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Amyotrophic Lateral Sclerosis

Progress and Prospects for Treatment

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Contents

Abstract

Fifteen years ago, a role for excitotoxic damage in the pathology of amyotrophic lateral sclerosis (ALS) was postulated. This stimulated the development of riluzole, the only available treatment for the disease. Since then, the identification of abnormal forms of superoxide dismutase as the genetic basis of certain familial forms of ALS has provided a huge impetus to the search for new effective treatments for this devastating disease. Transgenic mouse models have been developed expressing these aberrant mutants that develop a form of motor neurone disease the progress of which can be slowed by riluzole. Studies in these mice have provided evidence for a role for excitotoxic, apoptotic and oxidative processes in the development of pathology. The mice can be used for testing molecules targeting these processes as potential therapies, to allow the most promising to be evaluated in humans. Several such agents are currently in clinical trials.

Many previous clinical trials in ALS were insufficiently powered to demonstrate any relevant effect on disease progression. This situation has been to some extent remedied in the more recent trials, which have recruited many hundreds of patients. However, with the exception of studies with riluzole, the results of these have been disappointing. In particular, a number of large trials with neurotrophic agents have revealed no evidence for efficacy.

Nonetheless, the need for large multinational trials of long duration limits the number that can be carried out and makes important demands on investment. For this reason, surrogate markers that can be used for rapid screening in patients of potential treatments identified in the transgenic mice are urgently needed.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, involving the upper and lower motor neurones that control voluntary movement. The disease is characterised by progressive muscle weakness with death, usually resulting from respiratory failure, occurring 3–5 years from the first appearance of symptoms. The disease can be either sporadic (SALS; 90%) or familial (FALS; 10%) in nature, with an average age onset of 59 years, an incidence of 1–3 per 100 000 and a prevalence of 5–9 per 100 000.[1,2]

Although, at the present time, ALS is invariably fatal, at least two major advances, made in the last decade of the twentieth century, have opened up new prospects for its future treatment and management. Firstly, it was demonstrated that around 20% of patients with the autosomal dominant FALS have mutations in the anti-oxidant enzyme, Cu/Zn superoxide dismutase (SOD-1).[3-6] Secondly, the anti-glutamate drug, riluzole, which delays death and/or time to tracheostomy in ALS patients, became the first and still the only approved treatment for the disorder.[7-11] The subsequent creation of transgenic rodent models, over-expressing human SOD-1 (hSOD-1) mutant enzymes, that can be successfully treated with riluzole has provided the first animal model of ALS. Studies in these animals have provided evidence that glutamate-mediated excitotoxicity, free radical-mediated damage, mitochondrial dysfunction and apoptosis may be involved in the pathogenesis of ALS. In addition, the availability of these transgenic models has provided an experimental system in which to test potential therapeutic agents.[6,12-15]

Although there is still considerable debate on the relative importance of the above mechanisms in the pathogenesis of the disease, thanks to these recent advances there is now a bewildering array of molecules proposed as potential treatments for ALS. It would therefore seem to be an appropriate time to take stock of trials past to enable us to reflect on the best way forward for the future.

1. Animal Models in Amyotrophic Lateral Sclerosis (ALS)

1.1 Spontaneous Models

Several mouse strains, such as the Mnd, wobbler, wasted and pmn mouse, which spontaneously develop motor neurone pathology, have been used in the past as animal models for ALS. The advantages and disadvantages of these models are reviewed by Doble and Kennel, 2000.[16]

1.2 Transgenic Animals

The discovery that a subset of patients with FALS have point mutations in SOD-1, led to the development of transgenic mice over-expressing a mutated form of the human enzyme hSOD-1.[17,18] Unlike mice expressing wild-type hSOD-1,[12] those expressing mutated hSOD-1 enzymes develop ALS-like clinical symptoms after an asymptomatic period that varies with both the mutation expressed and the transgene copy number.[18,19] Cytopathological changes observed are similar to those observed in human ALS.[20-24] The most widely employed of the transgenic models are mice expressing around 18 copies of hSOD-1 with a glycine to alanine mutation at residue 93 (hSOD-1^{G93A}).[17,18] These animals develop ALS-like symptoms at around 3 months of age with death normally occurring between 4 to 5 months, while low expressor hSOD-1^{G93A} mice have a life span of around 8 months.[17-19]

A transgenic SOD-1 rat model of ALS has also been developed, with the animals either expressing hSOD-1^{G93A} or a histidine to arginine mutation at residue 46.[15] Again, these animals develop striking and selective motor neurone degeneration and paralysis. It has been proposed that the larger size of this model, as compared with the transgenic mice, will facilitate studies involving manipulations of spinal fluid and spinal cord.[15]

Oosthuyse et al., took a different approach to produce another mouse model of ALS.[25] In this instance, the hypoxia response element in the vascular endothelial growth factor (VGEF) promoter was deleted causing reduced expression of VGEF in the spinal cord and late-onset progressive motor neurone disease. This indicates that chronic vascular insufficiency and possibly insufficient VEGF-dependent neuroprotection lead to the select degeneration of motor neurones.

The availability of the transgenic animal models has facilitated investigations into the molecular events that lead to motor neurone death as well as providing a new model to evaluate potential treatments for ALS.

2. Pharmacopathological Changes in ALS

Since the first descriptions of ALS, much data has accumulated from post-mortem and biopsy studies on biochemical or physiological abnormalities in the disease. However, it has always been difficult to establish whether these correspond to consequences of the disease or potential aetiological factors involved in the pathophysiological process itself. The availability of transgenic animal models of ALS has been of critical importance in addressing these issues and in identifying the most promising biochemical mechanisms at which potential therapies could be directed. This following section reviews the progress that has been made in this field.

2.1 Excitotoxicity

2.1.1 Human Studies

Abnormally high synaptic concentrations of glutamate, the major excitatory amino acid in the nervous system, result in prolonged neuronal depolarisation leading to elevated intracellular Ca^{2+} concentrations that in turn can lead to mitochondrial damage, and the activation of enzymatic and nuclear mechanisms of cell death.[26-29] In ALS there is evidence, albeit indirect, that a defect in glutamate turnover leads to increased extracellular glutamate levels with resulting deleterious consequences.[29-33] The nature of the defect(s) that leads to excitotoxicity in ALS has yet to be elucidated, although there are indications that it may be mediated through the activation of Ca^{2+}-permeable alpha-amino-3-hydroxy-5-methyl-4-

isoxazole propionic acid (AMPA) glutamate receptors.[32,34] Transporter proteins that remove glutamate from the extracellular space[35] have also been implicated, with the selective loss of the dominant transporter, the astroglial-specific excitatory amino acid transporter 2 (EAAT2 or glutamate transporter [GLT]-1), in the motor cortex and spinal cord of ALS patients.[36,37] However, although alternative splicing of EAAT2 mRNA was initially proposed to account for this protein loss,[38,39] subsequent studies showed that this was not ALS-specific.[40-44] Likewise, mutations in the EAAT2 gene are infrequent.[45]

The notion of an excitotoxic process in ALS is also supported by the beneficial effect on disease progression of riluzole. Although the precise mechanism(s) of action of this drug is unknown, an anti-glutamate mechanism has been put forward.[10] Riluzole has anti-convulsant and sedative properties *in vivo*; *in vitro* it inhibits glutamate release and appears to be an indirect antagonist of NMDA (*N*-methyl-D-aspartate) glutamate receptors. In addition, riluzole may also have neurotrophic or anti-apoptotic properties in certain experimental paradigms.

2.1.2 Animal Models

Compared to controls, the hSOD-1 transgenic mice show signs of glutamate toxicity[22] with elevated levels of extracellular cortical glutamate,[46,47] and decreased glutamate transport in cerebral cortex[48] and spinal cord[49] preparations. In line with the results of the clinical tests,[7,8] treatment of the hSOD-1^{G93A} mouse with riluzole prolonged survival time by 12%[50] and, in a separate study, was also found to preserve motor function.[51] Another anti-glutamate drug, gabapentin also increased survival, although to a lesser extent (6%).[50] More recently, an antagonist of AMPA glutamate receptors, RPR-119990, has been reported to improve muscle strength and increase survival as well as preserving glutamate uptake in spinal cord preparations from hSOD-1^{G93A} mice.[52]

In the transgenic rat model, the hSOD-1^{G93A} animals showed focal loss of the EAAT2 glutamate transporter in the ventral horn of the spinal cord that coincided with gliosis, appeared before motor neurone degeneration and exceeded 90% at end-stage disease.[53]

2.2 Oxidative Stress

2.2.1 Human Studies

Oxidative stress occurs when cells are damaged by exposure to highly reactive free radicals, such as superoxide (O_2^-), the hydroxyl radical (OH) and the nitronium radical, peroxynitrite ($ONOO^-$) that irreversibly damage cellular components.[54-58] Under normal circumstances the levels and activity of these radicals are controlled by enzymatic defence mechanisms, such as the superoxide dismutases, glutathione peroxidase and catalase, and non-enzymatic defence mechanisms, such as ascorbic acid, α-tocopherol (vitamin E) and glutathione.[54] Oxidative damage arises when an imbalance occurs in the system allowing the free radicals to react with lipids, protein and DNA, often causing irreparable damage that can lead to cell death. Free radical-induced damage has been implicated in the aging process,[59] and has also been hypothesised to be involved in other neurodegenerative diseases such as Alzheimer's disease,[60-62] Parkinson's disease[61,63] and Huntington's disease.[61,62]

The first indications that oxidative damage might play a role in the pathogenesis of ALS came with the demonstration that a subgroup of patients with FALS had point mutations in the ubiquitous, cytosolic enzyme, SOD-1[3,4] and, to date, around 100 different mutations have been detected in the enzyme from between 14–23% of patients with FALS.[64] Epidemiological studies have also shown SOD-1 mutations in 3–7% of patients with apparently SALS, although some of these latter patients may be misdiagnosed FALS patients possibly due to low penetrance.[64]

SOD-1 is a homodimer, with each subunit binding a catalytically essential copper ion and a stabilising zinc ion, and protects against oxidative damage by converting O_2^- to hydrogen peroxide. However, many of the mutations observed in FALS neither abolish nor decrease this dismutase

activity and their deleterious consequences are thought to be due to a 'gain-of-function' rather than a loss-of-function. The hypotheses advanced for this gain of function include conformational and stability changes leading to enhanced free radical generation, possibly due to aberrant metal chemistry[65-77] and/or the formation of toxic aggregates.[78-82] This is further reviewed by Julien, 2001.[83]

Although there is no evidence that SOD-1 function is altered in the majority of patients with ALS, the similarity of the familial and sporadic forms in terms of clinical symptoms and disease course suggested that oxidative damage might be important in all patients with ALS. In line with this, evidence for oxidative damage has been found in SALS patients and FALS patients with or without mutations in SOD-1.[84-97]

2.2.2 Animal Models

Increased signs of oxidative damage have also been observed in hSOD-1 transgenic mouse models[48,67,98-104] and a number of anti-oxidant treatments have been tested, such as the free-radical scavenger α-tocopherol administered with selenium. This combination delayed disease onset, although not progression or survival of the SOD-1^{G93A} animals,[50] while carboxyfullerenes, which also act as free-radical scavengers, both delayed onset and increased survival time (6.4%).[105] Catalase is responsible for hydrogen peroxide removal and this enzyme, when modified with polyamine to increase its blood-brain barrier permeability, delayed onset and increased survival time (10.8%),[106] although putrescine-modified catalase had a significant effect on onset but not survival time.[107] The SOD/catalase mimetics, EUK-8 and EUK-134, increased survival time (7.7% and 10.4%, respectively) and reduced markers of oxidative stress in low-expressor hSOD-1^{G93A} mice.[108]

