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Central & Peripheral Nervous Systems

Emerging disease-modifying therapies for the treatment of motor neuron disease/amyotrophic lateral sclerosis

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It has been > 130 years since the first description of the upper and lower motor neuron disease called amyotrophic lateral sclerosis (ALS). Sadly, there has been little change in the long interval over which this disease is diagnosed, or in its poor prognosis. Significant gains have been made, however, in understanding its pathophysiology and in symptomatic care. Disease-causing mutations have been identified and used to create animal models. Other identified mutations may increase susceptibility and cause disease only in a particular environment and at a particular age. A number of 'downstream' molecular pathways have been implicated, including transcriptional disturbances, protein aggregation, excitotoxicity, mitochondrial dysfunction, oxidative stress, neuroinflammation, cytoskeletal and axonal transport derangements, growth factor dysregulation and apoptosis. This knowledge has led to an impressive pipeline of candidate therapies that offer hope for finally being able to alter ALS disease progression. These are described and prioritized herein, and suggestions are offered for efficiently sifting through them.

Keywords: ALS, amyotrophic lateral sclerosis, MND, motor neuron disease, neuroprotection

Expert Opin. Emerging Drugs (2007) 12(2):229-252

1. Background

In a strict anatomic sense, motor neuron disease (MND) refers to a heterogeneous group of conditions characterized by degeneration of lower motor neurons (those that have cell bodies in the cranial nerve nuclei or in the anterior horn of the spinal cord and synapse directly on muscle) and/or upper motor neurons (those that have cell bodies in the brain and synapse on lower motor neurons). Commonly though, MND is used to refer to a more specific neurodegenerative disease involving both populations of motor neurons [1]. This more specific disease is also called amyotrophic lateral sclerosis (ALS), a preferred term that is used for the remainder of this paper.

Jean Martin Charcot gave the first comprehensive description of ALS in 1874 [2]; unfortunately, the diagnostic interval and clinical course of this disease have changed little since. As lower motor neurons degenerate and die, patients with ALS develop widespread muscle atrophy, fasciculations and weakness (hence Charcot's term 'amyotrophic'). As upper motor neurons die, the spinal cord becomes sclerotic, in particular the lateral corticospinal tracts (hence Charcot's term 'lateral sclerosis'), and spastic tone and hyper-reflexia ensue. Diagnostic certainty increases with the number of body parts manifesting these 'lower and upper motor neuron signs' [3]; as a result, there can be considerable delay between initial symptoms and diagnosis [4-6]. ALS most often starts insidiously, with painless, asymmetric weakness in distal

Table 1. FALS subtypes [18-20,201].

| Name | Gene | Function | Locus | Inheritance | Phenotype |
|--------------------|---------------------------|--|---------|-------------|---|
| FALS1 [135] | <i>SOD1</i> | Catalyzes reduction of superoxide anions to oxygen and hydrogen peroxide | 21q22.1 | AD, AR | Similar to SALS, but variable onset and progression |
| FALS2 [21,22] | <i>Alsin</i> | Binds G proteins; possibly endosomal trafficking | 2q33 | AR | Childhood onset, slow progression |
| FALS3 [136] | ? | | 18q21 | AD | Similar to SALS |
| FALS4 [137] | <i>Senataxin</i> | RNA and DNA helicase; DNA repair | 9q34 | AD | Childhood onset, slow progression |
| FALS5 [23] | ? | | 15q15 | AR | Childhood onset, slow progression |
| FALS6 [138] | ? | | 16q12 | AD | Similar to SALS, but variable progression |
| FALS7 [139] | ? | | 20p13 | AD | Similar to SALS |
| FALS8 [140] | <i>VAPB</i> | Intracellular membrane protein involved in vesicle transport | 20q13 | AD | Some similar to SALS; others primarily lower motor neuron |
| FALS-ANG [141,142] | <i>Angiogenin</i> | Mediator of blood vessel formation | 14q11 | AD | Similar to SALS |
| FALS-NFH [95] | Neurofilament heavy chain | Contributes to axonal structure and caliber | 22q12 | AD | Variable |
| FALS-DCTN1 [96] | <i>Dynactin</i> | Microtubule-based motor protein | 2p13 | AD | Variable |
| FALS-FTD [143,144] | ? | | 9p13 | AD | SALS with frontotemporal dementia |
| FALS-FTDP [145] | <i>Tau</i> | Stabilizes microtubules and regulate their assembly | 17q21 | AD | Amyotrophy with frontotemporal dementia, and parkinsonism |

AD: Autosomal dominant; AR: Autosomal recessive; FALS: Familial amyotrophic lateral sclerosis; SALS: Sporadic amyotrophic lateral sclerosis.

limb muscles: a ‘bulbar onset’ in which the disease starts in speech and swallowing muscles occurs in ~ 30% of cases [7-10]. Unfortunately, relentless progression is the rule, with eventual limb paralysis, and loss of speech, swallowing and breathing functions. Occasionally, there will be prominent frontotemporal dementia [11] or, more rarely, Parkinsonism [12]. On average, patients with ALS will require a feeding tube, tracheostomy and mechanical ventilator to sustain life beyond 3 years of disease onset [13,14], although occasional patients may survive decades without these interventions [15]. ALS is a rare disease across most of the world, with an incidence of 1 – 2 per 100,000 per year (similar to that of multiple sclerosis [16]), and a prevalence of 1/20,000 [7-10]. These figures understate the impact of ALS, however, when one considers that the lifetime risk of developing the disease is roughly 1 in 400 [17].

Between 3 and 10% of patients with ALS have a family history of this disease [18], and are thus referred to as having ‘familial’ ALS (FALS). Several FALS-causing mutations have now been described (Table 1 [18-20,201]). These are usually transmissible via autosomal dominant inheritance, although recessive [21-23] and X-linked [24] patterns have also been described. Table 1 lists the known FALS subtypes. Mutations in some FALS genes, in particular many of those in the *SOD1* gene, typically cause a phenotype that is clinically indistinguishable from sporadic ALS (SALS). Mutations in other FALS genes,

however, produce disease in children (e.g., FALS2), disease that progresses much more slowly (e.g., FALS2, FALS4), or disease that affects primarily lower motor neurons (e.g., some FALS8 families or one FALS-DCTN1 family). To further complicate matters, different specific mutations within a given gene can lead to different phenotypes; for example, specific mutations in the *SOD1* gene can lead to a limb or bulbar onset, or to a faster or slower progressing disease [18]. Beyond FALS1, which accounts for 2% of all ALS [18-20], most of these ALS subtypes are incredibly rare conditions; in some cases, they occur in single families (e.g., FALS3, FALS7). Nonetheless, the study of FALS has been very useful. The identified disease-causing mutations point to specific upstream processes that may be relevant to the cause of SALS, especially with FALS subtypes that have a similar clinical profile to SALS (FALS1). Animal models of FALS, in particular FALS1, have yielded clues to potential downstream disease mechanisms (Table 2) that may also be part of SALS pathophysiology. FALS1 animal models are being used as one of several preclinical assays to screen drugs for human ALS trials [25,26], although it should be noted that these animals do not acquire, nor manifest, disease as humans do. They have multiple copies of the mutant gene, rather than just one as humans do. Their motor system is not organized in the same fashion as humans, and upper motor neuron signs are not as obvious.

Table 2. Putative downstream ALS mechanisms.

| Mechanism | References supporting involvement in FALS | References supporting involvement in SALS |
|---|---|---|
| Genetic and transcriptional disturbances | [68-70] | [29-32] |
| Protein aggregation | [146-148] | [146-148] |
| Excitotoxicity | [79] | [79,84] |
| Mitochondrial dysfunction | [87] | [87] |
| Oxidative stress | [71] | [71] |
| Neuroinflammation | [90] | [90] |
| Cytoskeletal derangements/impaired axonal transport | [94,98] | [94,98] |
| Growth factor dysregulation | [99] | [99] |
| Apoptosis | [102,103] | [104] |

ALS: Amyotrophic lateral sclerosis; FALS: Familial ALS; SALS: Sporadic ALS.

Rare patients with SALS will have one of the mutations described in Table 1 [27]. For the rest, and thus the great majority of all patients with ALS, the cause is unknown. Many believe SALS is caused by a combination of a genetic susceptibility and an environmental exposure [28]. Putative susceptibility genes have been identified using candidate-gene approaches [28], and more recent genome-wide association studies [29-32], but so far none appears constant across multiple studies and/or populations. A cluster of markedly increased ALS prevalence once occurred on Guam, but has recently normalized [33]. Here, specific Tau single-nucleotide polymorphisms (SNPs) may influence risk, perhaps by regulating *MAPT* expression [34]. A lively debate over the environmental cause of the Guamanian cluster continues in the literature, with recent evidence for and against the consumption of flying foxes, which may be concentrators of a neurotoxin found in cycad beans [35,36]. Smaller, still unverified, clusters appear to exist in Italian soccer players [37] and American veterans [38], especially those deployed to the Persian Gulf in the early 1990s [39,40]. Theories about the exact exposures responsible for these clusters abound, but none have yet been proven [41]. Case-control studies have also suggested environmental risks, but these are not consistent across different studies and/or populations [41]. The biology of ageing may interact as a third influence on disease development, as SALS becomes much more common with ageing [41,42]. Unfortunately, there is no animal model of SALS; thus, compared with FALS, there is less evidence for specific 'downstream' mechanistic events. At first glance, mechanisms that appear relevant to FALS also appear to play a role in SALS (Table 2). However, the fact that several drugs that slowed disease progression in FALS animals subsequently failed in trials of SALS patients [43-47] may suggest important mechanistic differences between these types of ALS.

2. Medical need/existing treatment

Despite numerous clinical trials over the past 50 years [48], only one medication has emerged that can prolong survival in patients with ALS: riluzole (2-amino-6-[trifluoromethoxy] benzothiazole; RP-54274). This drug, which was developed as an anticonvulsant, inhibits the presynaptic release of glutamate, and modulates sodium channels [49], potassium channels [50] and calcium currents [51]. In a mouse model of FALS1, riluzole delays disease onset and slows decline in motor function [52]. Two double-blind, placebo-controlled trials in humans with ALS show that treatment with 100 mg/day is associated with a statistically significant improvement in tracheostomy-free survival [53,54]. The effect size, however, is small: median tracheostomy-free survival improves by ~ 60 days. Furthermore, no consistent benefit is seen on secondary measures such as manual muscle testing and functional scores. Riluzole is generally well tolerated, with the most common side effects being gastrointestinal disorders, dizziness and asthenia. Elevations in liver enzymes may occur; thus, regular monitoring of liver function is required. Serious drug related side effects occur in < 2% of patients [55,56]. The cost of the medication is high, presently ~ US\$900/month [202].

In recent years, advances in supportive care have eased the symptom burden of ALS for patients and caregivers, and allowed some preservation of quality of life through the disease [57,58]. Recent reviews are available to guide clinicians on optimal methods for breaking the news, symptomatic therapy, nutrition and feeding tubes, managing respiratory therapy, palliative care and advanced directives [59-61]. Some supportive interventions such as feeding tubes [62], bilevel positive airway pressure [63], and even multidisciplinary care teams [64] may prolong survival in patients with ALS. Unfortunately, although potentially greater than riluzole, the size of these survival

benefits is still small. Thus, there is an urgent need for novel therapies that can meaningfully alter the development and/or course of ALS.

3. Current research goals

The research goal addressed herein is to use existing knowledge of ALS pathophysiology to generate new therapies that can meaningfully alter the development and/or course of ALS.

