Xie L., Banwait S., Marti H.H., Greenberg D.A., Jin K. Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. *J. Neurosci.*, **27**: 304-307, 2007.

Watanabe T., Yuki S., Egawa M., Nishi H. Protective effects of MCI-186 on cerebral ischemia: possible involvement of free radical scavenging and antioxidant actions. *J. Pharmacol. Exp. Ther.*, **268**: 1597-1604, 1994.

Westerheide S.D., Bosman J.D., Mbadugha B.N., Kawahara T.L., Matsumoto G., Kim S., Gu W., Devlin J.P., Silverman R.B., Morimoto R.I. Celastrols as inducers of the heat shock response and cytoprotection. *J. Biol. Chem.*, **279**: 56053-56060, 2004.

Wicklund M.P. Amyotrophic lateral sclerosis: possible role of environmental influences. *Neurol. Clin.*, **23**: 461-484, 2005.

Wood J.D., Beaujeux T.P., Shaw P.J. Protein aggregation in motor neurone disorders. *Neuropathol. Appl. Neurobiol.*, **29**: 529-545, 2003.

Wu D.C., Re D.B., Nagai M., Ischiropoulos H., Przedborski S. The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. *Proc. Natl. Acad. Sci. U. S. A.*, **103**: 12132-12137, 2006.

Xu J.X. Radical metabolism is partner to energy metabolism in mitochondria. *Ann. N.Y. Acad. Sci.*, **1011**: 57-60, 2004.

Yang Q. and Guan K.L. Expanding mTOR signaling. *Cell Res.*, **17**: 666-681, 2007.

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

Zheng C., Nennesmo I., Fadeel B., Henter J.I. Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. *Ann. Neurol.*, **56**: 564-567, 2004.