Acetylcysteine (N-acetyl-L-cysteine) increases plasma levels of cysteine, the rate-limiting precursor for glutathione synthesis, which in turn is the principle substrate for the detoxification of hydrogen peroxide and lipid peroxides. Although in one study acetylcysteine was found to have no effect on disease onset or death,[109] in another it was shown to improve motor performance and increase survival time (9.6%).[110] Thioctic acid (α-lipoic acid), a mitochondrial coenzyme also reported to enhance the activity of the glutathione system and increase ascorbic acid levels,[111] improved motor performance, delayed weight loss and increased survival time (6%),[112] while lysine acetylsalicylate delayed the appearance of motor deficits with no effect on the onset of paralysis or survival time.[113] *Panax quinquefolium* (American ginseng), which is thought to have anti-oxidant properties, delayed onset and increased survival time by 5.3%.[114] Results with inhibitors of neuronal nitric oxide synthase (NOS) have been variable. A selective inhibitor, ARR-17477, significantly increased survival time in both high and low expressor hSOD-1^{G97A} mice, while other less selective molecules had no beneficial effect.[115,116] In addition, reduced neuronal NOS expression had no effect on disease onset, progression or survival time.[115] However, this does not rule out a role for inducible NOS (iNOS), which is upregulated in the spinal cord of the transgenic mouse.[117] *Ginko biloba* extract increased survival but this only reached significance in male hSOD-1^{G97A} mice.[118]

Copper chelators have also been tested for potential beneficial effects in the transgenic mice model. Penicillamine delayed onset and increased survival time,[119] as did trientine, alone[112] or in combination with ascorbate.[120] In line with these results, metallothioneins I and II, which maintain intracellular concentrations of metals such as copper and zinc, are increased 2-fold in the spinal cord of low-expressor hSOD-1^{G93A} mice compared with controls,[121] and a combination of reduced metallothionein expression and hSOD-1^{G93A} overexpression led to both accelerated disease onset and a decrease in survival time.[121] However, ablating CCS, the copper chaperone for SOD-1 and necessary for the efficient incorporation of copper into the enzyme in motor neurones, in the transgenic mice had no effect on disease onset or progression.[122]

Thus, although the evidence for oxidative damage in the transgenic mice expressing mutated hSOD-1 has mounted over the last few years the precise mechanism by which this is brought about is still far from clear.

2.3 Links Between Excitotoxicity and Oxidative Damage

The excitotoxic and oxidative stress theories of ALS are not mutually exclusive. Free radical-mediated damage could sensitise neurones to glutamate-mediated excitotoxic mechanisms and, conversely, the increase in intracellular Ca^{2+} concentrations that accompanies excitotoxicity can increase free radical production.[17,29,32,123] In addition, two of the SOD-1 mutant enzymes found in FALS with, respectively, an alanine to valine change at residue 4 or an isoleucine to threonine at residue 113, *in vitro* selectively inactivate the glial glutamate transporter EAAT2[124] that, as discussed in section 2.1.1, has been implicated in excitotoxicity.[36,37]

2.4 Mitochondrial Dysfunction

2.4.1 Human Studies

Both glutamate neurotoxicity and oxidative stress can lead to mitochondrial dysfunction[29] and mitochondrial damage that might result from one or a combination of both mechanisms has also been reported in patients with ALS[91,94,125-129] and reviewed by Beal, 2000[130] and Swerdlow et al., 2000.[131]

2.4.2 Animal Models

Both hSOD-1^{G93A} and hSOD-1^{G37R} mice show mitochondrial vacuolisation and swelling,[12,132] and it has been suggested that massive mitochondrial degeneration is an early event in the development of the disease in the hSOD-1^{G93A} animals.[133] The animals also show increased vulnerability to mitochondrial toxins,[134] oxidative damage to spinal motor neurone mitochondrial DNA[104] and increased mitochondrial complex 1 activity.[126] A partial deficiency of manganese SOD, the main scavenger of oxygen radicals in the mitochondria, exacerbated the clinical phenotype and shortened survival time (7.9%).[135]

Both co-enzyme Q and creatine have been tested in the hSOD-1^{G93A} mouse for their potential to improve mitochondrial function. Co-enzyme Q, an electron transport chain cofactor and an antioxidant with neuroprotective effects,[136,137] increased survival time by 4.6%,[136] while in two separate studies creatine both improved motor performance and increased survival time by 18%[138] and 14.6%,[112] respectively. Creatine acts as an energy buffer by increasing muscle and brain phosphocreatine levels. It may also stabilise mitochondrial creatine kinase and inhibit the opening of the mitochondrial transition pore, and has been hypothesised to be of value in a variety of neurological disorders.[139]

2.5 Apoptosis

2.5.1 Human Studies

There is increasing evidence that motor neurone demise in ALS occurs via apoptosis (reviewed in Sathasivam et al.[140]), a programmed mechanism of cell death involving a variety of different, interactive pathways.[141,142] Some of the key events of apoptosis detected in susceptible tissue from patients with ALS compared with controls include: (i) cytochrome C release from mitochondria;[143] (ii) up-regulation of the tumour suppressor protein, p53;[144,145] (iii) modulation of the pro-apoptopic and anti-apoptopic Bcl-2 oncoprotein family;[146-151] (iv) increased levels of prostate-apoptosis response-4;[152] and (v) increased DNA fragmentation[147-150] and caspase activation. Caspases are intracellular cysteine proteases, synthesised as inactive pro-enzymes, primarily responsible for the morphological and biochemical changes associated with apoptosis.[141,153,154] Initiator caspases are activated in the early stages of cell death with effector caspases, responsible for proteolytic events that lead directly to apoptosis, being activated at a later stage. In patients with ALS, initiator caspase-1, formerly known as interleukin-1β-converting enzyme and responsible for inflammatory cytokine maturation, is activated in muscle fi-

bres,[147] spinal cord[155] and serum,[156] with the effector enzyme, caspase-3, being activated in the spinal cord anterior horn and motor cortex.[148]

2.5.2 Animal Models

Although there have been reports to the contrary,[157] a large number of studies have detected the signs of apoptopic cell death in the SOD-1 transgenic mice. These include: (i) DNA laddering;[158] (ii) a decrease in the anti-apoptopic survival signal proteins phosphatidy-linositol 3-kinase and Akt/protein kinase B in spinal motor neurones of pre-symptomatic animals;[142] and (iii) cleavage of the apoptosis inhibitor XIAP by caspases in end-stage disease.[143] There are also changes to the Bcl family of oncoproteins in symptomatic, but not asymptomatic hSOD-1^{G93A} mice;[159] furthermore, over expression of the apoptosis suppressor *Bcl-2* in these animals delayed disease onset and increased survival time by 12.5%.[160] In addition, intraspinal injection of *Bcl-2* encoded in an adeno-associated virus led to an improvement in motor performance and a significant increase in the number of surviving motor neurones at the end-stage of disease, although there was no effect on survival time.[161]

Further evidence that cell death in the transgenic mouse models proceeds via apoptosis comes from several studies demonstrating the activation of caspase-1,[162,163] -9,[143] -3[155,158,162,163] and -7[143] in regions affected by neurodegeneration in ALS in hSOD-1^{G93A},[155,158,163] hSOD-1^{G37R}[162] and hSOD-1^{G85R}[162] mice. The processing is sequential, with the initiator caspase-1 and -9 being activated before the effector caspase-3 and -7.[143,162,163] In addition, the expression of a dominant negative inhibitor of caspase-1 in hSOD-1^{G85R} mice increased survival time by 8.3%,[164] while administration of N-benzoyloxycarbonyl-Val-Asp-fluoromethylketone, a general inhibitor of caspase enzymes, increased survival time of hSOD-1^{G93A} mice by 22%.[155]

Even more impressive results were achieved with WHI-p13, an inhibitor of the tyrosine kinase janus kinase-3, which increased survival time of the hSOD-1^{G93A} mice by 49%.[165] It is thought that inhibition of this kinase might suppress expression of the proto-oncogene, *c-jun*.[166]

Another putative anti-apoptotic drug is cyclosporin, which is hypothesised to stabilise mitochondrial membranes, preventing the release of apoptogenic factors and assembly of mitochondrial permeability transition pore, increased survival time by 12 days in SOD-1^{G93A} mice.[167]

Minocycline is a tetracycline antibiotic that *in vivo* inhibits caspase-1, caspase-3, iNOS and p38 mitogen-activated protein kinase, and mediates neuroprotection in a variety of experimental models.[168,169] Inhibition of these enzymes is indirect and is mediated by inhibition of cytochrome C translation from the mitochondria, one of the key events in apoptosis and a potent stimulus for caspase activation.[169] In line with this, minocycline delayed disease onset and increased survival (10.3%) in the hSOD-1^{G93A} mice.[169] This is also in agreement with a previous observation that cytochrome C is translated from the mitochondria to the cytosol during disease progression in these animals.[143]

However, not all putative, anti-apoptotic strategies have had a beneficial effect in the transgenic mice. As discussed in section 2.5.1, levels of the transcription factor p53, thought to be involved in neuronal apoptosis, are increased in human ALS and a similar observation has been made in hSOD-1^{G86R} mice.[170] However, crossing knockout p53 mice with hSOD-1^{G93A} mice had no effect on disease progression in the offspring.[171,172] Similarly, CGP-3455 and desmethylselegiline, which have anti-apoptotic effects *in vitro* by preventing nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase, had no effect on disease development or progression in the hSOD-1^{G93A} mice.[112] It should also be noted that the anti-necrotic agent, 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly (ADP-ribose) polymerase that is activated as a consequence of oxidative damage to DNA, also had no significant effect in these animals.[112]

2.6 Inflammation

Differential gene expression studies in the transgenic mice have shown the upregulation of genes involved in inflammatory processes. In addition, mRNA expression and activity of the inducible form of cyclooxygenase (COX)-2, the rate-limiting enzyme in the production of prostaglandins, increase in parallel to disease progression in these animals.[173] In line with this, a selective COX-2 inhibitor SC-236 prolonged survival (20%) but had little effect on disease onset in hSOD-1^{G93A} mice.[174]

2.7 Neurotrophic Factors

As discussed in section 3.3, clinical trials involving neurotrophic factor have had little success and there have been few published reports on the effects of these factors in the SOD-1 transgenic mice. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor (GDNF) delayed disease onset and muscle deterioration in hSOD-1^{G93A} animals,[175] while intramuscular injection of an adenoviral vector encoding cardiotrophin-1 delayed the onset of motor impairment.[176] However, fibroblast growth factor had no beneficial effect.[177]

2.8 Cytoskeletal Defects

2.8.1 Human Studies

Neuropathological studies of the spinal cord of ALS patients have revealed the presence of specific inclusion bodies not commonly observed in other pathologies. These inclusion bodies are believed to represent aggregates of cytoskeletal proteins, raising the possibility that cytoskeletal pathology may be involved in the aetiology of ALS.[178] Moreover, mutations in genes encoding neurofilament proteins have been identified in a few patients with apparently sporadic ALS.[179,180]

2.8.2 Animal Studies

Transgenic mice have been bred that express human neurofilament genes,[181,182] either the human neurofilament heavy chain gene (*NF-H*), the human neurofilament light chain gene (*NF-L*), or the human *NF-L* gene carrying a L394P point-mutation. Although all these mice present, to a greater or lesser degree, cytological changes in anterior horn cells and clinical manifestations of motor neurone impairment, only in the *NF-L*[L394P] strain has loss of anterior horn cells been demonstrated unequivocally. Taken as a group, these transgenic models show that neurofilament accumulation can disrupt the functional and morphological integrity of motor neurones. Mice overexpressing the intermediate filament protein peripherin also develop a late-onset motor neurone disease.[183] Potential therapeutic interventions have not yet been evaluated in the neurofilament transgenic mice.