4. Scientific rationale

Studies on FALS (in particular FALS1 animal models) and, to a lesser extent, SALS have suggested at least 10 promising target pathways for ALS therapeutic agents. There is likely to be significant interaction between these. In this section, evidence that each target pathway occurs and is relevant in FALS and SALS is reviewed. The proposed upstream events leading to each target are also described and related to what is known about the mechanism or downstream events by which the target may lead to motor neuron degeneration, although it is not always clear whether these events occur in series or in parallel.

4.1 Disease-causing genes

As reviewed in Table 1, in recent years, a number of putative disease-causing mutations have been mapped out in FALS families [18-20]. Rare patients with SALS will also have one of these mutations [27]. Inserting these mutations into animals can result in an ALS phenotype [18-20,25,26]. Turning down the production of FALS gene products via RNA interference (RNAi) technology or antisense oligonucleotides can slow disease progression in animals [20].

4.1.1 Upstream events

These upstream events are likely to be at the top of the cascade. That is, they are the primary, most upstream event for patients in whom they occur. They are either inherited or arise as spontaneous mutations.

4.1.2 Downstream events

The most common and best understood of the ALS-causing mutations occur in the *SOD1* gene [65]. The product of this gene normally catalyzes the reduction of superoxide anions to oxygen and hydrogen peroxide. More than 100 different *SOD1* point mutations can cause an ALS phenotype [65]. These mutations do not appear to cause disease by a loss in function, but rather a toxic gain in function for the mutant SOD1 (mSOD1) protein. Evidence for this comes from the observation that mSOD1 catalytic potency does not correlate with disease in mice or humans, and that *SOD1*-null mice do not develop ALS [65]. Very recent work reveals more surprises. First, expression of mSOD1 must occur within both neuronal and non-neuronal cells in order for ALS to ensue, at least in mouse models [26,66]. Second, mSOD1 expression within different cell types may be responsible for different phases of disease [26].

For example, selective deletion of *mSOD1* within mouse motor neurons appears to slow disease onset and an early phase of progression; selective deletion within microglia and macrophages had little effect on early disease, but strikingly slows later disease progression [67]. Beyond these important observations, exactly how mSOD1 leads to selective motor neuron degeneration is still unknown; it probably acts through one or more of the following mechanisms: aggregation, facilitation of excitotoxicity, mitochondrial damage, oxidative stress, or immune dysregulation, or inducing a pro-apoptotic state.

4.2 Other genetic and transcriptional disturbances

Beyond the disease-causing mutations described in Section 4.1, candidate gene approaches and more recent genome-wide screens have shown differences in gene structure and/or expression in FALS animal models [68-70] and in patients with SALS [29-32] compared with healthy controls.

4.2.1 Upstream events

Mutations or polymorphisms in susceptibility genes may be inherited or spontaneous. Expression of mSOD1 in a motor neuron cell line can alter gene transcription [71], although the mechanism by which this occurs is not clear. The regulation of transcription is complex. In part, it is regulated in via histones, which ultimately affect the actions of RNA polymerase. Histones are, in turn, regulated by covalent modifications such as acetylation. In this light, the finding of hypoacetylation in FALS1 mice [72] may be relevant and may provide a specific pathway for reversing transcriptional dysregulation. Indeed, sodium phenylbutyrate, a histone deacetylase inhibitor, appears to reverse some of the transcriptional dysregulation in FALS1 animals, and prolongs survival [72].

4.2.2 Downstream events

In some cases, there are obvious mechanisms by which the altered genes might affect downstream ALS pathophysiology. For example, downregulation of genes associated with the cytoskeleton and axonal transport, and upregulation of genes associated with cell-death pathways in patients with SALS [31,32] could play into the mechanisms described below. In others, the relationship remains unclear. In a genome-wide association study of 276 American SALS patients and 276 American neurologically normal controls, the minor allele of 34 SNPs was associated with altered risk of developing ALS. Genes involved in the regulation and function of neuronal cytoarchitecture were over-represented in this list, thus suggesting that such proteins may be relevant to the pathogenesis of motor neuron degeneration [30].

4.3 Protein aggregation

Intracellular, cytoplasmic protein aggregates are a pathologic hallmark of FALS, of animal models of FALS, and of SALS [20,26,73]. These include: ubiquitinated inclusions, which are seen in a variety of neurodegenerative disorders; Bunina bodies, which stain for the protease inhibitor cystatin C and

are unique to ALS; and phosphorylated and non-phosphorylated neurofilament inclusions, which can be associated with immunoreactive SOD1, even in SALS [20,26]. Other than mere appearance, multiple lines of evidence suggest that protein aggregates may play a key role in ALS. First, there is the spatial correlation: aggregates are mainly found within motor neurons, and in some cases, within the astrocytes surrounding them [20,26,73]. In cell mSOD1 culture models, the propensity to form aggregates does not occur in hippocampal or dorsal root ganglia neurons, only in motor neurons [74]. Second there is the temporal correlation: aggregates in FALS1 animal models are evident prior to any other measure of disease [75,76]. The levels of mSOD1 expression required to produce disease in FALS1 animal models coincide with levels required to produce SOD1 aggregates [77]. Finally, and perhaps most impressively, there is the correlation between SOD1 stability and disease progression: in FALS1 patients, mutations leading to the most unstable mSOD1 are associated with fastest disease progression [78].

4.3.1 Upstream events

There is evidence that the structure of some mSOD1 proteins is unstable and prone to spontaneous misfolding and aggregation [1]. For other specific mutations, oxidative stress appears to increase the likelihood of mSOD1 aggregation [71]. Decreased heat-shock protein chaperone activity may further aggravate matters [26], and provides a specific pathway for attempting to counter act aggregation. In support, arimoclolomol, a co-inducer of heat-shock proteins, slows disease progression in FALS1 mice [20].

4.3.2 Downstream events

Aggregates may induce disease by overwhelming the cells' chaperone or ubiquitin-proteasome machinery [26], loss of other protein function through co-aggregation [26], impairing mitochondrial function (see below) and impairing axonal transport (see below). On the other hand, it is also possible that aggregates have a protective effect within neurons by decreasing the surface area of components exposed to the cytosol.

4.4 Excitotoxicity

Excitotoxic injury occurs when glutamate receptors (e.g., NMDA, AMPA) are inappropriately activated, resulting in excessive entry of intracellular calcium into neurons [1,79]. Several observations suggest that this may be part of FALS and SALS pathophysiology. Elevated levels of extracellular glutamate have been found in FALS1 mice [80], and in the cerebrospinal fluid of patients with SALS [81]. The spinal fluid of patients with SALS is toxic to cortical neurons in culture, and this effect can be blocked by AMPA antagonists [82]. Other specific forms of motor neuron disease in humans, such as lathyrism, can be caused by ingestion of glutamate agonists [83]. Finally, riluzole, the only medication that prolongs survival in FALS models and patients with SALS, acts partly by inhibiting the release of glutamate [53,54].

4.4.1 Upstream events

There appears to be a loss of a critical astroglial glutamate transporter called EAAT2/GLT1 in FALS1 animals and in some patients with either FALS or SALS [84,85]. Inducing a loss of this transporter via antisense oligonucleotides can result in motor neuron degeneration in cell cultures or in rats [79]. Cross-breeding FALS1 mice with those that overexpress EAAT2/GLT1 can delay disease onset [79]. Administration of ceftriaxone to the mSOD1 ALS rodent model increases EAAT2 mRNA and protein levels and prolongs survival [85]. Reactive oxygen species that occur in states of oxidative stress (see below) may further act to potentiate excitotoxicity, as glutamate uptake via glial and neuronal transporters is reduced in their presence [71].

4.4.2 Downstream events

Chronic, even mild, elevations in intracellular calcium can result in cell death [79]. In this light, motor neurons appear to be particularly sensitive to excitotoxicity for two reasons. First, motor neurons have a high proportion of AMPA receptors that are deficient in a particular subunit called GluR2; as a result these receptors are especially calcium permeable [79]. FALS1 models made to express high levels of calcium-impermeable GluR2 subunits have improved lifespan and preserved motor function [86]. A second reason for the selective vulnerability of motor neurons here may be their poor capacity for buffering intracellular calcium due to low expression of calcium-buffering proteins [79]. As a result, mitochondria are forced to take on a greater calcium-buffering role, which can lead to their own depolarization, impairment in energy production and generation of reactive oxygen species.

4.5 Mitochondrial dysfunction

There are morphological abnormalities in the mitochondria of humans with SALS [87]. Similar abnormalities occur in FALS1 models, and the appearance of them is temporally correlated with the onset of weakness [88]. Mitochondria in FALS1 models are not functioning normally in terms of energy production or calcium buffering and are spilling some of their contents into the cytoplasm [87].

4.5.1 Upstream events

The cause of the mitochondrial dysfunction remains unknown. One possibility is accumulation of aggregated proteins (such as mSOD1) within the mitochondria where they may clog protein translocation machinery and/or tie up antiapoptotic proteins such as Bcl-2 [87]. Other possibilities include mutations in mitochondrial DNA, and calcium overload occurring as a result of increased calcium-buffering requirements within neurons.

4.5.2 Downstream events

Dysfunctional mitochondria may result in impaired calcium buffering within neurons, impaired energy production, spillage of reactive oxygen species into the cytoplasm (which, in turn, creates oxidative stress), and release of cystatin C into

the cytoplasm, which may activate the apoptosis cascade. In FALS1 mice, administration of creatine, which may enhance mitochondrial energy production and reduce permeability by blocking a transition pore, prolongs survival [20]; unfortunately, at the dosages studied thus far, creatine does not seem to work in humans with ALS [20]. Minocycline, a drug that may block release of cytochrome *c* into the cytoplasm, produces a similar benefit in FALS1 models [20].

4.6 Oxidative stress

Immunohistochemical studies have found markers of increased oxidative stress in patients with FALS and SALS compared with controls. These include elevated protein carbonyl levels, increased 3-nitrotyrosine levels, increased levels of 8-hydroxy-2'-deoxyguanosine (indicative of oxidative damage to DNA), increased levels of 4-hydroxynonenal (indicative of lipid peroxidation), elevated levels of ascorbate free radical and elevated 3-nitrotyrosine [71]. A number of antioxidants, including coenzyme Q₁₀, AEOL10150, C3 and a green tea flavonoid, can prolong survival in FALS1 animal models [71].

4.6.1 Upstream events

As mentioned in Section 4.2.1, mSOD1 can alter gene expression in a motor neuron cell line; in particular, genes involved in antioxidant responses show decreased expression [89]. Another possibility is that part of the toxic gain in function of mSOD1 is to act as a peroxidase in reverse of its normal function, or to react with peroxynitrate to cause tyrosine nitration [71]. Mitochondrial dysfunction may contribute by spilling reactive oxygen species into the cytoplasm.

4.6.2 Downstream events

There is evidence that some *mSOD1* products, in particular those from A4V and G93A mutations, are more prone to aggregation under conditions of oxidative stress [71]. Reactive oxygen species may impair calcium buffering, thus potentiating excitotoxicity and accelerating mitochondrial damage [71]. There is even evidence that maintenance of the neurofilament network is impaired under conditions of oxidative stress [71].