2.8.3 Consequences for Therapy

Although there have been suggestions that cytoskeletal abnormalities may be a consequence of oxidative stress,[78-83] and thus be corrected by antioxidant drugs (see section 2.2), it is not clear how cytoskeletal defects themselves could be a potential target of therapeutic interventions. The importance of cytoskeletal proteins in ALS is discussed in several reviews.[179,184-187]

2.9 Other Mechanisms

Other treatments that have been tried in the transgenic mice include intravenously administered mononuclear cells from human umbilical cord to animals that have received either a lethal[188] or sublethal[189] dose of irradiation that increased survival time by 31.6% and 16.3%, respectively. It was suggested that these results indicate that ALS is an autoimmune disease; however, the immunosuppressant tacrolimus (FK-506) had no effect on disease progression or survival.[190] As in other neurodegenerative diseases, the use of embryonic stem cells may hold promise for the replacement of degenerated motor neurones but there have been no specific studies carried out in the SOD transgenic mice.

3. Clinical Trials: Past

3.1 Anti-Glutamate Agents

Drugs that have been investigated in clinical trials for ALS are listed in table I. To date, the only trials that have led to the licensing of a treatment for ALS are those carried out with riluzole, which showed that the drug increased survival time/time to tracheostomy. (Bensimon, 1994;[7] Lacomblez, 1996;[8] Meininger, 1997;[9] Miller, 2001[11]). Another anti-glutamate drug gabapentin showed promise in phase II trials with the suggestion that it slowed the rate of decline of patients with ALS.[191] However, a phase III trial employing a higher dose, a longer duration and a larger sample size, showed no evidence of a beneficial effect on either disease progression or symptoms.[192] Similar negative results have been reported for other potential anti-excitotoxic drugs, although in most cases small numbers of patients were employed. The compounds tested include threonine[193,194] and other branched-chain amino acids,[195,196] the glutamate-release inhibitor lamotrigine,[197] the glutamate-receptor antagonist dextromethorphan,[198-200] and the calcium channel antagonists nimodipine[201] and verapamil.[202] However, it should be pointed out that in the case of dextromethorphan at least, this compound is rapidly and completely metabolised by more than 90% of the population.

3.2 Anti-Oxidant Agents

In general, clinical trials with anti-oxidants have yielded similar negative results. The molecules tested include acetylcysteine,[203] reduced glutathione[204] and selegiline, a monoamine oxidase inhibitor with anti-oxidant properties.[205-207] However, promising results were obtained in a study looking at the effect of α-tocopherol in patients taking riluzole. There was no significant difference between treatment groups on survival or on the Norris scale. However, after 12-months treatment a statistically significantly higher proportion of patients receiving tocopherol plus riluzole remained in a milder ALS health state compared with those who received riluzole plus placebo.[208] In addition, after 3-months treatment, plasma malondialdehyde levels in the group receiving α-tocopherol plus riluzole fell to below those of young adults and this was coupled with a significant increase in plasma glutathione peroxidase activity.[95,208] The feasibility of intrathecal administration of recombinant human superoxide dismutase has also been investigated,[225] but no efficacy data are available.

3.3 Neurotrophic Agents

Several neurotrophic factors that might stimulate surviving motor neurones into compensating for the function of lost neurones have undergone clinical trials as potential ALS treatments. Brain-derived neurotrophic factor (BDNF) promotes motor neurone survival in a number of animal models,[226,227] and in particular slows the loss of motor function and motor neurones in the wobbler mouse.[228-230] In a phase I–II study, subcutaneous recombinant human methionyl BDNF appeared to increase survival and retard loss of pulmonary function in patients with ALS.[209] Although a phase III study did not confirm these findings,[210] the results were thought promising enough to continue trials with either higher subcutaneous doses or intrathecal administration. However, both these trials have since been discontinued, since no evidence of efficacy was obtained.[231]

Ciliary neurotrophic factor (CNTF) also showed potential as an ALS treatment in two animal models, the pmn mouse[232] and the wobbler mouse.[228] However, negative results were obtained with subcutaneously administered, recombinant human CNTF in two large double-blind, placebo-controlled trials with significant adverse effects being observed in each case, including an increase in the death rate.[211,212]

Mecasermin (recombinant insulin-like growth factor 1) promotes motor neurone survival in an excitotoxic model of neurodegeneration and improves muscle strength in the wobbler mouse.[214,233] Subcutaneous mecasermin showed promising results in a US trial,[213] however, the results of a similar European trial were negative[214] and

Table I. Clinical trials for amyotrophic lateral sclerosis (ALS)

Drug	Phase	No. of pts	Duration (mo)	Trial design	Evaluation criteria	Results	Reference
Anti-excitotoxic							
Riluzole	II	155	12	DB, PC	Survival, functional status	Significant increase in survival	7
Riluzole	III	959	18	DB, PC, 4 arms	Survival	Significant increase in survival	8
Gabapentin	II	152	6	DB, PC	Muscle strength	Non-significant positive trend	191
Gabapentin	III	204	9	DB, PC	Muscle strength	No beneficial effect	192
Threonine	II	23	12	DB, PC	Norris scale	No beneficial effect	193
Threonine	II	30	12	OL, R	Norris scale	No beneficial effect	194
BCAA	II	126	12	DB, PC, 2PG	MMT, Norris & Appel scales, FVC	No beneficial effect	195
BCAA/threonine	II	95	6	DB, PC, 3PG	MMT & Z scores	No beneficial effect	196
Lamotrigine	II	67	18	DB, PC	Survival	No beneficial effect	197
Dextromethorphan	II	14	7 (+6)	DB, PC, CO (OL)	Norris & bulbar scales, Electrophysiological parameters	No beneficial effect	198
Dextromethorphan	II	45	12	DB, PC	Survival, FVC, ALS severity scale	No beneficial effect	199
Dextromethorphan	II	49	12	DB, PC	Norris scale	No beneficial effect	200
Nimodipine	II	87	3+1	DB, PC, CO	FVC, TQNE	No beneficial effect	201
Verapamil	II	72	12	OL, HC	Pulmonary & limb function	No beneficial effect	202
Anti-oxidant							
N-acetylcysteine	II	110	12	DB, PC	Survival	No beneficial effect	203
Reduced glutathione	II	32	37	R, OL, CO	Norris & bulbar scales, FVC, muscle strength	No beneficial effect	204
Selegiline	II	111	6	DB, open control	Survival, disability score, treatment withdrawal	No beneficial effect	205
Selegiline	II	10	6	DB, PC, CO	Appel score	No beneficial effect	206
Selegiline	II	133	9	DB, PC, CO	Norris score	No beneficial effect	207
α-Tocopherol	III	289	12	DB, PC	Norris limb scale and survival	Non significant positive effect on progression	208
Neurotrophic factor							
BDNF	I/II	283	6	DB, PC	Survival		209
BDNF	III	1135	9	DB, PC, 3 arms	Survival	No beneficial effect	210
CNTF	II/III	730	9	DB, PC, 3 arms	Muscle strength	No beneficial effect	211
CNTF	II/III	570	6	DB, PC, 4 arms	MVIC, pulmonary function	No beneficial effect, Increased mortality	212
Mecasermin (rhIGF-1)	II/III	266	9	DB, PC, 3 arms	Appel scale	Decreased rate of decline	213
Mecasermin (rhIGF-1)	II/III	183	9	DB PC	Appel scale	No beneficial effect	214
Growth hormone	II/III	75	12-18	DB, PC	Survival, TQNE, MRC score	No beneficial effect	215

Table I continued

mecasermin has not been approved as a treatment for ALS. The results of a phase II/III trial of growth hormone that acts through mecasermin also showed no beneficial effect[215] and neither did the synthetic growth hormone protropin.[234]

Some of the problems encountered above may be due to the fact that BDNF, CTNF and mecasermin do not cross the blood brain barrier. Interest is therefore being shown in orally active compounds that can mimic the actions of the neurotrophic factors, possibly by stimulating their synthesis or release. One of these molecules is xaliproden, (SR-57746A), a 5-HT_{1A} receptor agonist, that stimulates BDNF, CDNF and nerve growth factor (NGF) synthesis by an undetermined mechanism. Xaliproden also promotes mouse motor neurone survival *in vitro*,[235] and *in vivo* in the axotomised developing rat spinal cord[236] and pmn mice.[237] Two double-blind, placebo-controlled trials were undertaken in 1997 to evaluate safety, tolerability and effectiveness either with xaliproden in monotherapy (n = 800) or in combination with riluzole (1000). Although, a preliminary analysis showed that xaliproden is well tolerated,[238] no further details are available.

3.4 Immunomodulators

No clinical benefit was observed with immunosuppression by total lymphoid irradiation[220] or treatment with interferon β-1α.[217] In one uncontrolled study, cyclophosphamide treatment was reported to lead to a mild, although transient, improvement in bulbar and motor function in a subset of patients,[218] whereas, in another study, it was found to have no beneficial effect[219] (table I).

4. Clinical Trials: Present

4.1 Anti-Glutamate Drugs

Anti-glutamate drugs reported to be undergoing evaluation as potential ALS treatments at the present time include topiramate (Topamax™ **1**) cur-

1 Use of tradenames is for product identification only and does not imply endorsement.

TRH	II	36	13	DB, CO	Motor unit loss	No beneficial effect	216
Immune modifier							
Interferon β-1α	II	61	12	DB, PC	MRC, Norris bulbar, FVC	No beneficial effect	217
Cyclophosphamide	II	44	3	OL	Bulbar and motor scores	Inconclusive, mild, transient improvement	218
Cyclophosphamide	II	18	6	OL, HC	Muscle strength, motor co-ordination, pulmonary function	No beneficial effect	219
Lymphoid irradiation	II	61	24	DB, PC	Motor function	No beneficial effect	220
Immunoglobulin	II	7	3	OL	MRC, bulbar & Rankin scores	No beneficial effect	221
Immunoglobulin	II	9	3	OL	Muscle strength	No beneficial effect	222
Anticholinergic							
3,4-diaminopyridine	II	9	1.5	DB, PC, CO	FIM, nerve conduction, speech assessment, motor impairment	No beneficial effect	223
Methylcobalbumin	II	24	1	DB, OL, 2 arms	CMAP	CMAP increased at high dose	224

BCAA = branched chain amino acids; **BDNF** = brain-derived neurotrophic; **CMAP** = compound muscle action potential; **CNTF** = ciliary neurotrophic factor; **CO** = cross-over; **DB** = double blind; **FIM** = functional independence measurement; **FVC** = forced vital capacity; **HC** = historical controls; **MMT** = manual muscle testing; **MRC** = Medical Research Council; **MVIC** = maximum voluntary isometric contraction; **OL** = open label; **PC** = placebo controlled; **PG** = parallel group; **R** = randomised; **rhIGF** = recombinant insulin-like growth factor; **TQNE** = Tufts quantitative neuromuscular exam; **TRH** = thyrotropin releasing hormone; **Z score** = maximal voluntary isometric contraction.

rently used to treat epilepsy[239] and a dextromethorphan derivative. Although, as discussed in section 3.1, dextromethorphan itself has previously shown no beneficial effect in clinical trials (table I), this may have been due to its rapid metabolism and a derivative that is more stable *in vivo* is being evaluated for efficacy in the control of emotional lability in ALS patients. Inhibitors of N-acetylated-α-linked acidic dipeptidase (NAALADase), the enzyme responsible for the hydrolysis of N-acetylaspartylglutamate (a major peptidic component of the brain) into N-acetylaspartate and glutamate, are still in the preclinical stage of development.[240]

4.2 Anti-Oxidants, Anti-Apoptotic Agents and Energy Stimulators

Following the publication of the encouraging results obtained with creatine in the transgenic mice,[138] several studies are underway to assess its safety and efficacy, and a preliminary report has indicated that it may have a temporary benefit in situations such as high intensity activity.[241] However, creatine is freely available as a dietary supplement and is likely to be taken by a large percentage patients with ALS, whether prescribed or not.[185] Similar studies are under way with coenzyme Q that also had a beneficial, although smaller, effect in the transgenic mouse.