4.7 Immune dysregulation

There is morphological and biochemical evidence that microglia (the resident macrophages of the CNS) are increased in numbers and are 'activated' in FALS1 animals and patients with SALS [90]. Microglial activation appears to occur prior to, or at the onset of, disease in FALS1 animals [91]. Selectively diminishing mSOD1 activity in microglia and peripheral macrophages significantly slows disease progression in FALS1 animals [67]. Minocycline, which can inhibit microglial activation (as well as favorably affecting mitochondrial function), prolongs survival in FALS1 animals [20,90]. COX-2 activity is also increased in FALS1 models and in the spinal cords of patients with SALS [20,47]. In FALS1 models, COX-2 expression temporally parallels motor neuron loss. Treatment of these animals with celecoxib, a COX-2 inhibitor, prolongs their survival as

well [92]; unfortunately, a similar effect was not observed in human trials [20,47]. Activated T cells, antineuronal antibodies and antibodies against voltage-gated calcium channels have all been found in patients with SALS [1].

4.7.1 Upstream events

Pro-inflammatory genes are upregulated in FALS1 models; these include COX-2, TNF- α and IL-1B [20]. Recent work has shown that mSOD1 binds to chromogranins in secretory vesicles in neurons and glial; when released into the extracellular space, these appear to provoke microglial activation [93]. Microglia derived from FALS1 mice may have increased cytotoxic abilities even when removed and placed in cell cultures [90].

4.7.2 Downstream events

Activated microglia can induce apoptosis by releasing products such as nitric oxide, Fas ligand or TNF- α [90]. COX-2 is a key enzyme in the synthesis of prostenoids, which are potent mediators of downstream inflammatory cascades [20]. Antibodies against voltage-gated calcium channels may disrupt the regulation of intracellular calcium [1], leading to mitochondrial and cytoskeletal damage.

4.8 Cytoskeletal and axonal transport defects

The largest caliber, most neurofilament-rich lower motor neurons are the most severely affected in animal models of FALS1 and in patients with SALS [20,26,94]. Neurofilaments and peripherin are found within intracellular inclusions in FALS and SALS [94]. Neurofilament accumulations and defects in axonal transport occur early in the course of disease in FALS1 models [94]. Transgenic mice that express a mutation in a neurofilament gene [94], overexpress peripherin [1], or have a mutation in the dynein/dynactin axonal transport motor [26] will develop motor neuron disease (although not classical ALS). Genetically removing neurofilaments or removing their crosslinking regions can prolong survival in FALS1 animals [26].

4.8.1 Upstream events

Mutations in neurofilaments have been observed in rare patients with FALS and with SALS [95]. Mutations in peripherin have been observed in rare patients with SALS, and mutations in the dynein/dynactin complex have been observed in rare patients with SALS as well [94,96]. Inflammatory cytokines may be able to increase peripherin levels [97]. Glutamate exposure leads to increased phosphorylation of neurofilaments and slows axonal transport [94,98].

4.8.2 Downstream events

Neurofilament disorganization, crosslinking and aggregation could disrupt axonal transport, resulting in 'strangulation' of the axons [1].

4.9 Growth factor dysregulation

Brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, and IGF-1 can each support the growth and survival

of developing motor neurons *in vitro*, and can promote motor neuron survival in animal models; unfortunately, trials of each of these failed to show any benefit for SALS patients [99]. VEGF can also promote motor neuron survival in animal models. VEGF has received much recent attention because, unlike the other growth factors mentioned, patients with FALS and SALS have a significant reduction in plasma levels of VEGF [99]. Case-control studies on patients with FALS and SALS in Belgium and the UK reveal that variations in the *VEGF* gene associated with particularly low plasma levels of VEGF are also associated with ALS [100]. Mice expressing a mutant VEGF develop motor neuron disease; administration of VEGF to FALS mice or rats can delay disease onset and prolong survival [100].

4.9.1 Upstream events

Variations in the *VEGF* gene that may confer susceptibility to ALS could potentially be inherited or acquired as new mutations.

4.9.2 Downstream events

Theoretically, reduced VEGF levels could contribute to motor neuron death by ischemia. Extracellular chromogranins [93], which are antiangiogenic, could exacerbate this mechanism [101].

4.10 Apoptosis

Apoptosis, or programmed cell death, is a terminal sequence of events initiated by cysteine-aspartate proteases called caspases. Histological and biochemical evidence of apoptosis has been found in FALS1 models [102,103]. There is some similar evidence that it also occurs in SALS patients [104], although this is still being debated [105]. Caspase inhibitors can extend survival in FALS1 mice [1].

4.10.1 Upstream events

Mutant proteins including mSOD1 may sequester anti-apoptotic proteins such as Bcl-2. Transcriptional dysregulation could result in overexpression of pro-apoptotic genes and underexpression of antiapoptotic genes. Mitochondrial spillage of cystatin C and microglial activation can activate the caspase cascade.

4.10.2 Downstream events

Apoptosis is likely to be the final sequence of events in motor neuron death. This has not precluded attempts to slow ALS progression by targeting this downstream pathway.

5. Competitive environment

A diverse group of potential therapeutic agents have been brought into the ALS pipeline based on the above mechanisms. These are reviewed in alphabetical order, along with an opinion on the advantages and disadvantages of each (Table 3).

5.1 AEOL-10150

AEOL-10150 (Aeolus Pharmaceuticals) is a manganese porphyrin compound that acts as a catalytic antioxidant. When administered at symptom onset in FALS1 mice, it prolongs survival [20,106,203]. It was recently administered subcutaneously as a single dose to patients with ALS; in this design it was safe and well tolerated [203]. A multiple dose Phase II safety study is underway. Even when administered at symptom onset, most preclinical studies with this compound show a large effect size in FALS mice. However, it hits a mechanism that is likely to be downstream in ALS pathophysiology, and there are limited safety data in humans with ALS so far.

5.2 AM-1241

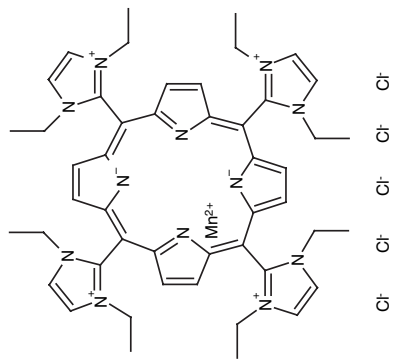
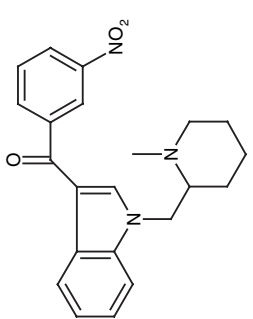
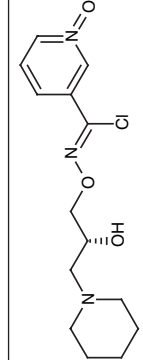
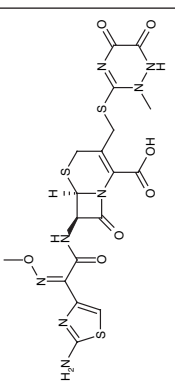
AM-1241 is a selective agonist at the CB2 cannabinoid receptor, and is reported to have anti-inflammatory properties. Injections at symptom onset can dramatically prolong survival in FALS1 mice [107]. The size of the survival benefit in FALS1 mice can be large, but inflammation may be a downstream mechanism; there is no human experience with this compound and administration is likely to be parenteral (it was delivered via intraperitoneal injection in the animal study).

5.3 Antisense oligonucleotide to mSOD1

Recently, an antisense oligonucleotide to mSOD1 from Isis Pharmaceuticals was administered intraventricularly to FALS1 rats, beginning 1 month prior to disease onset; levels of mSOD1 were widely reduced and survival was increased [108]. This particular compound has not been tested in humans. It hits a mechanism that is likely to be at or near the top of FALS SOD1 pathophysiology and may be preferable to RNAi due to stability and lack of 'off target' deleterious effects [108]. It may be a targeted therapy for mSOD1-mediated disease. However, it is unclear whether this mechanism plays a role in most cases of SALS. There are limited safety data in humans with ALS. Fomiviresen is an antisense oligonucleotide drug approved by the FDA, but administration is limited to ocular delivery for cytomegalovirus retinitis rather than systemically. For treatment of ALS, intrathecal or intraventricular administration may be required and little is known about the systemic effects of administering this class of agents in this manner.

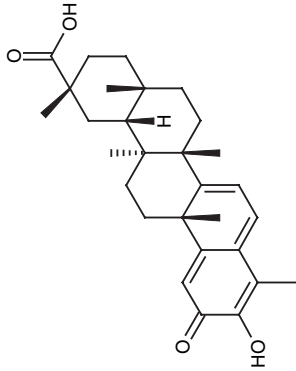
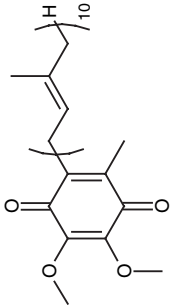
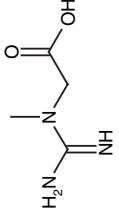
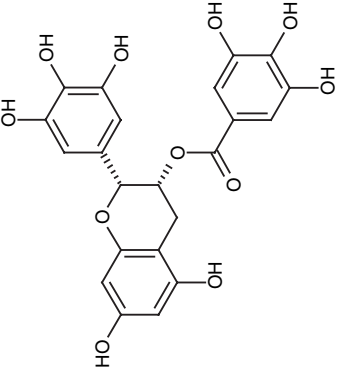
5.4 Arimoclomol

Arimoclomol (CytRx) is co-inducer of heat-shock proteins that prolongs survival in FALS mice; the effect on survival occurs whether the drug is started before or at symptom onset [20,106]. It was recently administered orally at different dosages to 84 patients with ALS over 12 weeks; it appears safe and well tolerated and crosses the blood-brain barrier [109]. A Phase IIb study is about to start. By inducing heat-shock proteins, this drug may augment their ability to chaperone protein aggregates; protein aggregation is likely to be relevant in FALS and SALS, and likely to be upstream. Oral dosing, cerebrospinal fluid penetration and excellent safety data in humans with ALS are pluses. However, there is no proof that

| Table 3. ALS competitive environment. | | | | | | |
|---------------------------------------|---------|---|----------------------|--------------------|--------------------------------------|--|
| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism | |
| AEOL-10150 | Aeolus |  | None | Phase Ib | Antioxidant | |
| AM-1241 | NA |  | None | Preclinical | Anti-inflammatory | |
| Antisense oligonucleotide to mSOD1 | Isis | NA | None | Preclinical | Decrease production of mSOD1 protein | |
| Arimoclomol | CytRx |  | None | Phase IIb | Heat-shock protein inducer | |
| Ceftriaxone | Generic |  | Antibiotic | Phase I – III | Increases EAAT2/GLT1 activity | |

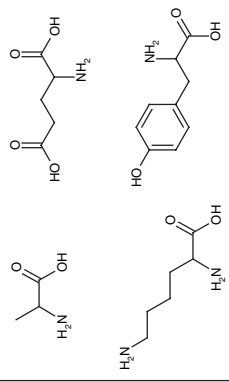
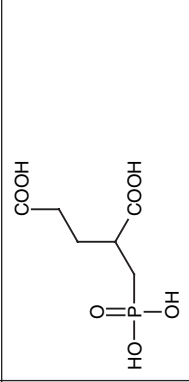
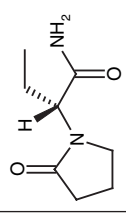
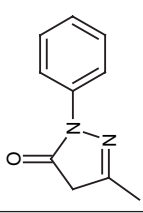
ALS: Amyotrophic lateral sclerosis; AAV: Adeno-associated virus; BDNF: Brain-derived neurotrophic factor; ECGC: Catechin (-) epigallocatechin-3-gallate; EPO: Recombinant human erythropoietin; HGF: Recombinant human hepatocyte growth factor; IGF-I: Recombinant human insulin-like growth factor; mSOD: Mutant SOD; NAALADase: N-acetylated, α -linked acidic dipeptidase; RNAi: RNA interference.