A 6-month study of minocycline in 20 patients with ALS has been undertaken to investigate safety, tolerability, and its effect muscle strength and breathing capacity. As discussed above (in section 2.5.2), minocycline extends survival in the transgenic SOD-1 mice, probably by inhibiting a key stage in apoptosis.[169]

4.3 Neurotrophic Agents

Molecules under investigation for ALS that may cross the blood brain barrier and stimulate neurotrophin-like action include buspirone and leteprinim potassium (AIT-082; Neotrofin™). Buspirone is a 5-HT_{1A} receptor agonist and a commonly used anxiolytic agent that may mimic or stimulate the activity of neurotrophins such NGF and BDNF,[242] and leteprinim potassium is a lead compound of a series of purine derivatives that is currently in phase II/III clinical trials for Alzheimer's disease.[243]

4.4 Other Investigations

An ALS-like syndrome has been described recently as an HIV-related neurological complication.[244] Following reports of the successful treatment of this syndrome with antiretroviral therapy,[244,245] a study is underway to look at the effects of the protease inhibitor indinavir in patients with ALS.

Other clinical trials currently or shortly to be underway include: (i) a phase II trial to look at the effect of androgen suppression; (ii) an epidemiological study to look at the incidence of ALS among Gulf-war veterans; (iii) an observational, retrospective study looking at exogenous toxicants and genetic susceptibility and factors that influence disease severity; and (iv) a screening/diagnostic study looking at determinants of disease severity, including changes in free radical damage and anti-oxidant defences.

Further details on current clinical trials in ALS can be found at www.clinicaltrials.gov and www.alsa.org.research/drug.

5. Clinical Trials: Future

Since the first reported clinical trial 1944, over 50 compounds have been documented as having been tested as potential treatments for ALS[246,247] (table I), while, undoubtedly, many others have not reached publication stage. If the success of these trials is judged by the licensing of a drug to treat the disease then only those carried out with riluzole can be said to have achieved their objective.[7,8] However, there has been a great deal of recent debate and criticism over the design and aims of many past clinical trials for ALS.[247-251] A recent survey of those trials carried out in the 1990s showed that approximately half involved 18 patients or less with a mean duration of 24 weeks, evaluation criteria varied widely and, in many cases, the data reported were scant.[249] It has also

been estimated that to obtain any meaningful results the minimum number of participants should be 120,[248] although even this may be an underestimate, and in our opinion an effective clinical trial for a potential disease-modifying drug should involve at least 400 patients per group and last for a minimum of 1 year with survival as the primary endpoint to demonstrate any significant effect that is additional to riluzole. The use of such a large number of patients in a disease that is relatively rare poses significant logistical problems and necessitates large multi-national trials. Although expensive, the successful execution of recent trials with riluzole and certain growth factors demonstrates that it is possible to carry out such large trials with sufficient statistical power to demonstrate effects on the evolution of the disease. Trials for a putative symptomatic treatment may require fewer patients and should be designed following the hypothesis that a functional and not survival effect is expected.

However, it is difficult to make hard and fast rules, as improving the quality of life of patients with ALS is just as important as extending life. A molecule that does not have an impact on survival but ameliorates muscular force could, in the absence of any aggravating effects, be a suitable candidate for symptomatic treatment. Therefore, clinical trials should not necessarily be stopped if survival is not increased.

There has also been an appeal for the full publication of all results, as a negative result in a well designed trial may give vital information.[249] The need for a consensus in choosing endpoints as well as in design is inescapable and in an attempt to rectify these problems, consensus guidelines for clinical trials in ALS have been drawn up by the World Federation of Neurology[252] and are available at their website.[253]

6. Methodological Issues

6.1 Need for Early Diagnosis

The mean time from onset of symptoms to a confirmed diagnosis of ALS is currently around 16–18 months.[254,255] The importance of early diagnosis and its associated problems, both ethical and technical, are addressed in several articles.[256-260] The transgenic mouse models suggest a long, clinically silent period for the disease,[261,262] although, as pointed out,[258] this does not necessarily imply a similar time course in humans. However, electromyographic measurements have shown that altered patterns of muscle innervation appear long before clinical weakness becomes apparent[263,264] and it has been suggested that up to 80% of neurones have to fail for clinical signs to become overt.[265] In line with this, the effect of riluzole in preserving motor function in the transgenic mouse was greater the earlier treatment was started.[51] Similarly, the beneficial effects of lysine acetylsalicylate on motor performance in the transgenic mice were observed if treatment was started at 5 weeks of age, before the onset of motor neurone dysfunction, but not if it was started at 13 weeks at the onset of motor neurone death.[113] Early treatment of patients with ALS, preferably during the non-symptomatic stage, might therefore prove advantageous. This of course needs to be balanced against the consequences of false diagnoses, with all the attendant consequences, both social and economical, that at present may seem to outweigh any potential benefits. However, as a strategy for the future, early accurate diagnosis and early effective treatment should surely be amongst our goals.

6.2 The Need for Biological Markers

So far no reliable biological marker has been established for ALS,[266] although several indicators of oxidative damage have been proposed as potential candidates. One of these is nitrotyrosine, formed by the interaction of peroxynitrite with the side chains of tyrosine residues and found to be elevated 7-fold in the cerebrospinal fluid from ALS patients compared with controls.[92] However, as pointed out,[267] determining the levels of a marker in the cerebrospinal fluid (CSF) would involve a lumbar puncture and thus would not be suitable for routine screening. Perhaps more prom-

ising is malondialdehyde,[267] a measure of lipid peroxidation,[54] the levels of which are significantly higher in the plasma of ALS patients than in age-matched controls or young adults.[89,208] In addition, a significant positive correlation has been found between malondialdehyde levels and scores for fatigue and stiffness whilst a negative correlation was found with muscle testing scores.[208] Another advantage is that measuring plasma malondialdehyde levels in is a relatively simple laboratory procedure.[267] Another indicator of oxidative damage proposed as a possible ALS marker is the concentration of protein-associated carbonyl groups in red blood cells that shows a positive correlation with the onset of clinical symptoms.[89]

Imaging techniques also have a potential role in detecting ALS markers.[268] For instance, measuring metabolic changes in N-acetylaspartate, choline and creatine by magnetic resonance imaging has also been proposed as a surrogate marker for ALS that might aid early detection and monitor progression and treatment response.[269] These initial results are promising but the technique requires validation before it's full potential can be assessed.

The identification of markers that could detect the disease in either its pre-clinical or early clinical stages would aid early diagnosis of ALS as well as clinical trials and epidemiological studies. The ideal marker should be measurable by a test that is inexpensive, reliable and simple to perform, making it easy to incorporate into clinical trials. The advantages of such a test are clear. At present, large numbers of patients are required to see an effect using survival, clinical rating scales, positron emission tomography etc. A marker would allow rapid screening of potential treatments before initiating huge clinical trials with clinically important endpoints. As discussed earlier in this section, one promising candidate is plasma malondialdehyde. The results obtained with this indicator of lipid peroxidation suggest that pre-screening of peripheral malondialdehyde levels, or similar markers of oxidative damage, in an 'at risk' population might increase the chances of successful early diagnosis. However, it should be borne in mind that oxidative damage is not likely to be specific to ALS and, for instance, increased malondialdehyde levels have been reported in the erythrocytes of patients with Alzheimer's disease.[270] Indeed, oxidative stress seems to be a general feature of a number of degenerative neurological disorders.[32,60-62,271,272] However, coupled with other diagnostic techniques it could provide a useful early indication of a problem.

6.3 Reliability of the Transgenic Mouse Model

Very few of the drugs tested in SOD-1 mice have been used in clinical trials so it is difficult to ascertain the reliability of the animals as an effective model. The fact that gabapentin had no effect in human trials, while it increased survival in the $hSOD^{G93A}$ mice, has led to the suggestion that the mice are not a reliable screening vehicle for ALS drugs.[185] However, it has been pointed out that the effect of gabapentin in the transgenic mice was less pronounced than that of riluzole (6% increase in survival versus 13%, respectively).[192] In addition, the effects of α-tocopherol in the SOD mice in delaying onset but not increasing survival were mirrored in the clinical trial where patients receiving α-tocopherol supplements stayed in a milder health state for a longer period.[208]

No data have yet been published on the effects of BDNF, CNTF and mecasermin in the SOD-1 transgenic mice, although it has been reported that these studies have been carried out with negative results.[251] If this is the case, then the SOD-1 animal model would seem to reflect the human situation better than the pmn and wobbler mice models, where promising results were reported with the neurotrophic factors.[214,228-230,232,233]

As pointed out,[16] no animal model can be expected to reproduce human disease with total accuracy. Nevertheless the SOD-1 transgenic mouse model would seem to have advantages over previously used animal models and may point the way to new agents for clinical trials, such as anti-apoptotic drugs.

7. Conclusions: The Way Forward

Prevention of ALS may seem a utopian dream, as the only currently known cause of the disease is point mutations in SOD-1, which occurs in around only 2% of patients. However, genetic linkage analysis has shown that there are at least five FALS genes to be found,[273] the identification of which will be crucial to our further understanding of the disease. Thus, progressive genetic advances will point the way to new potential therapies that will need to be tested by classical pharmacological methods as outlined in this review. In addition, genetic advances, coupled with the development of reliable disease markers, should also enable the benefits of treatment in the pre-symptomatic phase to be tested, in at least potential FALS patients. Although it is early days, advances in stem cell research may also one day offer hope for patients with ALS.[187,274]

What we can safely conclude today is that the pathology of ALS is a complex interaction of more than one factor and combination therapy is undoubtedly the way forward. This could include molecules that mitigate the deleterious effects of glutamate and free radicals combined with neuroprotective agents and/or anti-apoptotic drugs.