Table 3. ALS competitive environment (continued).

| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism |
|--------------------------|-----------------|--|----------------------------------|--------------------|--|
| Celastrrol | Schering-Plough |  | None | Preclinical | Suppresses TNF- α , IL-1 β , nitric oxide; heat-shock protein inducer |
| Coenzyme Q ₁₀ | Generic |  | None | Phase II | Antioxidant; facilitates mitochondrial respiration |
| Creatine (high dose) | Generic |  | None | Phase II | Antioxidant; stabilizes mitochondrial transition pore; facilitates mitochondrial ATP synthesis |
| C3 | Merck, C Sixty | | None | Preclinical | Antioxidant |
| EGCG | Generic |  | None | Preclinical | Antioxidant |
| EPO | Multiple | NA | Accelerates recovery from anemia | Phase II completed | Augments BDNF release; anti-inflammatory; antiapoptotic |

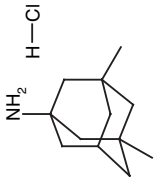
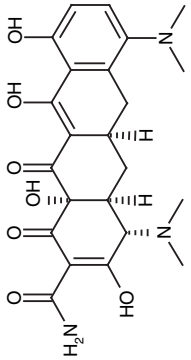
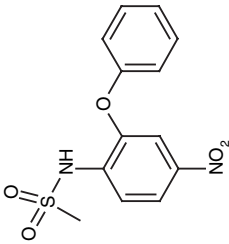
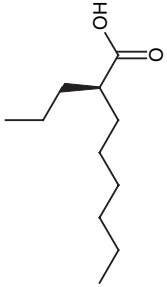
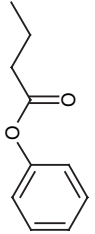
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Table 3. ALS competitive environment (continued).

| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism |
|--------------------|-------------------|---|--|--------------------|--|
| Glatiramer acetate | Teva |  | Multiple sclerosis | Phase II | Evokes neuroprotective T-cell response; protects against glutamate toxicity; stimulates release of growth factors |
| GPI-16072 | Guilford |  | None | Preclinical | NAALADase inhibitor (decreases glutamate production and inhibits glutamate release) |
| G-CSF | Amgen | NA | Accelerates recovery from leucopenia in chemotherapy | Phase II | Neurotropic; antiapoptotic |
| HGF | Multiple | NA | None | Preclinical | Growth factor; antiapoptotic |
| IGF-1 | Cephalon | NA | None | Phase III | Growth factor |
| IGF-1/AAV | Ceregene | As above | None | Phase I | Growth factor delivered by viral vector |
| Levetiracetam | UCB Pharma |  | Epilepsy | Phase II | Calcium-channel modulator; reduces oxidative stress; promotes release of BDNF from astrocytes; histone deacetylase inhibitor |
| MCL-186 | Mitsubishi Pharma |  | Stroke | Phase III | Free radical scavenger; blocks mitochondrial transition pore; upregulates bcl-2 expression |

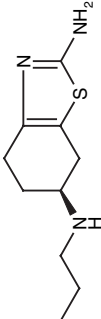
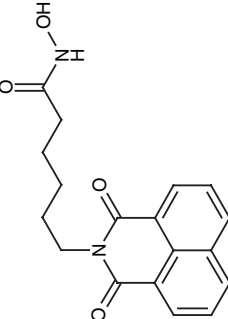
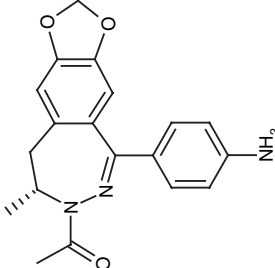
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Table 3. ALS competitive environment (continued).

| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism |
|----------------|---|---|---|--------------------|---|
| Memantine | Merz |  | Alzheimer's disease | Phase II | AMPA antagonist |
| Minocycline | Johnson & Johnson |  | Antibiotic | Phase III | Prevents microglial activation; prevents caspase activation |
| Nimesulide | Helsinn |  | Acute pain; fever; osteoarthritis; dysmenorrhea | Pre-clinical | COX-2 inhibition; prostaglandin synthase inhibition; metalloprotease inhibition |
| ONO-2506 | Ono Pharmaceuticals |  | None | Phase III | Prevents reactive astrocytosis; glutamate antagonist; COX-2 inhibitor |
| Phenylbutyrate | Scandinavian Formulae, Ucyclid, Medicis |  | Urea cycle disorders | Phase II | Histone deacetylase inhibitor; GABA agonist; sodium channel antagonist |

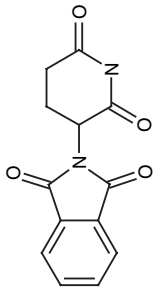
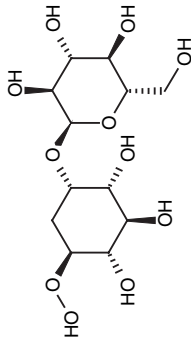
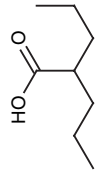
ALS: Amyotrophic lateral sclerosis; AAV: Adeno-associated virus; BDNF: Brain-derived neurotrophic factor; ECGG: Catechin (-) epigallocatechin-3-gallate; EPO: Recombinant human erythropoietin; HGF: Recombinant human hepatocyte growth factor; IGF-I: Recombinant human insulin-like growth factor; mSOD: Mutant SOD; NAALADase: N-acetylated, α -linked acidic dipeptidase; RNAi: RNA interference.

Table 3. ALS competitive environment (continued).

| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism |
|--------------------------|---------------------|--|--|--------------------|--|
| <i>R</i> (+) pramipexole | NA |  | Pramipexole is used in Parkinson's disease and restless leg syndrome | Phase II | Antioxidant |
| RNAi for mSOD1 | NA | NA | None | Preclinical | Decrease production of mSOD1 protein |
| RO-26-2853 | Roche | NA | None | Preclinical | Anti-inflammatory; inhibits matrix metalloproteases |
| Scriptaid | Alexis Biochemicals |  | None | Preclinical | Disrupts aggregation; histone deacetylase inhibitor |
| Stem cells | NA | NA | None | Preclinical | Replace damaged neurons or glial cells; Augment glutamate uptake |
| Talampanel | Teva |  | None | Phase III | AMPA modulator |

ALS: Amyotrophic lateral sclerosis; AAV: Adeno-associated virus; BDNF: Brain-derived neurotrophic factor; EGCG: Catechin (-) epigallocatechin-3-gallate; EPO: Recombinant human erythropoietin; HGF: Recombinant human hepatocyte growth factor; IGF-I: Recombinant human insulin-like growth factor; mSOD: Mutant SOD; NAALADase: N-acetylated, α-linked acidic dipeptidase; RNAi: RNA interference.

Table 3. ALS competitive environment (continued).

| Compound | Company | Structure | Approved indications | ALS research stage | Mechanism |
|---------------|-------------|---|----------------------------|--------------------|--|
| Tamoxifen | AstraZeneca |  | Breast cancer | Phase II | Estrogen receptor modulator; inhibits PKC |
| Thalidomide | Celgene |  | Leprosy, myeloma; cachexia | Phase II | TNF- α inhibitor; angiogenesis inhibitor |
| Trehalose | Carghill |  | None | Preclinical | May prevent protein aggregation |
| TRO-19622 | Trophos | NA | None | Phase II | Inhibits opening of mitochondrial transition pore; glutamate antagonist; antiapoptotic |
| Valproic acid | Teva |  | Epilepsy | Phase III | Histone deacetylase inhibitor, which may modulate transcription |
| VEGF | NA | NA | None | Preclinical | Growth factor |

ALS: Amyotrophic lateral sclerosis; AAV: Adeno-associated virus; BDNF: Brain-derived neurotrophic factor; EGCG: Catechin (-) epigallocatechin-3-gallate; EPO: Recombinant human erythropoietin; HGF: Recombinant human hepatocyte growth factor; IGF-I: Recombinant human insulin-like growth factor; mSOD: Mutant SOD; NAALADase: N-acetylated, α -linked acidic dipeptidase; RNAi: RNA interference.

arimoclomol affects protein aggregation in humans with ALS at dosages studied. Long-term safety experience in humans is limited.

5.5 Ceftriaxone

Ceftriaxone is a cephalosporin antibiotic, which is at present off patent and sold as a generic drug. It increases expression of EAAT2/GLT1 activity in rats, and prolongs survival in FALS1 mice [20,85,106]. An innovative Phase I – III adaptive design pharmacokinetic, safety and efficacy study is underway in ALS patients. Ceftriaxone acts to mediate excitotoxicity; the importance of this mechanism in SALS is presumably illustrated by riluzole's success. This drug has been used extensively in humans for other indications, has good brain penetration and has a good short-term safety record. However, intravenous administration is required and there is limited safety experience in humans.

5.6 Celastrol

Celastrol (Schering-Plough) has multiple effects relevant to ALS, including suppression of TNF- α , IL-1B and nitric oxide, and induction of heat-shock protein [106,110]. Administration prior to symptom onset prolongs survival in FALS1 mice [111]. Celastrol potentially hits multiple downstream mechanisms. A form of it has long been used in humans as a naturopathic remedy for 'inflammation'. Oral administration is also a plus. However, there is a lack of safety and pharmacokinetic data in humans with ALS. It is not clear that this drug can cross the blood–brain barrier [106].

5.7 Coenzyme Q₁₀

Coenzyme Q₁₀ is a generic antioxidant, which can also facilitate mitochondrial respiration [106]. It can prolong survival in FALS1 mice [111], is administered orally and crosses the blood–brain barrier. In an open-label, dose-escalation study in humans, it was safe and well tolerated at doses up to 3 g/day over 8 months [20]. A randomized Phase II study in patients with ALS has begun. Coenzyme Q₁₀ has multiple relevant mechanisms, oral administration, brain penetration and some safety data in humans with ALS. However, there are limited long-term safety data in humans so far.

5.8 Creatine (high dose)

Creatine has multiple effects that might be relevant in ALS, including its ability to act as antioxidant, stabilize the mitochondrial transition pore and facilitate mitochondrial ATP synthesis. When given prior to disease onset, creatine prolongs survival in FALS1 mice [45,112,113]. Phase II trials in patients with ALS failed to show a benefit at two different dosing regimens [45,112,113]. However, these trials may not have used doses that optimize brain phosphocreatine levels. Furthermore, creatine can be used in combination with other therapies to maximize its benefit, at least in FALS1 models [114], thus suggesting that it might be useful as part of a 'cocktail'. An innovative Phase II 'selection' trial in which higher dose

creatine (20 g/day) is used in combination with either minocycline or celecoxib is underway. Advantages of creatine are its multiple relevant mechanisms, oral administration, brain penetration and excellent safety profile. However, there have been multiple negative studies in humans with ALS when used as monotherapy at doses of 10 g/day.

5.9 C3

C3 is a small molecule from Merck and C Sixty. It is a fullerene or 'buckyball' that acts as a potent antioxidant, and when delivered via intraperitoneal pumps, it can prolong survival in FALS1 mice [71]. Its advantages are unclear, but on the downside it hits a mechanism that is likely to be downstream in SALS. Other problems include intraperitoneal delivery, lack of data on brain penetration and lack of safety data in humans with ALS.

5.10 Catechin (-) epigallocatechin-3-gallate

Catechin (-) epigallocatechin-3-gallate is a generic compound derived from green tea, a flavonoid with antioxidant effects. When administered orally to FALS1 mice, it had a large beneficial effect on survival [71]. Oral dosing and size of effect in FALS1 models are pluses with this compound. Its disadvantages are similar to those of C3.