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References

1. Havercamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population: validation of a scoring system and a model for survival prediction. Brain 1995; 118: 707-19
2. Swash M. Clinical features and diagnosis of amyotrophic lateral sclerosis. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz; 2000: 3-30
3. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutasegene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362: 59-62
4. Deng H-X, Hentati A, Tainer JA, et al. Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. Science 1993; 261: 1047-51
5. Cudkowicz ME, McKenna-Yasek D, Sapp PE, et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997; 41: 210-21
6. Gurney ME, Liu R, Althaus JS, et al. Mutant CuZn superoxide dismutase in motor neuron disease. J Inherit Metab Dis 1998; 21: 587-97
7. Bensimon G, Lacomblez L, Meininger V, et al. A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 1994; 330: 585-91
8. Lacomblez L, Bensimon G, Leigh PN, et al. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Lancet 1996; 347: 1425-31
9. Meininger V. Efficacy of riluzole in the treatment of amyotrophic lateral sclerosis. Rev Contemp Pharmacol 1997; 8: 255-64
10. Doble A. Effects of riluzole on glutaminergic neurotransmission in the mammalian central nervous system and other pharmacological effects. Rev Contemp Pharmacol 1997; 8: 213-26
11. Miller R, Mitchell J, Lyon M, et al. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Available in The Cochrane Library [database on disk and CD ROM]. Updated quarterly. The Cochrane Collaboration; issue 2. Oxford: Update Software, 2002: CD001447
12. Gurney ME, Pu H, Chiu AY. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 1994; 264: 1772-5
13. Price DL, Sisodia SS, Borchelt DR. Genetic neurodegenerative diseases: the human illness and transgenic models. Science 1998; 282: 1079-83
14. Green SL, Tolwani RJ. Animal models for motor neuron disease. Lab Anim Sci 1999; 49: 480-7
15. Nagai M, Aoki M, Miyoshi I, et al. Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. J Neurosci 2001; 21: 9246-54
16. Doble A, Kennel P. Animal models of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1: 301-12
17. Gurney ME. Transgenic animal models of amyotrophic lateral sclerosis. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000: 251-62
18. Shibata N. Transgenic mouse model for familial amyotrophic lateral sclerosis with superoxide dismutase-1 mutation. Neuropathology 2001; 21: 82-92
19. Dal Canto MC, Gurney ME. A low expressor line of transgenic mice carrying a mutant human Cu,Zn superoxide dismutase (SOD1) gene develops pathological changes that most closely resemble those in human amyotrophic lateral sclerosis. Acta Neuropathol (Berl) 1997; 93: 537-50
20. Dal Canto MC, Gurney ME. Development of central nervous system pathology in a murine transgenic model of amyotrophic lateral sclerosis. Am J Pathol 1994; 145: 1271-9
21. Dal Canto MC, Gurney ME. Neuropathological changes in 2 lines of mice carrying a transgene for mutant human Cu, Zn, SOD and in mice overexpressing wild-type human SOD-a model of familial amyotrophic lateral sclerosis (FALS). Brain Res 1995; 676: 25-40
22. Ikonomidou C, Qin Qin Y, Labruyere J, et al. Motor neuron dehgeneration induced by excitotoxin agonists has features in common with those seen in the SOD-1 transgenic mouse model of amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 1996; 55: 211-24

23. Tu PH, Raju P, Robinson KA, et al. Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 1996; 93: 3155-60
24. Kato S, Nakashima K, Horiuchi S, et al. Formation of advanced glycation end-product-modified superoxide dismutase-1 (SOD1) is one of the mechanisms responsible for inclusions common to familial amyotrophic lateral sclerosis patients with SOD1 gene mutation, and transgenic mice expressing human SOD1 gene mutation. Neuropathology 2001; 21: 67-81
25. Oosthuyse B, Moons L, Storkebaum E, et al. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet 2001; 28: 131-8
26. Olney JW. Excitatory amino acids and neuropsychiatric disorders. Biol Psychiatry 1989; 26: 505-25
27. Choi DW. Excitotoxic cell death. J Neurol 1992; 23: 1261-76
28. Regan RF, Panter S, Witz A, et al. Ultrasructure of excitotoxic neuronal death in murine cortical culture. Brain Res 1995; 705: 188-9
29. Atlante A, Calissano P, Bobba A, et al. Glutamate neurotoxicity, oxidative stress and mitochondria. FEBS Lett 2001; 497: 1-5
30. Leigh PN. Excitotoxicity in ALS. Neurology 1996; 47 Suppl. 4: S221-7
31. Ince PG, Eggett CJ, Shaw PJ. The role of excitotoxicity in neurological disease. Rev Contemp Pharmacol 1997; 8: 195-212
32. Doble A. The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacol Ther 1999; 81: 163-221
33. Jackson M, Rothsrein JD. Excitotoxicity in amyotrophic lateral sclerosis. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000: 263-77
34. Van Den Bosch L, Vandenberghe W, Klaassen H, et al. Ca(2+)-permeable AMPA receptors and selective vulnerability of motor neurons. J Neurol Sci 2000; 180: 29-34
35. Danbolt NC. Glutamate uptake. Prog Neurobiol 2001; 65: 1-105
36. Rothstein JD, Van Kammen M, Levey AI, et al. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 1995; 38: 73-84
37. Bristol LA, Rothstein JD. Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. Ann Neurol 1996; 39: 676-9
38. Lin CL, Bristol LA, Jin L, et al. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter in amyotrophic lateral sclerosis. Neuron 1998; 20: 589-602
39. Meyer T, Munch C, Knappenberger B, et al. Alternative splicing of the glutamate transporter EAAT2 (GLT-1). Neurosci Lett 1998; 241: 68-70
40. Nagai M, Abe K, Okamoto K, et al. Identification of alternative splicing forms of GLT-1 mRNA in the spinal cord of amyotrophic lateral sclerosis patients. Neurosci Lett 1998; 244: 165-8
41. Jackson M, Steers G, Leigh PN, et al. Polymorphisms in the glutamate transporter gene EAAT2 in European ALS patients. J Neurol 1999; 246: 1140-4
42. Meyer T, Fromm A, Munch C, et al. The RNA of the glutamate transporter EAAT2 is variably spliced in amyotrophic lateral sclerosis and normal individuals. J Neurol Sci 1999; 170: 45-50
43. Honig LS, Chambliss DD, Bigio EH, et al. Glutamate transporter EAAT2 splice variants occur not only in ALS, but also in AD and controls. Neurology 2000; 55: 1082-8
44. Flowers JM, Powell JF, Leigh PN, et al. Intron 7 retention and exon 9 skipping EAAT2 mRNA variants are not associated with amyotrophic lateral sclerosis. Ann Neurol 2001; 49: 643-9
45. Aoki M, Lin CL, Rothstein JD, et al. Mutations in the glutamate transporter EAAT2 gene do not cause abnormal EAAT2 transcripts in amyotrophic lateral sclerosis. Ann Neurol 1998; 43: 645-53
46. Alexander GM, Deitch JS, Seeburger JL, et al. Elevated cortical extracellular fluid glutamate in transgenic mice expressing human mutant (G93A) Cu/Zn superoxide dismutase. J Neurochem 2000; 74: 1666-73
47. Andreassen OA, Jenkins BG, Dedeoglu A, et al. Increases in cortical glutamate concentrations in transgenic amyotrophic lateral sclerosis mice are attenuated by creatine supplementation. J Neurochem 2001; 77: 383-90
48. Guo Z, Kindy MS, Kruman I, et al. ALS-linked Cu/Zn-SOD mutation impairs cerebral synaptic glucose and glutamate transport and exacerbates ischemic brain injury. J Cereb Blood Flow Metab 2000; 20: 463-8
49. Canton T, Pratt J, Stutzmann JM, et al. Glutamate uptake is decreased tardively in the spinal cord of FALS mice. Neuroreport 1998; 9: 775-8
50. Gurney ME, Cutting FB, Zhai P, et al. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. Ann Neurol 1996; 39: 147-57
51. Gurney ME, Fleck TJ, Himes CS, et al. Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. Neurology 1998; 50: 62-6
52. Canton T, Bohme GA, Boireau A, et al. RPR 119990, a novel alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid antagonist: synthesis, pharmacological properties, and activity in an animal model of amyotrophic lateral sclerosis. J Pharmacol Exp Ther 2001; 299: 314-22
53. Howland DS, Liu J, She Y, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci U S A 2002; 99: 1604-9
54. Benzie IF. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. Int J Food Sci Nutr 1996; 47: 233-61
55. Onorato JM, Thorpe SR, Baynes JW. Immunohistochemial and ELISA assays for biomarkers of oxidative stress in aging and disease. Ann N Y Acad Sci 1998; 20: 277-90
56. Keller JN, Mattson MP. Roles of lipid peroxidation in modulation of cellular signalling pathways, cell dysfunction and death in the nervous system. Rev Neurosci 1998; 9: 105-60
57. Cookson MR, Shaw PJ. Oxidative stress and motor neurone disease. Brain Pathol 1999; 9: 135-86
58. Torreilles F, Salman-Tabcheh S, Guerin M, et al. Neurodegenerative disorders: the role of peroxynitrite. Brain Res Brain Res Rev 1999; 30: 153-6
59. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998; 78: 547-81
60. Floyd RA. Antioxidants, oxidative stress and degenerative neurological disorders. Proc Soc Exp Biol Med 1999; 222: 236-45
61. Albers DS, Beal MF. Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease. J Neural Transm Suppl 2000; 59: 133-54
62. Butterfield DA, Howard BJ, LaFontaine MA. Brain oxidative stress in animal models of accelerated aging and the age-related neurodegenerative disorders, Alzheimer's disease and Huntington's disease. Curr Med Chem 2001; 8: 815-28
63. Schapira AH. Causes of neuronal death in Parkinson's disease. Adv Neurol 2001; 86: 155-62

64. Andersen PM. Genetics of sporadic ALS. Amyotroph Lateral Scler Other Motor Neuron Disord 2001; 2 Suppl. 1: S37-42
65. Wiedau-Pazos M, Goto J, Rabizadeh S, et al. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. Science 1996; 271: 515-8
66. Yim MB, Kang J-H, Yim H-S. A gain-of-function for an amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutant: an enhancement of free radical formation due to a decrease in Km for hydrogen peroxide. Proc Natl Acad Sci U S A 1996; 93: 5709-14
67. Bogdanov MB, Ramos LE, Xu Z, et al. Elevated "hydroxyl radical" generation in vivo in an animal model of amyotrophic lateral sclerosis. J Neurochem 1998; 71: 1321-4
68. Crow JP, Sampson JB, Zhuang YX, et al. Decreased zinc affinity of amyotrophic lateral sclerosis-associated superoxide dismutase mutants leads to enhanced catalysis of tyrosine nitration by peroxynitrite. J Neurochem 1997; 69: 1936-44
69. Ogawa Y, Kosaka H, Nakanishi T, et al. Stability of mutant superoxide dismutase-1 associated with familial amyotrophic lateral sclerosis determines the manner of copper release and induction of thioredoxin in erythrocytes. Biochem Biophys Res Commun 1997; 241: 251-7
70. Yim HS, Kang JH, Chock PB, et al. A familial amyotrophic lateral sclerosis-associated A4V Cu, Zn-superoxide dismutase mutant has a lower Km for hydrogen peroxide. J Biol Chem 1997; 272: 8861-3
71. Kim SM, Eum WS, Kwon OB, et al. The free radical-generating function of a familial amyotrophic lateral sclerosis-associated D90A Cu,Zn-superoxide dismutase mutant. Biochem Mol Biol Int 1998; 46: 1191-200
72. Estevez A, Crow JP, Sampson JB, et al. Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. Science 1999; 286: 2498-500
73. Eum WS, Kang JH. Release of copper ions from the familial amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutants. Mol Cells 1999; 9: 110-4
74. Gabbianelli R, Ferri A, Rotilio G, et al. Aberrant copper chemistry as a major mediator of oxidative stress in a human cellular model of amyotrophic lateral sclerosis. J Neurochem 1999; 73: 1175-80
75. Goto JJ, Zhu H, Snachez RJ, et al. Loss of in vitro metal ion binding specificity in mutant copper-zinc superoxide dismutase associated with familial amyotrophic lateral sclerosis. J Biol Chem 2000; 275: 1007-14
76. Kang JH, Eum WS. Enhanced oxidative damage by the familial amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutants. Biochim Biophys Acta 2000; 1524: 162-70
77. Lyons TJ, Nersissian A, Huang H, et al. The metal binding properties of the zinc site of yeast copper-zinc superoxide dismutase: implications for amyotrophic lateral sclerosis. J Biol Inorg Chem 2000; 5: 189-203
78. Briujn LI, Houseweart MK, Kato S, et al. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independant from wild-type SOD1. Science 1998; 281: 1851-4
79. Johnston JA, Dalton MJ, Gurney ME, et al. Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2000; 97: 12571-6
80. Okado-Matsumoto A, Myint T, Fujii J, et al. Gain in function of mutant Cu, Zn-superoxide dismutases as a causative factor in familial amyotrophic lateral sclerosis: less reactive oxidant formation but high spontaneous aggregation and precipitation. Free Radic Res 2000; 33: 65-73
81. Kato S, Sumi-Akamaru H, Fujimura H, et al. Copper chaperone for superoxide dismutase co-aggregates with superoxide dismutase 1 (SOD1) in neuronal Lewy body-like hyaline inclusions: an immunohistochemical study on familial amyotrophic lateral sclerosis with SOD1 gene mutation. Acta Neuropathol (Berl) 2001; 102: 233-8
82. Oeda T, Shimohama S, Kitagawa N, et al. Oxidative stress causes abnormal accumulation of familial amyotrophic lateral sclerosis-related mutant SOD1 in transgenic Caenorhabditis elegans. Hum Mol Genet 2001; 10: 2013-23
83. Julien JP. Amyotrophic lateral sclerosis. unfolding the toxicity of the misfolded. Cell 2001; 104: 581-91
84. Bowling AC, Schulz JB, Brown RHJ, et al. Superoxide dismutase activity, oxidative damage and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J Neurochem 1993; 61: 2322-5
85. Shaw PJ, Ince PG, Falkous G, et al. Oxidative damage to protein in sporadic motor neuron disease spinal cord. Ann Neurol 1995; 38: 691-5
86. Abe K, Pan LH, Watanabe M, et al. Upregulation of protein-tyrosine nitration in the anterior horn of cells of amyotrophic lateral sclerosis. Neurol Res 1997; 19: 124-8
87. Beal MF, Ferrante RJ, Browne SE, et al. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. Ann Neurol 1997; 42: 646-54
88. Ferrante RJ, Browne SE, Shinobu LA, et al. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. J Neurochem 1997; 69: 2064-74
89. Oteiza PI, Uchitel OD, Carrasquedo F, et al. Evaluation of antioxidants, protein and lipid oxidation in blood from sporadic amyotrophic lateral sclerosis patients. Neurochem Res 1997; 22: 535-9
90. Smith G, Henry YK, Mattson MP, et al. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Ann Neurol 1998; 44: 696-9
91. Borthwick GM, Johnson MA, Ince PG, et al. Mitochondrial enzyme activity in amyotrophic lateral sclerosis: implications for the role of mitochondria in neuronal cell death. Ann Neurol 1999; 46: 787-90
92. Toghi H, Abe T, Yamazaki K, et al. Remarkable increase in cerebrospinal fluid 3-nitrotyrosine in patients with amyotrophic lateral sclerosis. Ann Neurol 1999; 46: 129-31
93. Bogdanov M, Brown RH, Matson W, et al. Increased oxidative damage to DNA in ALS patients. Free Radic Biol Med 2000; 29: 652-8
94. Aoyama K, Matsubara K, Fujikawa Y, et al. Nitration of manganese superoxide dismutase in cerebrospinal fluids is a marker for peroxynitrite-mediated oxidative stress in neurodegenerative diseases. Ann Neurol 2000; 47: 524-7
95. Bonnefont-Rousselot D, Lacomblez L, Jaudon M, et al. Blood oxidative stress in amyotrophic lateral sclerosis. J Neurol Sci 2000; 178: 57-62
96. Sasaki S, Shibata N, Komori T, et al. iNOS and nitrotyrosine immunoreactivity in amyotrophic lateral sclerosis. Neurosci Lett 2000; 291: 44-8
97. Shibata N, Nagai R, Uchida K, et al. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. Brain Res 2001; 917: 97-104
98. Ferrante RJ, Shinobu LA, Schulz JB, et al. Increased 3-nitrotyrosine and oxidative damage in mice with a copper/zinc superoxide dismutase mutation. Ann Neurol 1997; 42: 326-34

99. Oostveen JA, Gurney ME, Hall ED. Immunocytochemical evidence of spinal cord peroxidation, peroxynitrite formation and astrocyte and microglial activation in the transgenic model of familial amyotrophic lateral sclerosis [abstract]. Soc Neurosci Abs 1997; 23: 13
100. Andrus PK, Andersen PM, Nilsson P, et al. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1998; 71: 2041-8
101. Hall ED, Andrus PK, Oostveen JA, et al. Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. J Neurosci Res 1998; 53: 66-77
102. Liu D, Wen J, Liu J, et al. The roles of free radicles in amyotrophic lateral sclerosis: reactive oxygen species and elevated oxidation of protein, DNA and membrane phospholipids. FASEB J 1999; 13: 2318-28
103. Cha CI, Chung YH, Shin C, et al. Immunocytochemical study on the distribution of nitrotyrosine in the brain of the transgenic mice expressing a human Cu/Zn SOD mutation. Brain Res 2000; 853: 156-61
104. Warita H, Hayashi T, Murakami T, et al. Oxidative damage to mitochondrial DNA in spinal motoneurons of transgenic ALS mice. Brain Res Mol Brain Res 2001; 89: 147-52
105. Dugan LL, Turetsky DM, Du C, et al. Carboxyfullerenes as neuroprotective agents. Proc Natl Acad Sci U S A 1997; 94: 9434-9
106. Poduslo JF, Whelan SL, Curran GL, et al. Therapeutic benefit of polyamine-modified catalase as a scavenger of hydrogen peroxide and nitric oxide in familial amyotrophic lateral sclerosis transgenics. Ann Neurol 2000; 48: 943-7
107. Reinholz MM, Merkle CM, Poduslo JF. Therapeutic benefits of putrescine-modified catalase in a transgenic mouse model of familial amyotrophic lateral sclerosis. Exp Neurol 1999; 159: 204-16
108. Jung C, Rong Y, Doctrow S, et al. Synthetic superoxide dismutase/catalase mimetics reduce oxidative stress and prolong survival in a mouse amyotrophic lateral sclerosis model. Neurosci Lett 2001; 304: 157-60
109. Jaarsma D, Guchelaar HJ, Haasdijk E, et al. The antioxidant N-acetylcysteine does not delay disease onset and death in a transgenic mouse model of amyotrophic lateral sclerosis [abstract]. Ann Neurol 1998; 44: 293
110. Andreassen OA, Dedeoglu A, Klivenyi P, et al. N-acetyl-cysteine improves survival and preserves motor performance in an animal model of familial amyotrophic lateral sclerosis. Neuroreport 2000; 11: 2491-3
111. Hagen TM, Ingersoll RT, Lykkesfeldt J, et al. (R)-alpha-lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. FASEB J 1999; 13: 411-8
112. Andreassen OA, Dedeoglu A, Friedlich A, et al. Effects of an inhibitor of poly(ADP-ribose) polymerase, desmethylselegiline, trientine, and lipoic acid in transgenic ALS mice. Exp Neurol 2001; 168: 419-24
113. Barneoud P, Curet O. Beneficial effects of lysine acetylsalicylate, a soluble salt of aspirin, on motor performance in a transgenic model of amyotrophic lateral sclerosis. Exp Neurol 1999; 155: 243-51
114. Jiang F, DeSilva S, Turnbull J. Beneficial effect of ginseng root in SOD-1 (G93A) transgenic mice. J Neurol Sci 2000; 180: 52-4
115. Facchinetti F, Sasaki M, Cutting FB, et al. Lack of involvement of neuronal nitric oxide synthase in the pathogenesis of a transgenic mouse model of familial amyotrophic lateral sclerosis. Neuroscience 1999; 90: 1483-92
116. Upton-Rice MN, Cudkowicz ME, Mathew RK, et al. Administration of nitric oxide synthase inhibitors does not alter disease course of amyotrophic lateral sclerosis SOD1 mutant transgenic mice. Ann Neurol 1999; 45: 413-4
117. Almer G, Vukosavic S, Romero N, et al. Inducible nitric oxide synthase up-regulation in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1999; 72: 2415-25
118. Ferrante RJ, Klein AM, Dedeoglu A, et al. Therapeutic efficacy of EGb761 (Gingko biloba extract) in a transgenic mouse model of amyotrophic lateral sclerosis. J Mol Neurosci 2001; 17: 89-96
119. Hottinger AF, Fine EG, Gurney ME, et al. The copper chelator d-penicillamine delays onset of disease and extends survival in a transgenic mouse model of familial amyotrophic lateral sclerosis. Eur J Neurosci 1997; 9: 1548-51
120. Nagano S, Ogawa Y, Yanagihara T, et al. Benefit of a combined treatment with trientine and ascorbate in familial amyotrophic lateral sclerosis model mice. Neurosci Lett 1999; 265: 159-62
121. Nagano S, Satoh M, Sumi H, et al. Reduction of metallothioneins promotes the disease expression of familial amyotrophic lateral sclerosis mice in a dose-dependent manner. Eur J Neurosci 2001; 13: 1363-70
122. Subramaniam JR, Lyons WE, Liu J, et al. Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. Nat Neurosci 2002; 5: 301-7
123. Gurney ME. Transgenic models of familial amyotrophic lateral sclerosis. J Neurol 1997; 244 Suppl. 2: S15-20
124. Trotti D, Rolfs A, Danbolt NC, et al. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. Nat Neurosci 1999; 2: 427-33
125. Beal MF. Mitochondrial dysfunction in neurodegenerative diseases. Biochem Biophys Acta 1998; 1366: 211-23
126. Browne SE, Bowling AC, Baik MJ, et al. Metabolic dysfunction in familial, but not sporadic amypotrophic lateral sclerosis. J Neurochem 1998; 71: 281-7
127. Swerdlow RH, Parks JK, Cassarino DS, et al. Mitochondria in sporadic amyotrophic lateral sclerosis. Exp Neurol 1998; 153: 135-42
128. Wiedemann FR, Winkler K, Kuznetsov AV, et al. Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. J Neurol Sci 1998; 156: 65-72
129. Dhaliwal GK, Grewal RP. Mitochondrial DNA deletion mutation levels are elevated in ALS brains. Neuroreport 2000; 11: 2507-9
130. Beal MF. Energetics in the pathogenesis of neurodegenerative diseases. Trends Neurosci 2000; 23: 298-304
131. Swerdlow RH, Parks JK, Pattee G, et al. Role of mitochondria in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1: 185-90
132. Wong PC, Pardo CA, Borchelt DR, et al. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron 1995; 14: 1105-16
133. Kong J, Xu Z. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice carrying a mutant SOD1. J Neurosci 1998; 18: 3241-50
134. Andreassen OA, Ferrante RJ, Klivenyi P, et al. Transgenic ALS mice show increased vulnerability to the mitochondrial toxins MPTP and 3-nitropropionic acid. Exp Neurol 2001; 168: 356-63
135. Andreassen OA, Ferrante RJ, Klivenyi P, et al. Partial deficiency of manganese superoxide dismutase exacerbates a transgenic mouse model of amyotrophic lateral sclerosis. Ann Neurol 2000; 47: 447-55