5.11 Recombinant human erythropoietin

Recombinant human erythropoietin (EPO), which is used to stimulate red blood cell production in patients with anemia, has interesting anti-inflammatory and antiapoptotic effects, and can augment BDNF release *in vitro* and in animal models. In a recent open-label safety study, subcutaneous administration was well tolerated in 25 patients with ALS over two injections given a month apart [115]. EPO can theoretically affect multiple relevant mechanisms and has extensive safety data in humans without ALS. However, it is administered subcutaneously and has not yet been shown to affect relevant mechanisms in patients with ALS when administered at safe or tolerable doses.

5.12 Glatiramer acetate

Glatiramer acetate, a synthetic compound from TEVA, is a combination of four amino acids. It is used to reduce the frequency of relapses in patients with multiple sclerosis, in which it is believed to act by boosting regulatory T-cell immunity; in addition to this ultimately anti-inflammatory property, its antiglutamatergic and growth factor-stimulating effects [116] may be relevant in ALS. It prolonged survival in some, but not other, studies in FALS1 mice, and in a Phase II trial it was safe, well tolerated and affected the immune system at the dosage studied [117]. It has multiple potentially relevant mechanisms. This compound affects a disease-relevant mechanism when administered at a tolerable dosage to patients with ALS. However, preclinical data are limited and discordant; it hits a mechanism that may be downstream in ALS, and requires subcutaneous administration.

5.13 Recombinant human granulocyte-stimulating factor

Recombinant human granulocyte-stimulating factor, which is used to stimulate white blood cell production in patients with leucopenia, has neurotropic and antiapoptotic effects. It has recently been shown to prolong survival in a FALS1 animal model [118] and Phase II trials are about to begin. Its advantages and disadvantages are identical to those of EPO.

5.14 GPI-16072

GPI-16072 (Guilford, Inc.) inhibits *N*-acetylated α -linked acidic dipeptidase, and thus can decrease glutamate production and release. It can prolong survival in FALS1 mice [106]. It mediates excitotoxicity; the importance of this mechanism in SALS is presumably illustrated by riluzole's success. Effects apparently only occur during excessive stimulation of glutamate, which should minimize the side effects typically seen with antiglutamate therapies [106]. However, this class has never been administered to humans.

5.15 Recombinant human hepatocyte growth factor

Recombinant human hepatocyte growth factor is reportedly neurotropic, antiapoptotic, and able to optimize levels of EAAT2/GLT1 in animals [119,120]. When administered via intrathecal pump, it prolongs survival in FALS1 rats [119] and when delivered via gene therapy it can do so in FALS1 mice [120]. It has demonstrated positive effects on survival in two different animal species, with two different delivery systems, even if delivered at symptom onset. However, this compound has no safety or efficacy data in patients with ALS and may require intrathecal delivery.

5.16 IGF-1

Recombinant human IGF-1 from Cephalon has been extensively tested in patients with ALS, with conflicting results. One study showed a slowing in functional decline; no benefit was seen in another [106]; a third Phase III study is underway. This compound is well tolerated, and so far is the only agent other than riluzole to show any benefit against any marker of disease progression in patients with ALS. However, the benefit is inconsistent between studies. Even if the most positive study can be replicated, the benefit, like that of riluzole, is likely to be small. Because it does not cross the blood–brain barrier, this compound may eventually require intrathecal delivery.

5.17 Adeno-associated virus engineered to contain the gene for IGF-1 (IGF-1/AAV)

Ceregene has engineered the adeno-associated virus to contain the gene for IGF-1. This can prolong survival in FALS1 mice if given prior to disease onset [106] and a Phase II study in patients with ALS is about to begin. Theoretically, after intramuscular injection, this vector could allow targeted delivery of IGF-1 to motor neurons [106]. However, there are no data on safety, tolerability or pharmacokinetics in humans with ALS. The best delivery mechanism is not yet determined.

5.18 Levetiracetam

Levetiracetam is a widely prescribed antiepileptic drug from UCB Pharma. It has multiple proposed mechanisms of action that might be relevant in ALS: it is a calcium-channel modulator; it can mediate oxidative stress *in vitro*; it can induce the release of BDNF from astrocytes and it is a histone deacetylase inhibitor [121]. An open-label study is underway to determine its safety and tolerability in patients with motor neuron diseases including ALS [121]. It is orally administered, and been extensively used in epilepsy patients with excellent safety and tolerability. There are few drug–drug interactions and no requirement for monitoring; it can hit multiple ALS relevant mechanisms; it offers the possibility of improving symptoms such as cramps and spasticity, as well as slowing disease progression [121]. However, it does not appear to delay onset or improve survival in FALS1 mice [122] and safety or tolerability data in ALS patients are scant at present.

5.19 MCI-186

Also called edavarone, MCI-186 is a small molecule from Mitsubishi Pharma used in Asia to treat stroke. It has multiple ALS-relevant mechanisms of action, including free radical scavenging, blocking the mitochondrial transition pore and upregulating bcl-2 expression [123]. When administered intravenously in an open-label Phase II study of 20 patients with ALS, MCI-186 was safe and well tolerated. Markers of oxidative stress were favorably affected, and there was a suggestion of slowed disease progression as measured by the ALS functional rating scale [123]. A Phase III study is underway.

MCI-186 has multiple mechanisms of action and some preliminary safety and efficacy data in patients with ALS. This compound affects a disease-relevant mechanism when administered at a tolerable dosage to patients with ALS. However, its intravenous administration is less desirable than oral administration.

5.20 Memantine

Memantine from Merz is used to treat patients with Alzheimer's disease. It has excellent CNS penetration and acts as an NMDA-receptor antagonist [106]. It is administered orally and has a good safety and tolerability record in humans. However, as yet there are no data from FALS animal models or from patients with ALS.

5.21 Minocycline

Originally from Johnson & Johnson and now available as a generic, minocycline is a second-generation tetracycline antibiotic that can prevent microglial activation and caspase activation [106]. It prolongs survival in FALS1 mice and has been reasonably safe and well tolerated in ALS patients in Phase II studies [106], but these small studies were not powered to look for efficacy. A Phase III study is underway. It is administered orally, has a wealth of preclinical data in FALS1 mice, and safety and tolerability are all favorable. However, it presumably acts via mechanisms that may occur late in the ALS cascade

and there is no proof that it affects these mechanisms in humans with ALS at the dosages being used.

5.22 Nimesulide

Nimesulide (Helsinn), which is now available as a generic, is a COX-2 inhibitor with antioxidant properties. It can preserve motor skills in FALS1 mice [124] and can be administered via multiple routes, including orally. However, it acts via the same mechanism as celecoxib, which failed in human ALS trials, and questions about its safety in humans have been raised [106].

5.23 ONO-2506

ONO-2506 is an enantiomer of valproic acid from Ono Pharmaceuticals, which has antiglutamate and anti-inflammatory functions, and may be able to inhibit reactive astrocytosis [106]. A Phase II study in humans with ALS has been completed, but results are not yet available. A Phase III study has begun. ONO-2506 works on multiple mechanisms, and has been used in humans with ALS; however, the pathways it acts on may be downstream and it is not yet clear if it affects any relevant pathway in humans with ALS at the dosages being studied.

5.24 Phenylbutyrate

Slight variations of the histone deacetylase inhibitor phenylbutyrate are produced by Scandinavian Formulae, Ucylyd and Medicis. One form has been used for many years in children with urea cycle disorders; in this population, it appears to be safe and well tolerated. It can alter transcription and prolong survival in FALS1 mice [72] and a recent Phase II study suggests that it is safe and well tolerated in patients with ALS at dosages that should alter transcription [125]. It is orally administered, has effects on an upstream pathway, and appears safe and well tolerated in humans with ALS. It should soon be known whether it affects transcription in humans with ALS at doses studied. However, further trials in ALS require completion of long-term animal toxicology studies in rodent and non-rodent species.

5.25 R(+) pramipexole

Pramipexole is a dopamine agonist used in Parkinson's disease that can reduce oxidative stress in patients with ALS [126]. In cell culture and animal studies, this enantiomer of pramipexole can block the mitochondrial transition pore and caspase activation. As it has less affinity for dopamine receptors than pramipexole, it should have fewer side effects. In FALS1 mice, R(+) pramipexole prolongs survival [126]. A small open-label study in humans is underway. R(+) pramipexole have multiple relevant mechanisms of action; however, its proposed mechanisms of action are possibly downstream in ALS pathophysiology and there is little published preclinical data in ALS using this enantiomer.

5.26 RNAi for mSOD1

Like antisense oligonucleotides, RNAi is a means for shutting off the product of *mSOD1*. Recent work suggests that this approach can prolong survival and preserve motor function

when given to presymptomatic FALS1 mice [20,26]. RNAi for mSOD1 hits a mechanism that is likely to be at or near the top of FALS pathophysiology. It is likely to be a highly relevant therapy for SOD1-mediated disease. However, it is not clear that SOD1 plays a role in SALS. Optimal method for delivery is not yet known. Rapid turnover and 'off target' effects make this less desirable compared with antisense oligonucleotides [109].

5.27 RO-26-2853

RO-26-2853 from Roche acts as an anti-inflammatory agent by specifically inhibiting the activation of matrix metalloproteases – enzymes that digest the extracellular matrix. It prolongs survival in FALS1 mice if given presymptomatically, but not if given at symptom onset [127]. RO-26-2853 has a unique mechanism of action among ALS relevant therapies. However, it probably acts on events that are downstream in the ALS cascade, and there is a lack of safety or efficacy data for this agent in humans.

5.28 Scriptaid

Scriptaid is a small molecule from Alexis Biochemicals that can interfere with protein aggregation *in vitro* [20,106]. FALS1 animal studies are underway. Scriptaid targets a mechanism that is probably upstream and relevant to FALS and SALS, but there are limited preclinical data.

5.29 Stem cells

There are a number of ways in which stem cells could be beneficial in ALS. These include replacement of dying motor neurons, replacement of defective glial cells, sources of growth factor production, and 'sinks' for excitotoxins such as glutamate [20]. Recently, embryonic neural stem cells were transplanted into the spinal cord of FALS1 rats. These cells differentiated into neurons, formed synaptic contacts, released growth factors and extended the lifespan of the animals [128]. Even more recently, eight patients with ALS were given intrathecal injections of autologous mesenchymal stem cells, along with intravenous erythropoietin [115]. During 9 months of follow up, no significant side effects were reported, although transient benefits on strength, and a trend toward a reduced slope of decline in ALS functional rating scale was reported. The lack of placebo comparison group makes this data difficult to interpret.

The multiple mechanisms, including the ability to augment or replace neuronal and non-neuronal cells, are a plus. Theoretically, stem cell therapy offers the unique hope of restoration of function, rather than merely slowing or arresting disease progression. However, regulations in the US limit federal funding for embryonic stem cell research.

5.30 Talampanel

Talampanel, which was originally under development by Ivax for epilepsy, is a non-competitive modulator of AMPA receptors. It can prolong survival in FALS1 mice, and was safe

and well tolerated in a Phase II study in patients with ALS [106]. It crosses the blood–brain barrier and has demonstrated safety and tolerability in patients with ALS. However, it targets a mechanism that is probably downstream, and has not been shown to affect AMPA receptors in humans with ALS at dosages studied so far.

5.31 Tamoxifen

Tamoxifen (AstraZeneca) is used to treat breast cancer. It acts as an anti-inflammatory through inhibition of PKC. In a Phase II study of patients with ALS, it was safe and well tolerated; furthermore, there was a suggestion of efficacy with increased survival at certain dosages [106]. A Phase III trial is planned. Tamoxifen has been extensively used in humans and has a good safety profile. Aside from riluzole, this is one of the only agents to potentially show efficacy in a human ALS trial. However, there are no FALS1 animal data, and the Phase II study has not been published yet.

5.32 Thalidomide

Thalidomide, the infamous drug from Celgene, was originally marketed as a sedative, but teratogenicity forced its withdrawal from the world market in the 1960s. It is now being used again in the treatment of leprosy, myeloma and cachexia. It crosses the blood–brain barrier and has a number of interesting mechanisms of action, including suppression of TNF- α . When administered orally to FALS1 mice, it prolongs survival [129]. A Phase II study is underway in patients with ALS. There is extensive experience with this in humans, but the putative mechanism is probably downstream. Effects on TNF- α have not been demonstrated in patients with ALS at the dosages being studied, and significant side effects are seen in humans, including sedation, peripheral neuropathy and teratogenicity. Lenalidomide may offer a safer alternative [106].

5.33 Trehalose

Trehalose (Cargill) may have the ability to modulate protein aggregation [106]. It may attack an upstream mechanism relevant to FALS and SALS, but there are no data in FALS1 models, or in humans with ALS.

5.34 TRO-19622

TRO-19622 from Trophos, which is also being tested in neuropathic pain, is reported to inhibit the opening of the mitochondrial transition pore, act as a non-competitive glutamate antagonist and have antiapoptotic properties. It can maintain weight and motor function in FALS1 mice, and can prolong survival in models of other motor neuron diseases [130]. It appears safe and well tolerated in a small preliminary study of patients with ALS [130]. A larger Phase II study is under consideration. Its advantages include oral administration, multiple mechanisms of action and safety and tolerability in ALS patients. However, the proposed mechanisms of action are probably downstream, and there are limited data on use in patients with ALS.

5.35 Valproic acid

Valproic acid is a well-known antiepileptic drug from Teva that may modulate transcriptional dysregulation by acting as a histone deacetylase inhibitor. Included in this is an upregulation of the antiapoptotic protein bcl-2 and a downregulation of glycogen synthase kinase 3-B [131]. In FALS1 mice, it prolongs survival if given before or at symptom onset [131]. Phase II/III studies are underway in patients with ALS. This orally administered drug has a history of extensive use in humans with good safety and tolerability, but there are no data in humans with ALS yet.

5.36 VEGF

VEGF has been administered to FALS1 models intraventricularly and via viral vectors. Either way, it prolongs survival, but more impressively if given prior to rather than at symptom onset [26]. There are good reasons to suspect that VEGF deficiency plays a part in some patients with FALS and SALS, as described above, but, as with other growth factors, because it does not cross the blood–brain barrier, administration will have to be invasive. There are no data regarding safety, tolerability or efficacy in humans. VEGF may only be part of the pathophysiology in select patients with ALS, as VEGF polymorphisms are a risk factor in some populations and not others.

6. Potential development issues

The development of ALS therapies tends to follow a certain path. Targets are identified, most often from *in vitro* preclinical assays and FALS1 models. Therapies that may affect these targets are tested, sometimes *in vitro*, usually in FALS1 animal models. If the therapy is novel, long-term animal toxicology studies are carried out, typically in two species, followed by Phase I safety studies in healthy human volunteers. These are followed by Phase II safety studies and then Phase III efficacy studies in patients with ALS. Study inclusion criteria typically require enough upper and lower motor neuron signs to be confident of an ALS diagnosis, but also enough respiratory function to survive the duration of the study. Study duration and sample size depend on choice of primary outcome measure and desired effect size. Based on the signal-to-noise ratio, and the rate of change of available ALS outcome measures, Phase II studies usually require ~ 50 – 100 patients and last a few months; Phase III studies usually require at least 400 patients and to last 1 year. There are a number of significant problems with this approach that slow down the development of new therapeutics.

First, we lack a comprehensive understanding of the pathophysiology for any type of ALS. Ideas for new therapies, in part, come from observations made in FALS1 animal models, yet FALS1 patients typically account for < 5% of our trial populations. Indeed, FALS1 patients have even been excluded from participating in some recent trials [204]. Disagreement between FALS1 animal trial results and human SALS results, as seen with gabapentin, creatine and celecoxib [106], might

have multiple explanations, but nonetheless should raise concern over this development schema. Most therapies tested so far in humans with ALS, however, have been based on preclinical data, *in vitro* models of motor neuron dysfunction, and on pathways shown to be abnormal also in SALS.

Second, ALS is a rapidly progressive, devastating, uniformly fatal disease. The FDA requirement for long-term animal toxicology studies in two species, and for Phase I studies in healthy patients must be questioned.

Third, ALS is a rare disease. Our present Phase II and III designs are too resource intensive in terms of patient numbers and durations. Many patients will only get a chance at one trial over the course of their disease, meaning trial costs can also be measured in patient commitment, life investment and loss of hope. More sensitive ALS outcome measures and improved trial designs are needed.

Fourth, the diagnosis of ALS remains primitive and time consuming. The interval between first symptoms and a diagnosis that is certain enough to allow entry into a trial is typically ~ 1 year [4-6], at which point possibly more than half the motor neurons have been lost. In FALS1 animal models, earlier administration of therapies is often far more effective than later administration. A rapid diagnostic test is thus needed.

Aside from these methodological problems, there is the issue of sponsorship. ALS is considered an orphan disease. The small number of patients affected and the limited lifespan of these patients are likely to deter industry research funding.

Finally, it should be noted that most therapies in development have the goal of slowing or arresting progression. Restoration of function should also be a goal.

7. Expert opinion and conclusion

Although symptom control has improved over the past 130 years, ALS remains a devastating disease that dramatically limits function and lifespan. No existing drug therapies preserve function or prolong survival in a meaningful way. Tremendous gains have been made in understanding the mechanisms of FALS subtypes, in particular FALS1. However, a comprehensive mechanistic picture of any FALS subtype, much less SALS, has yet to emerge. The present pathway by which ALS therapies are developed has a number of problems; these include the use of FALS1 animal models as preclinical screens, the requirement for standard animal toxicology and Phase I safety studies, the resource-intensive nature of Phase II and III studies and the cumbersome nature of making a trial-acceptable diagnosis of ALS. In spite of all these problems, and the orphan nature of the disease itself, there is an impressive pipeline of potential therapies in various states of development.

In the next decade, we should focus on the most 'upstream' mechanisms for developing therapies or attempt to hit as many 'downstream' mechanisms as possible. We need to optimize the pathway by which potential therapies are developed. Finally, we need to find ways to attract more industry research sponsorship.

How will we accomplish these goals? In our present understanding of ALS, the most upstream events are probably disease-causing mutations, other genetic and transcriptional dysregulation and protein aggregation. Thus, RNAi and anti-sense oligonucleotides (which can shut off harmful genes) for mSOD1-mediated disease, phenylbutyrate (which may reverse transcriptional dysregulation) and arimoclomol (which may mediate protein aggregation) for FALS and SALS deserve immediate attention. Although some of these require more invasive, parenteral delivery, this should not preclude their use. Indeed, invasive delivery has been accepted for other agents used to treat other diseases having similarly dismal prognoses, such as brain tumors.

Riluzole is the one therapy that has shown the ability to slow disease progression in patients with ALS [53,54]. Development of more potent riluzole analogs should be undertaken.

Downstream events in ALS may occur in series or in parallel. Until this is teased out, attempts to mediate downstream events should occur via cocktail therapies that hit as many at once as possible. Trials should be designed as add-on therapies to riluzole. Indeed, there is evidence from FALS1 animal studies that therapy combinations work better than individual agents [114].

Continued work on the FALS1 model should lead to a more comprehensive picture of the mechanism for this disease subtype. Agents that appear promising in this model should be taken into trials specific for FALS1 patients. This approach has recently been considered and appears feasible [132].

Until we have a true model of SALS, or a drug that slows its progression, there will be uncertainty regarding the best preclinical screening assays. One conservative option would be to require new therapies to work in several *in vitro* preclinical models and in multiple different animal models and before bringing them into SALS clinical trials. Alternatively, standardization of SOD1 copy number, background genetics, gender and timing of therapy in experimental models should be considered. In particular, therapies that show efficacy at or after disease onset may be of higher interest as this approach reflects the way they will be used in humans.

Identification of SALS pathophysiology should be aided by the creation of new, very large, national and international ALS databases, and by new SNP genotyping technology that allows whole-genome screens. If the unexpected recent findings of this new whole-genome approach [30] are replicated, we must identify the functions of the genes most commonly altered with SALS and develop therapeutics that address these functions.

The requirement for long-term animal toxicology studies in two species and for Phase I studies in healthy volunteers should be reconsidered in ALS due to the devastating nature of this disease. Therapies for ALS require the same level of animal toxicology data prior to initiating treatments in humans as those developed for much more benign disorders such as headache. There should be a dialog with the FDA about modifying these requirements.

Biomarkers are being [133] and should continue to be developed that can be used to speed ALS diagnosis. New biomarkers will need to be carefully compared with other available outcome measures such as muscle strength, functional rating scales, pulmonary function, and electrodiagnostic studies such as motor unit estimation to determine which are most sensitive for future clinical trials. Innovative Phase II designs, including 'futility' and 'selection' designs [134,204], should continue to be explored with the goal of reducing duration and patient number requirements for our clinical trial pathway.

We should strive for 'proof of concept' in our Phase II trials. In other words, our compounds should clearly affect an ALS-relevant mechanism in ALS patients at the dosages studied prior to moving them into a Phase III study. It is not often possible to measure the effect of therapy on desired biochemistry in humans. However, if it is measurable, this approach should be taken early in drug development. The clinical trial of celecoxib attempted to do this by measuring prostaglandin E2 levels in cerebrospinal fluid [47]. Glatiramer

acetate is an example in which this proof of concept has been demonstrated in Phase II studies [117].

Trial design and execution will be further optimized by the formulation of consortiums, consisting of large numbers of ALS clinics working together with centralized data collection, monitoring and analysis. The Northeast ALS Consortium is an example of this [205].

Finally, we should continue attempts to relate the pathophysiology of ALS to that of other neurodegenerative diseases, and perhaps to that of ageing itself. Establishing such relationships will allow us to reap knowledge from multiple fields, and to attract much needed industry research sponsorship.