136. Matthews RT, Yang L, Browne S, et al. Coenzyme Q administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc Natl Acad Sci U S A 1998; 95: 8892-7
137. Beal MF. Coenzyme Q10 administration and its potential for treatment of neurodegenerative diseases. Biofactors 1999; 9: 261-6
138. Kliveny P, Ferrante RJ, Matthews RT, et al. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. Nature Med 1999; 5: 347-50
139. Tarnopolsky MA, Beal MF. Potential for creatine and other therapies targeting cellular energy dysfunction in neurological disorders. Ann Neurol 2001; 49: 561-73
140. Sathasivam S, Ince PG, Shaw PJ. Apoptosis in amyotrophic lateral sclerosis: a review of the evidence. Neuropathol Appl Neurobiol 2001; 27: 257-74
141. Bratton SB, Cohen GM. Apoptotic death sensor: an organelle's alter ego? Trends Pharmacol Sci 2001; 22: 306-15
142. Warita H, Manabe Y, Murakami T, et al. Early decrease of survival signal-related proteins in spinal motor neurons of presymptomatic transgenic mice with a mutant SOD1 gene. Apoptosis 2001; 6: 345-52
143. Guegan C, Vila M, Rosoklija G, et al. Recruitment of the mitochondrial-dependent apoptotic pathway in amyotrophic lateral sclerosis. J Neurosci 2001; 21: 6569-76
144. de la Monte SM, Sohn YK, Ganju N, et al. P53- and CD95-associated apoptosis in neurodegenerative diseases. Lab Invest 1998; 78: 401-11
145. Martin LJ. p53 is abnormally elevated and active in the CNS of patients with amyotrophic lateral sclerosis. Neurobiol Dis 2000; 7: 613-22
146. Mu X, He J, Anderson DW, et al. Altered expression of bcl-2 and bax mRNA in amyotrophic lateral sclerosis spinal cord motor neurons. Ann Neurol 1996; 40: 379-86
147. Tews DS, Goebel HH, Meinck HM. DNA-fragmentation and apoptosis-related proteins of muscle cells in motor neuron disorders. Acta Neurol Scand 1997; 96: 380-6
148. Martin LJ. Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution of a programmed cell death mechanism. J Neuropathol Exp Neurol 1999; 58: 459-71
149. Ekegren T, Grundstrom E, Lindholm D, et al. Upregulation of Bax protein and increased DNA degradation in ALS spinal cord motor neurons. Acta Neurol Scand 1999; 100: 317-21
150. Schoser BG, Wehling S, Blottner D. Cell death and apoptosis-related proteins in muscle biopsies of sporadic amyotrophic lateral sclerosis and polyneuropathy. Muscle Nerve 2001; 24: 1083-9
151. Shinoe T, Wanaka A, Nikaido T, et al. Upregulation of the pro-apoptotic BH3-only peptide harakiri in spinal neurons of amyotrophic lateral sclerosis patients. Neurosci Lett 2001; 313: 153-7
152. Pedersen WA, Luo H, Kruman I, et al. The prostate apoptosis response-4 protein participates in motor neuron degeneration in amyotrophic lateral sclerosis. FASEB J 2000; 14: 913-24
153. Yuan J, Yanker BA. Apoptosis in the nervous system. Nature 2000; 407: 802-9
154. Nicholson DW. From bench to clinic with apoptosis-based therapeutic agents. Nature 2000; 407: 810-6
155. Li M, Ona VO, Guegan C, et al. Functional role of capsase-1 and capsase-3 in an ALS transgenic mouse model. Science 2000; 288: 335-9
156. Ilzecka J, Stelmasiak Z, Dobosz B. Interleukin-1beta converting enzyme/Caspase-1 (ICE/Caspase-1) and soluble APO-1/Fas/CD 95 receptor in amyotrophic lateral sclerosis patients. Acta Neurol Scand 2001; 103: 255-8
157. Migheli A, Atzori C, Piva R, et al. Lack of apoptosis in mice with ALS. Nat Med 1999; 5: 966-7
158. Spooren WP, Hengerer B. DNA laddering and caspase 3-like activity in the spinal cord of a mouse model of familial amyotrophic lateral sclerosis. Cell Mol Biol (Noisy-le-grand) 2000; 46: 63-9
159. Vukosavic S, Dubois-Dauphin M, Romero N, et al. Bax and Bcl-2 interaction in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1999; 73: 2460-8
160. Kostic V, Jackson-Lewis V, de Bilbao F, et al. Bcl-2: prolonging life in a transgenic mouse model of amyotrophic lateral sclerosis. Science 1997; 277: 559-62
161. Azzouz M, Hottinger A, Paterna JC, et al. Increased motorneuron survival and improved neuromusclar function in transgenic ALS mice after intraspinal injection of an adeno-associated virus encoding Bcl-2. Hum Mol Genet 2000; 9: 803-11
162. Pasinelli P, Houseweart MK, Brown RHJ, et al. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu,Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2000; 97: 13901-6
163. Vukosavic S, Stefanis L, Jackson-Lewis V, et al. Delaying caspase activation by Bcl-2: a clue to disease retardation in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci 2000; 20: 9119-25
164. Friedlander RM, Brown RH, Gagliardini V, et al. Inhibition of ICE slows ALS in mice [letter]. Nature 1997; 388: 31
165. Trieu VN, Lui R, Liu X-P, et al. A specific inhibitor of Janus kinase-3 increases survival in a transgenic mouse model of amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2000; 267: 22-5
166. Goodman PA, Niehoff LB, Uckun FM. Role of tyrosine kinases in induction of the c-jun proto-oncogene in irradiated B-lineage lymphoid cells. J Biol Chem 1998; 273: 27028-38
167. Keep M, Elmer E, Fong KS, et al. Intrathecal cyclosporin prolongs survival of late-stage ALS mice. Brain Res 2001; 894: 327-31
168. Chen M, Ona VO, Li M, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med 2000; 6: 797-801
169. Zhu S, Stavrovskaya IG, Drozda M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 2002; 417: 74-8
170. Gonzalez de Aguilar JL, Gordon JW, Rene F, et al. Alteration of the Bcl-x/Bax ratio in a transgenic mouse model of amyotrophic lateral sclerosis: evidence for the implication of the p53 signaling pathway. Neurobiol Dis 2000; 7: 406-15
171. Kuntz C, Kinoshita Y, Beal MF, et al. Absence of p53: no effect in a transgenic mouse model of familial amyotrophic lateral sclerosis. Exp Neurol 2000; 165: 184-90
172. Prudlo J, Koenig J, Graser J, et al. Motor neuron cell death in a mouse model of FALS is not mediated by the p53 cell survival regulator. Brain Res 2000; 879: 183-7
173. Almer G, Guegan C, Teismann P, et al. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. Ann Neurol 2001; 49: 176-85
174. Frank KM, Coccia C, Drachman DB, et al. COX-2 inhibition prolongs survival in atransgenic mouse model of ALS [abstract]. Soc Neurosci Abstracts 2001; 2001: 296
175. Mohajeri MH, Figlewicz DA, Bohn MC. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and dis-

ease progression in a mouse model of familial amyotrophic lateral sclerosis. Hum Gene Ther 1999; 10: 1853-66
176. Bordet T, Lesbordes JC, Rouhani S, et al. Protective effects of cardiotrophin-1 adenoviral gene transfer on neuromuscular degeneration in transgenic ALS mice. Hum Mol Genet 2001; 10: 1925-33
177. Upton-Rice MN, Cudkowicz ME, Warren L, et al. Basic fibroblast growth factor does not prolong survival in a transgenic model of familial amyotrophic lateral sclerosis [abstract]. Ann Neurol 1999; 46: 934
178. Leigh PN, Swash M. Cytoskeletal pathology in motor neuron diseases. Adv Neurol 1991; 56: 115-24
179. Figlewicz DA, Krizus A, Martinoli MG, et al. Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. Hum Mol Genet 1994; 3: 1757-61
180. Al-Chalabi A, Andersen PM, Nilsson P, et al. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. Hum Mol Genet 1999; 8: 157-64
181. Julien JP, Beaulieu JM. Cytoskeletal abnormalities in amyotrophic lateral sclerosis: beneficial or detrimental effects? J Neurol Sci 2000; 180: 7-14
182. Lee MK, Cleveland DW. Neuronal intermediate filaments. Annu Rev Neurosci 1996; 19: 187-217
183. Beaulieu JM, Nguyen MD, Julien JP. Late onset death of motor neurons in mice overexpressing wild-type peripherin. J Cell Biol 1999; 147: 531-44
184. Al-Chalabi A, Leigh PN. Recent advances in amyotrophic lateral sclerosis. Curr Opin Neurol 2000; 13: 397-405
185. Rowland LP. Six important themes in amyotrophic lateral sclerosis (ALS) research, 1999. J Neurol Sci 2000; 180: 2-6
186. Hand CK, Rouleau GA. Familial amyotrophic lateral sclerosis. Muscle Nerve 2002; 25: 135-59
187. Morrison KE. Therapies in amyotrophic lateral sclerosis-beyond riluzole. Curr Opin Pharmacol 2002; 2: 302-9
188. Chen R, Ende N. The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. J Med 2000; 31: 21-30
189. Ende N, Weinstein F, Chen R, et al. Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). Life Sci 2000; 67: 53-9
190. Anneser JM, Gmerek A, Gerkrath J, et al. Immunosuppressant FK506 does not exert beneficial effects in symptomatic G93A superoxide dismutase-1 transgenic mice. Neuroreport 2001; 12: 2663-5
191. Miller RG, Moore D, Young A. Placebo-controlled trial of gabapentin in patients with amyotrophic lateral sclerosis: WALS Study Group. Neurology 1996; 47: 1383-8
192. Miller RG, Moore 2nd DH, Gelinas DF, et al. Phase III randomized trial of gabapentin in patients with amyotrophic lateral sclerosis. Neurology 2001; 56: 843-8
193. Blin O, Pouget J, Aubrespy G, et al. A double-blind placebo-controlled trial of L-threonine in amyotrophic lateral sclerosis. J Neurol 1992; 239: 79-81
194. Testa D, Caraceni T, Fetoni V, et al. Chronic treatment with L-threonine in amyotrophic lateral sclerosis: a pilot study. Clin Neurol Neurosurg 1992; 94: 7-9
195. Group TIAS. Branched-chain amino acids and amyotrophic lateral sclerosis: a treatment failure? Neurology 1993; 43: 2466-70
196. Tandan R, Bromberg MB, Forshew D, et al. A controlled trial of amino acid therapy in amyotrophic lateral sclerosis: I. clinical, functional, and maximum isometric torque data. Neurology 1996; 47: 1220-6
197. Eisen A, Stewart H, Schulzer M, et al. Anti-glutamate therapy in amyotrophic lateral sclerosis: a trial using lamotrigine. Can J Neurol Sci 1993; 20: 297-301
198. Askmark H, Aquilonius SM, Gillberg PG, et al. A pilot trial of dextromethorphan in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 1993; 56: 197-200
199. Gredal O, Werdelin L, Bak S, et al. A clinical trial of dextromethorphan in amyotrophic lateral sclerosis. Acta Neurol Scand 1997; 96: 8-13
200. Blin O, Azulay JP, Desnuelle C, et al. A controlled one-year trial of dextromethorphan in amyotrophic lateral sclerosis. Clin Neuropharmacol 1996; 19: 189-92
201. Miller RG, Shepherd R, Dao H, et al. Controlled trial of nimodipine in amyotrophic lateral sclerosis. Neuromuscul Disord 1996; 6: 101-4
202. Miller RG, Smith SA, Murphy JR, et al. A clinical trial of verapamil in amyotrophic lateral sclerosis. Muscle Nerve 1996; 19: 511-5
203. Louwerse ES, Weverling GJ, Bossuyt PMM. Randomised, double-blind, controlled trial of acetylcysteine in amyotrophic lateral sclerosis. Arch Neurol 1995; 52: 559-64
204. Chio A, Cucatto A, Terreni AA, et al. Reduced glutathione in amyotrophic lateral sclerosis: an open, crossover, randomized trial. Ital J Neurol Sci 1998; 19: 363-6
205. Mazzini L, Testa D, Balzarini C, et al. An open-randomized clinical trial of selegiline in amyotrophic lateral sclerosis. J Neurol 1994; 241: 223-7
206. Jossan SS, Ekblom J, Gudjonsson O, et al. Double blind cross over trial with deprenyl in amyotrophic lateral sclerosis. J Neural Transm Suppl 1994; 41: 237-41
207. Lange DJ, Murphy PL, Diamond B, et al. Selegiline is ineffective in a collaborative double-blind, placebo-controlled trial for treatment of amyotrophic lateral sclerosis. Arch Neurol 1998; 55: 93-6
208. Desnuelle C, Dib M, Garrel C, et al. A double-blind, placebo-controlled randomized clinical trial of alpha-tocopherol (vitamin E) in the treatment of amyotrophic lateral sclerosis: ALS riluzole-tocopherol Study Group. Amyotroph Lateral Scler Other Motor Neuron Disord 2001; 2: 9-18
209. Bradley WG. A phase I/II study of recombinant brain-derived neutrotrophic factor in patients with ALS [abstract]. Ann Neurol 1995; 38: 971
210. Group TBS. A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). Neurology 1999; 52: 1427-33
211. ALS CNTF Treatment Study Group. A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group. Neurology 1996; 46: 1244-9
212. Miller RG, Petajan JH, Bryan WW, et al. A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis. rhCNTF ALS Study Group. Ann Neurol 1996; 39: 256-60
213. Lai EC, Felice KJ, Festoff BW, et al. 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 1997; 49: 1621-30
214. Borasio GD, Robberecht W, Leigh PN, et al. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis: European ALS/IGF-I Study Group. Neurology 1998; 51: 583-6