Acknowledgements

This research was supported (in part) by the Intramural Program of the NIMH (BJT). R Bedlack is a speaker for Pfizer and Lilly, and has a research grant from UCB Pharma. ME Cudkowicz has consulted for Dembio, Teva and Ono Pharmaceuticals, and has a research grant from CytRx.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. ROWLAND LP, SCHNEIDER N: Amyotrophic lateral sclerosis. *New. Engl. J. Med.* (2001) **344**(22):1691-1700.
2. ROWLAND LP: How amyotrophic lateral sclerosis got its name. *Arch. Neurol.* (2001) **58**:512-515.
3. BROOKS BR: El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. *J. Neurol. Sci.* (1994) **124**(s):96-107.
4. HOUSEHAM E, SWASH M: Diagnostic delay in amyotrophic lateral sclerosis: what scope for improvement? *J. Neurol. Sci.* (2000) **180**:76-81.
5. CHIO A: ISIS survey: an international study on the diagnostic process and its implications in amyotrophic lateral sclerosis. *J. Neurol.* (1999) **246**(SIII):1-5.
6. CHIO A: Update on ISI survey: europe, north america and south america. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* (2000) **1**(S1):9-11.
7. SWINGLER RJ, FRASER H, WARLOW CP: Motor neuron disease and polio in Scotland. *JNNP* (1992) **55**:1116-1120.
8. TRAYNOR BJ, CODD MB, CORR B, FORDE C, FROST BA, HARDIMAN O: Incidence and prevalence of ALS in Ireland, 1995-1997. *Neurology* (1999) **52**:504.
9. PARALS: Incidence of ALS in Italy. *Neurology* (2001) **56**:239-244.
10. LOGROSCINO G, BEGHI E, ZOCCOLELLA S *et al.*: Incidence of amyotrophic lateral sclerosis in southern Italy: a population based study. *JNNP* (2005) **76**:1094-1098.
11. NEARY D, SNOWDEN JS, MANN D: Cognitive change in motor neurone disease/amyotrophic lateral sclerosis (MND/ALS). *J. Neurol. Sci.* (2000) **180**:15-20.
12. ZOCCOLELLA SG, PALAGANO G, FRADDOSIO A *et al.*: ALS-plus: 5 cases of concomitant amyotrophic lateral sclerosis and parkinsonism. *Neurol. Sci.* (2004) **23**(s2):123-124.
13. DEL AGUILA MA, LONGSTRETH WT JR, MCGUIRE V, KOEPEL TD, VAN BELLE G: Prognosis in amyotrophic lateral sclerosis. A population-based study. *Neurology* (2003) **60**:813-819.
14. MILLUL A, BEGHI E, LOGROSCINO G, MICHELI A, VITELLI E, ZARDI A: Survival of patients with amyotrophic lateral sclerosis in a population-based registry. *Neuroepidemiology* (2005) **25**(3):114-119.
15. GROHME K, MARAVIC MV, GASSER T, BORASIO GD: A case of amyotrophic lateral sclerosis with very slow progression over 44 years. *Neuromuscul. Disord.* (2001) **11**(4):414-416.
16. PUGLIATTI M, ROSATI G, CARTON H: The epidemiology of multiple sclerosis in Europe. *Eur. J. Neurol* (2006) **13**:700-722.
17. JOHNSON CA, STANTON BR, TURNER MR *et al.*: Amyotrophic lateral sclerosis in an urban setting: a population based study of inner city london. *J. Neurol.* (2006) **253**:1642-1643.
18. ANDERSEN P: Amyotrophic lateral sclerosis genetics with mendelian inheritance. In: *Amyotrophic Lateral Sclerosis*. Brown RH, Swash M, Pasinelli P (Eds), Informa Healthcare, London UK (2006):187-208.
- **Comprehensive recent review of FALS.**
19. MAJOR-KRAKAUER D, WILLEMS PJ, HOFMAN A: Genetic epidemiology of amyotrophic lateral sclerosis. *Clin. Genet.* (2003) **63**(2):83-101.
20. BRUIJN L, CUDKOWICZ M: Therapeutic targets for amyotrophic lateral sclerosis: current treatments and prospects for more effective therapies.

- Expert Rev. Neurotherapeutics* (2006) 6(3):417-428.
21. YANG Y, HENTATI A, DENG H *et al.*: The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* (2001) 29:160-165.
 22. HADANO S, HAND C, OSUGA H *et al.*: A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* (2001) 29:166-173.
 23. HENTATI A, OUAHCHI K, PERICAK-VANCE MA *et al.*: Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. *Neurogenetics* (1998) 2(1):55-60.
 24. HONG S, BROOKS SR, HUNG W *et al.*: X-linked dominant locus for late-onset familial amyotrophic lateral sclerosis. *Soc. Neurosci. Abst.* (1998) 24:478.
 25. JULIEN J, KRIZ J: Transgenic mouse models of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta* (2006) 1762:1013-1024.
 26. BOILLEE S, VANDE VELDE C, CLEVELAND D: ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* (2006) 52:39-59.
 - **Outstanding review, focussing on role of multiple cell types.**
 27. ALEXANDER MD, TRAYNOR BJ, MILLER N *et al.*: "True" sporadic als associated with a novel SOD-1 mutation. *Ann. Neurol.* (2002) 52(5):680-683.
 28. SIMPSON C, AL-CHALABI A: Amyotrophic lateral sclerosis as a complex genetic disease. *Biochimica et Biophysica Acta* (2006) 1762:973-985.
 - **Recent comprehensive review of genetic susceptibility.**
 29. ISHIGAKI S, NIWA J, ANDO Y *et al.*: Differentially expressed genes in sporadic amyotrophic lateral sclerosis spinal cords screening by molecular indexing and subsequent cDNA microarray analysis. *FEBS Lett.* (2002) 531:354-358.
 30. SCHYMICK J, SCHATZ S, HON-CHUNG F *et al.*: Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: first stage analysis and public release of data. *Lancet Neurol.* (2007) 6(4):322-328.
 31. TANAKA F, JIANG YM, YAMAMOTO M *et al.*: Gene expression profile of spinal motor neurons in sporadic amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* (2006) 7(S1):9.
 32. SARIS C, VAN VUGHT P, YIGITTOP H *et al.*: Molecular pathway analysis of amyotrophic lateral sclerosis by genome-wide expression profiling of human blood. *Amyotroph. Lateral Scler.* (2006) 7(S1):11.
 33. PLATO CC, GARRUTO RM, GALASK D *et al.*: Amyotrophic lateral sclerosis-parkinsonism-dementia complex of Guam: changing incidence rates during the past 60 years. *Am. J. Epidemiol.* (2003) 157:149-157.
 34. SUNDAR P, CHANG-EN Y, WEIVA S *et al.*: Two sites in the MAPT region confer genetic risk for Guam ALS/PDC and dementia. *Hum. Mol. Genet.* (2007) 16(3):295-306.
 35. COX PA, SACKS OW: Cycad neurotoxin, consumption of flying foxes, and ALS/PDC disease in guam. *Neurology* (2002) 58:956-959.
 36. KWANG C, CRAIG U, LEE C *et al.*: Cycad neurotoxin, consumption of flying foxes, and ALS/PDC disease in guam. *Neurology* (2002) 59:1664-1665.
 37. CHO A, BENZI G, DOSSENA M *et al.*: Severely increased risk for amyotrophic lateral sclerosis among Italian professional football players. *Brain* (2005) 128:472-476.
 38. WEISSKOPF M, O'REILLY E, MCCULLOUGH M *et al.*: Prospective study of military service and risk of amyotrophic lateral sclerosis. *Neurology* (2005) 64:32-37.
 39. HALEY RW: Excess incidence of ALS in young Gulf War veterans. *Neurology* (2003) 61:750-756.
 40. HORNER RD, KAMINS KG, FEUSSNER JR *et al.*: Excess incidence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology* (2003) 61:742-749.
 41. NELSON L, MCGUIRE V: Epidemiology of amyotrophic lateral sclerosis. In: *Amyotrophic Lateral Sclerosis*. Brown RH, Swash M, Pasinelli P (Eds), Informa Healthcare, London UK (2006):25-42.
 - **Recent comprehensive review of possible environmental triggers.**
 42. MCGUIRE V, LONGSTRETH W, KOEPEL T, VAN BELLE G: Incidence of amyotrophic lateral sclerosis in three counties in western Washington state. *Neurology* (1996) 12:219-228.
 43. MILLER RG, MOORE DH, GELINAS D *et al.*: Phase III randomized trial of gabapentin in patients with amyotrophic lateral sclerosis. *Neurology* (2001) 56:843-848.
 44. DESNUELLE C, DIB M, GARREL C *et al.*: A double-blind, placebo-controlled trial of α -tocopherol (vitamin E) in the treatment of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Mot. Neuron Disord.* (2001) 2:9-18.
 45. SHEFNER J, CUDKOWICZ M, SCHOENFELD D *et al.*: A clinical trial of creatine in ALS. *Neurology* (2004) 63:1656-1661.
 46. CUDKOWICZ M, SHEFNER J, SCHOENFELD D *et al.*: A randomized, placebo-controlled trial of topiramate in amyotrophic lateral sclerosis. *Neurology* (2003) 61:456-464.
 47. CUDKOWICZ M, SHEFNER J, SCHOENFELD D *et al.*: Trial of celecoxib in amyotrophic lateral sclerosis. *Ann. Neurol.* (2006) 60:22-31.
 48. LAU J, CONWIT R, MILLER R, CUDKOWICZ M: Therapeutic trials in amyotrophic lateral sclerosis: past, present, future. In: *Amyotrophic Lateral Sclerosis*. Brown RH, Swash M, Pasinelli P (Eds), Informa Healthcare, London, UK (2006):335-356.
 49. BENOIT E, ESCANDE D: Riluzole specifically blocks inactivated Na channels in myelinated nerve fiber. *Pflugers Arch.* (1991) 419:603-609.
 50. ZONA C, SINISCALCHI A, MERCURI NB *et al.*: Riluzole interacts with voltage-activated sodium and potassium currents in cultured rat cortical neurons. *Neuroscience* (1998) 85:931-938.
 51. STEFANI A, SPADONI F, BERNARDINI G: Differential inhibition by riluzole, lamotrigine and phenytoin of sodium and potassium currents in cultured rat cortical neurons. *Neuroscience* (1998) 85:931-938.
 52. GURNEY ME, FLECK TJ, HIMES CS, HALL ED: Riluzole preserves motor function in a transgenic model of familial amyotrophic lateral sclerosis. *Neurology* (1998) 50(1):62-66.
 53. BENSIMON G, LACOMBLEZ L, MEININGER V: A controlled trial of riluzole in amyotrophic lateral sclerosis. *N. Eng. J. Med.* (1994) 330:585-591.