215. Smith RA, Melmed S, Sherman B, et al. Recombinant growth hormone treatment of amyotrophic lateral sclerosis. Muscle Nerve 1993; 16: 624-33
216. Munsat TL, Taft J, Jackson IM, et al. Intrathecal thyrotropin-releasing hormone does not alter the progressive course of ALS: experience with an intrathecal drug delivery system. Neurology 1992; 42: 1049-53
217. Beghi E, Chio A, Inghilleri M, et al. A randomized controlled trial of recombinant interferon beta-1a in ALS: Italian Amyotrophic Lateral Sclerosis Study Group. Neurology 2000; 54: 469-74
218. Gourie-Devi M, Nalini A, Subbakrishna DK. Temporary amelioration of symptoms with intravenous cyclophosphamide in amyotrophic lateral sclerosis. J Neurol Sci 1997; 150: 167-72
219. Smith SA, Miller RG, Murphy JR, et al. Treatment of ALS with high dose pulse cyclophosphamide. J Neurol Sci 1994; 124 Suppl.: 84-7
220. Drachman DB, Chaudhry V, Cornblath D, et al. Trial of immunosuppression in amyotrophic lateral sclerosis using total lymphoid irradiation. Ann Neurol 1994; 35: 142-50
221. Meucci N, Nobile-Orazio E, Scarlato G. Intravenous immunoglobulin therapy in amyotrophic lateral sclerosis. J Neurol 1996; 243: 117-20
222. Dalakas MC, Stein DP, Otero C, et al. Effect of high-dose intravenous immunoglobulin on amyotrophic lateral sclerosis and multifocal motor neuropathy. Arch Neurol 1994; 51: 861-4
223. Aisen ML, Sevilla D, Edelstein L, et al. A double-blind placebo-controlled study of 3,4-diaminopyridine in amytrophic lateral sclerosis patients on a rehabilitation unit. J Neurol Sci 1996; 138: 93-6
224. Kaji R, Kodama M, Imamura A, et al. Effect of ultrahigh-dose methylcobalamin on compound muscle action potentials in amyotrophic lateral sclerosis: a double-blind controlled study. Muscle Nerve 1998; 21: 1775-8
225. Cudkowicz ME, Warren L, Francis JW, et al. Intrathecal administration of recombinant human superoxide dismutase 1 in amyotrophic lateral sclerosis: a preliminary safety and pharmacokinetic study. Neurology 1997; 49: 213-22
226. Koliatsos VE, Clatterbuck RE, Winslow JW, et al. Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. Neuron 1993; 10: 359-67
227. Kishino A, Ishige Y, Tatsuno T, et al. BDNF prevents and reverses adult rat motor neuron degeneration and induces axonal outgrowth. Exp Neurol 1997; 144: 273-86
228. Mitsumoto H, Ikeda K, Klinkosz B, et al. Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. Science 1994; 265: 1107-10
229. Ikeda K, Klinkosz B, Greene T, et al. Effects of brain-derived neurotrophic factor on motor dysfunction in wobbler mouse motor neuron disease. Ann Neurol 1995; 37: 505-11
230. Tsuzaka K, Ishiyama T, Pioro EP, et al. Role of brain-derived neurotrophic factor in wobbler mouse motor neuron disease. Muscle Nerve 2001; 24: 474-80
231. ALS association website: drug clinical news. Arugen-Regeneron partners discontinuing all cinical development of BDNF. Available from URL: http://www.alsa.org/news/news012801.cfm [Accessed 2002 Nov 20]
232. Sagot Y, Tan SA, Baetge E, Schmalbruch H, Kato AC, Aebischer Polymer encapsulated cell lines genetically engineered to release ciliary neurotrophic factor can slow down progressive motor neuronopathy in the mouse. Eur J Neurosci 1995; 7: 1313-1322
233. Hantai D, Akaaboune M, Lagord C, et al. Beneficial effects of insulin-like growth factor-I on wobbler mouse motoneuron disease. J Neurol Sci 1995; 129 Suppl.: 122-6
234. Armon C, Graves MC, Moses D, et al. Linear estimates of disease progression predict survival in patients with amyotrophic lateral sclerosis. Muscle Nerve 2000; 23: 874-82
235. Duong FH, Warter JM, Poindron P, et al. Effect of the nonpeptide neurotrophic compound SR 57746A on the phenotypic survival of purified mouse motoneurons. Br J Pharmacol 1999; 128: 1385-92
236. Iwasaki Y, Shiojima T, Kinoshita M, et al. SR57746A: a survival factor for motor neurons in vivo. J Neurol Sci 1998; 160 Suppl. 1: S92-6
237. Duong F, Fournier J, Keane PE, et al. The effect of the nonpeptide neurotrophic compound SR 57746A on the progression of the disease state of the pmn mouse. Br J Pharmacol 1998; 124: 811-7
238. Sanofi-Synthelabous US affiliate site: press releases. First half 2000 results. Available from URL: www.sanofi-synthelabous.com/news/20000906.htm [Accessed 2002 Nov 20]
239. Rosenfeld WE. Topiramate: a review of preclinical, pharmacokinetic, and clinical data. Clin Ther 1997; 19: 1294-308
240. Jackson PF, Slusher BS. Design of naaladase inhibitors: a novel neuroprotective strategy. Curr Med Chem 2001; 8: 949-57
241. Mazzini L, Balzarini C, Colombo R, et al. Effects of creatine supplementation on exercise performance and muscular strength in amyotrophic lateral sclerosis: preliminary results. J Neurol Sci 2001; 191: 139-44
242. Bronowska A, Les A, Chilmonczyk Z, et al. Molecular dynamics of buspirone analogues interacting with the 5-HT1A and 5-HT2A serotonin receptors. Bioorg Med Chem 2001; 9: 881-95
243. Neotrofin™. Available from URL: http://www.alsa.org/research/drugdev9.cfm [Accessed 2002 Nov 20]
244. Moulignier A, Moulonguet A, Pialoux G, et al. Reversible ALS-like disorder in HIV infection. Neurology 2001; 57: 995-1001
245. MacGowan DJ, Scelsa SN, Waldron M. An ALS-like syndrome with new HIV infection and complete response to antiretroviral therapy. Neurology 2001; 57: 1094-7
246. Louvel E, Hugon J, Doble A. Therapeutic advances in amyotrophic lateral sclerosis. Trends Pharmacol Sci 1997; 18: 196-203
247. Turner MR, Parton MJ, Leigh PN. Clinical trials in ALS: an overview. Semin Neurol 2001; 21: 167-75
248. Munsat TL. Issues in amyotrophic lateral sclerosis clinical trial design. Adv Neurol 1995; 68: 209-18
249. Meininger V, Salachas F. Review of clinical trials. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000: 389-402
250. Meininger V. Clinical trials: the past, a lesson for the future. Amyotroph Lateral Scler Other Motor Neuron Disord 2001; 2 Suppl. 1: S15-8
251. Mitsumoto H. Clinical trials: present and future. Amyotroph Lateral Scler Other Motor Neuron Disord 2001; 2 Suppl. 1: S10-4
252. Miller RG, Munsat TL, Swash M, et al. Consensus guidelines for the design and implementation of clinical trials in ALS: World Federation of Neurology committee on Research. J Neurol Sci 1999; 169: 2-12
253. World Federation of Neurology. Consensus guidelines for clinical trials in ALS. Available from URL: http://www.wfnals.org/Articles/airlie1998guidelines.htm [Accessed 2002 Nov 20]

254. Gelinas D. Conceptual approach to diagnostic delay in ALS: a United States Perspective. Neurolgy 1999; 53 Suppl. 5: S17-9
255. Dengler R. Current treatment pathways in ALS: a European perspective. Neurology 1999; 53 Suppl. 5: S4-S10
256. Belsh JM. Diagnostic challenges in ALS. Neurology 1999; 53 Suppl. 5: S26-30
257. Brooks BR. What are the implications of early diagnosis?: maintaining optimal health as long as possible. Neurology 1999; 53 Suppl. 5: S43-5
258. Ludolph AC, Riepe MW. Do the benefits of currently available treatments justify early diagnosis and treatment of amyotrophic lateral sclerosis?: arguments against. Neurology 1999; 53: S46-9
259. Cashman NR. Do the benefits of currently available treatments justify early diagnosis and treatment of amyotrophic lateral sclerosis?: arguments for. Neurology 1999; 53: S50-2
260. Brooks BR. Earlier is better: the benefits of early diagnosis. Neurology 1999; 53 Suppl. 5: S53-4
261. Morrison BM, Janssen WG, Gordon JW, et al. Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice. J Comp Neurol 1999; 391: 64-77
262. Williamson TL, Cleveland DW. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. Nat Neurosci 1999; 2: 50-6
263. Hansen S, Ballantyne JP. A quantitative electrophysiological study of motor neurone disease. J Neurol Neurosurg Psychiatry 1978; 41: 773-83
264. Sobue G, Sahashi K, Takahashi A, et al. Degenerating compartment and functioning compartment of motor neurons in ALS: possible process of motor neuron loss. Neurology 1983; 33: 654-7
265. Brooks BR, Sanjak M, Belden D, Juhasz-Poscine K, Waclawik A. Natural history of amyotrophic lateral sclerosis-impairment, disability, handicap. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000: 31-58
266. Shaw PJ, Williams R. Serum and cerebrospinal fluid biochemical markers of ALS. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1 Suppl. 2: S61-7
267. Dib M, Garrel C, Favier A, et al. Can Malondialdehyde be used as a biological marker of progression in neurodegenerative disease? J Neurol 2002; 249: 367-74
268. Karitzky J, Ludolph AC. Imaging and neurochemical markers for diagnosis and disease progression in ALS. J Neurol Sci 2001; 191: 35-41
269. Suhy J, Miller RG, Rule R, et al. Early detection and longitudinal changes in amyotrophic lateral sclerosis by (1)H MRSI. Neurology 2002; 58: 773-9
270. Bermejo P, Gomez-Serranillos P, Santos J, et al. Determination of malonaldehyde in Alzheimer's disease: a comparative study of high-performance liquid chromatography and thiobarbituric test. Gerontology 1997; 43: 218-22
271. Markesbery WR. The role of oxidative stress in Alzheimer disease. Arch Neurol 1999; 56: 1449-52
272. Sayre LM, Smith MA, Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. Curr Med Chem 2001; 8: 721-38
273. Andersen PM, Morita M, Brown RHJ. Genetics of amyotrophic lateral sclerosis: an overview. In: Brown Jr RH, Meininger V, Swash M, editors. Amyotrophic lateral sclerosis. London: Martin Dunitz, 2000: 145-160
274. Vastag B. Stem cells step closer to the clinic: paralysis partially reversed in rats with ALS-like disease. JAMA 2001; 285: 1691-3

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