54. LACOMBLEZ L, BENSIMON G, LEIGH P *et al.*: Dose ranging study of riluzole in amyotrophic lateral sclerosis. *Lancet* (1996) 347:1425-1431.
55. SORENSON E: An acute, life-threatening, hypersensitivity reaction to riluzole. *Neurology* (2006) 67:2260-2261.
56. PONGRATZ D, NEUNDORFER B, FISCHER W: German open label trial of riluzole 50mg b.i.d. in amyotrophic lateral sclerosis (ALS). *J. Neurol. Sci.* (2000) 180:82-85.
57. OLIVER D, BORASIO G: Palliative care and quality of life in amyotrophic lateral sclerosis. In: *Amyotrophic Lateral Sclerosis*. Brown RH, Swash M, Pasinelli P (Eds), Informa Healthcare, London, UK (2006):357-362.
58. VAN DEN BERG JP, KALMIJN S, LINDEMAN E *et al.*: Multidisciplinary ALS care improves quality of life in patients with ALS. *Neurology* (2005) 65(8):1264-1267.
59. MILLER RG, ROSENBERG JA, GELINAS DF *et al.*: Practice parameter: the care of the patient with amyotrophic lateral sclerosis (an evidence based review), report of the quality standards subcommittee of the american academy of neurology: ALS practice parameters task force. *Neurology* (1999) 52(7):1311-1323.
- **Guide to optimal care of patients with ALS.**
60. LEIGH N, ABRAHAMS S, AL-CHALABI A *et al.*: The management of motor neurone disease. *J. Neurol. Neurosurg. Psychiatry* (2003) 74:32-47.
- **Guide to optimal care of patients with ALS.**
61. ENG D: Management guidelines for motor neurone disease patients on non-invasive ventilation at home. *Palliat. Med.* (2006) 20:69-79.
62. MAZZINI L, CORRA T, ZACCALA M, MORA G, DEL PIANO M, GALANTE M: Percutaneous endoscopic gastrostomy and enteral nutrition in amyotrophic lateral sclerosis. *Neurology* (1995) 242:695-698.
63. ABOUSSOUAN LS, KHAN SU, MEEKER DP, STELMACH K, MITSUMOTO H: Effect of noninvasive positive-pressure ventilation on survival in amyotrophic lateral sclerosis. *Ann. Intern. Med.* (1997) 127:450-453.
64. TRAYNOR BJ, ALEXANDER M, CORR B, FROST E, HARDIMAN O: Effect of a multidisciplinary amyotrophic (ALS) clinic on ALS survival: a population based registry 1996-2000. *J. Neurol. Neurosurg. Psychiatry* (2003) 74(9):1258-1261.
65. ANDERSEN PM: Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr. Neurol. Neurosci. Rep.* (2006) 6(1):37-46.
66. CLEMENT AM, NGUYEN MD, ROBERTS EA *et al.*: Wild-type non-neuronal cells extend survival of SOD-1 mutant motor neurons in ALS mice. *Science* (2003) 302:113-117.
67. BOILEE S, YAMANAKA K, LOBSIGER C *et al.*: Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* (2006) 312:1389-1392.
68. YOSHIHARA T, ISHIGAKI S, YAMAMOTO M *et al.*: Differential expression of inflammation-and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem.* (2002) 80:158-167.
69. FERRAIULO L, HEATH P, HOLDEN H *et al.*: Gene expression profile of spinal motor neurons in the G93A SOD1 mouse model of ALS at different time points. *Amyotroph. Lateral Scler.* (2006) 7(S1):10.
70. KUDO L, KARSTEN S, WIEDAU-PAZOS M: Whole genome microarray analysis of motor neuron vulnerability in G93A-SOD1 and P301L-Tau transgenic mouse models of als. *Amyotroph. Lateral Scler.* (2001) 7(S1):10.
71. BARBER S, MEAD R, SHAW P: Oxidative stress in als: a mechanism of neurodegeneration and a therapeutic target. *Biochimica et Biophysica Acta* (2006) 1762:1051-1067.
- **Recent comprehensive review of oxidative stress hypothesis.**
72. RYU H, SMITH K, CAMELO S *et al.*: Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice. *J. Neurochem.* (2005) 93(5):1087-1098.
73. BRUIJN L, HOUSEWEART MK, KATO S *et al.*: Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* (1998) 281:1851-1854.
74. DURHAM HD, ROY J, DONG L, FIGLEWICZ DA: Aggregation of mutant Cu/Zn superoxide dismutase proteins in a culture model of ALS. *J. Neuropathol. Exp. Neurol.* (1997) 56:523-530.
75. CLEVELAND DW, LIU J: Oxidation versus aggregation - how do SOD1 mutants cause ALS? *Nat. Med.* (2000) 6:1320-1321.
76. JOHNSTON J, DALTON M, GURNEY M, KOPITO R: 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. USA* (2000) 97:12571-12576.
77. WANG J, XU G, SLUNT HH: Coincident thresholds of mutant protein for paralytic disease and protein aggregation caused by restrictively expressed superoxide dismutase cDNA. *Neurobiol. Dis.* (2005) 20:943-952.
78. SATO T, NAKANISHI T, YAMAMOTO Y *et al.*: Rapid disease progression correlates with instability of mutant SOD1 in familial ALS. *Neurology* (2001) 65:1954-1957.
79. VAN DEN BOSCH L, VAN DAMME P, BOGAERT E, ROBBERECHT W: The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta* (2006) 1762:1068-1082.
- **Recent comprehensive review of excitotoxicity hypothesis.**
80. ALEXANDER G, DEITCH J, SEEBURGER J, DEL VALLE L, HEIMAN-PATTERSON T: Elevated cortical extracellular fluid glutamate in transgenic mice expressing human mutant (G93A) superoxide dismutase. *J. Neurochem.* (2000) 74:1666-1673.
81. SHAW P, FORREST V, RICHARDSON J, WASTELL H: CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration* (1995) 4:209-216.
82. COURATIER P, HUGON J, SINDOU P, VALLAT J, DUMAS M: Cell culture evidence for neuronal degeneration in amyotrophic lateral

- sclerosis being linked to glutamate AMPA/kainate receptors. *Lancet* (1993) 341:265-268.
83. SPENCER P, ROY D, LUDOLPH A, HUGON J, DWIVEDI M, SCHAUMBURG H: Lathyrism: evidence for role of the neuroexcitatory amino acid BOAA. *Lancet* (1986) 2:1066-1067.
84. ROTHSTEIN JD, VAN KAMMEN M, LEVEY A *et al.*: Selective loss of glutamate transporter GLT-1 in ALS. *Ann. Neurol.* (1995) 38:73-84.
85. ROTHSTEIN JD, PATEL S, REGAN MR *et al.*: β -lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* (2005) 433:73-77.
86. TATENO M, SADAKATA H, TANAKA M *et al.*: Calcium-permeable AMPA receptors promote misfolding of mutant SOD1 protein and development of amyotrophic lateral sclerosis in a transgenic mouse model. *Hum. Mol. Genet.* (2004) 13:2183-2196.
87. MANFREDI G, BEAL M: Mitochondrial dysfunction and energy metabolism in amyotrophic lateral sclerosis. In: *Amyotrophic Lateral Sclerosis*. Brown RH, Swash M, Pasinelli P (Eds), Informa Healthcare, London, UK (2006):323-331.
- **Recent comprehensive review of mitochondrial hypothesis.**
88. KONG J, XU Z: Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J. Neurosci.* (1998) 18:3241-3250.
89. KIRBY J, HALLIGAN E, BAPTISTA M *et al.*: Mutant SOD1 alters the motor neuron transcriptase: implications for familial ALS. *Brain* (2005) 128:1686-1706.
90. MOISSE K, STRONG M: Innate immunity in amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta* (2006) 1762:1083-1093.
- **Recent comprehensive review of neuroinflammation hypothesis.**
91. ALEXIANU M, KOZOVSKA M, APPEL S: Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* (2001) 57:1282.
92. DRACHMAN D, ROTHSTEIN J: Inhibition of cyclooxygenase-2 protects motor neurons in an organotypic model of amyotrophic lateral sclerosis. *Ann. Neurol.* (2000) 48(5):792-795.
93. URUSHITANI M, SILK A, SAKURAI T, NUKINA N, TAKAHASHI R, JULIEN J: Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. *Nat. Neurosci.* (2005) 9:108-118.
94. XIAO S, MCLEAN J, ROBERTSON J: Neuron intermediate filaments and ALS: a new look at an old question. *Biochimica et Biophysica Acta* (2006) 1762:1001-1012.
- **Recent comprehensive review of cytoskeletal derangement hypothesis.**
95. AL-CHALABI A, ANDERSEN P, NILSSON P *et al.*: Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* (1999) 8:157-164.
96. PULS I, JONNAKUTY C, LAMONTE B *et al.*: Mutant dynactin in motor neuron disease. *Nat. Genet.* (2003) 33:455-456.
97. STERNECK E, KAPLAN DR, JOHNSON P: Interleukin-6 induces expression of peripherin and cooperates with Trk receptor signaling to promote neuronal differentiation in PC12 cells. *J. Neurochem.* (1996) 67:1365.
98. CHEVALIER-LARSEN E, HOLZBAUR E: Axonal transport and neurodegenerative disease. *Biochimica et Biophysica Acta* (2006) 1762:1094-1108.
- **Recent comprehensive review of defective axonal transport hypothesis.**
99. EKESTERN E: Neurotrophic factors and amyotrophic lateral sclerosis. *Neurodegener. Dis.* (2004) 1:88-100.
100. STORKEBAUM E, LAMBRECHTS D, DEWERCHIN M *et al.*: Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat. Neurosci.* (2004) 8:85-92.
101. SENDTNER M: Damaging secretions: chromogranins team up with mutant SOD1. *Nat. Neurosci.* (2006) 9:12-14.
102. LI M, ONA VO, GUEGAN C *et al.*: Functional role of caspase 1 and 3 in ALS. *Science* (2000) 288:335-339.
103. SPOOREN W, 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.* (2000) 46:63.
104. MARTIN LJ: Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution to a programmed cell death mechanism. *J. Neuropathol. Exp. Neurol.* (1999) 58:459-471.
105. HE B, STRONG M: Motor neuronal death in sporadic amyotrophic lateral sclerosis (ALS) is not apoptotic: a comparative study of ALS and chronic aluminum chloride neurotoxicity in new Zealand white rabbits. *Neuropathol. Appl. Neurobiol.* (2000) 26:150-160.
106. TRAYNOR BJ, BRUIJN L, CONWIT F *et al.*: Neuroprotective agents for clinical trials in ALS: a systematic assessment. *Neurology* (2006) 67:20-27.
- **Recent review of potential ALS therapies.**
107. SHOEMAKER JL, SEELY KA, REED R, CROW P, PRATHER L: The cb2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *Atlanta USA Society for Neuroscience Meeting* (2006).
108. SMITH RA, MILLER TM, YAMANAKA K *et al.*: Antisense oligonucleotide therapy for neurodegenerative disease. *J. Clin. Invest.* (2006) 116(8):2290-2297.
109. CUDKOWICZ M, SHEFNER M, SIMPSON E *et al.*: A multicenter, dose ranking safety and pharmacokinetic study of arimoclomol in ALS. *Amyotrophic Lateral Sclerosis* (2006) 7(S1):113.
110. KIPANI K, KIAEI M, CHEN J, CALINGASAN N, BEAL M: Celastrol blocks motor neuron cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. *J. Neurochem.* (2004) 90(S1):92.
111. MATTHEWS RT, YANG L, BROWNE S, BAIK M, BEAL M: Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proc. Natl. Acad. Sci. USA* (1998) 95:8892-8897.
112. ROTHSTEIN J: Of mice and men: reconciling preclinical ALS mouse studies and human clinical trials. *Ann. Neurol.* (2003) 53:423-426.
113. GROENVELD G, VELDINK J, VAN DER TWEEL I *et al.*: A randomized, sequential trial of creatine in amyotrophic lateral sclerosis. *Ann. Neurol.* (2003) 53:437-445.

114. KLIVENYI P, KIAEI M, GARDIAN G *et al.*: Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of ALS. *J. Neurochem.* (2004) **88**:576-582.
115. KIM S, KIM H, KOH S *et al.*: Effectiveness of recombinant human erythropoietin therapy in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* (2006) **7**(S1):9.
116. BLAIR M, PEASE M, HAMMOND J *et al.*: Effect of glatiramer acetate on primary and secondary degeneration of retinal ganglion cells in the rat. *Investig. Ophthalmol. and Vis. Sci.* (2005) **46**:884-890.
117. GORDON PH, DOORISH C, MONTES J *et al.*: Randomized, controlled phase II trial of glatiramer acetate in ALS. *Neurology* (2006) **66**:1117-1119.
118. PITZER C, KRUGER C, KIRSCH F *et al.*: G-CSF protects motoneurons and counteracts denervation atrophy in a mouse model of ALS. *Atlanta USA Society for Neuroscience Meeting* (2006).
119. AOKI M, ISHIGAKI I, NAGAI M *et al.*: Intrathecal delivery of hepatocyte growth factor at the onset of paralysis slows disease progression in a rat model of ALS. *Amyotrophic Lateral Sclerosis* (2006) **7**(S1):41.
120. FUNAKOSHI H, NAKAMURA T: Hepatocyte growth factor as a novel neurotrophic factor for amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* (2006) **7**(S1):41.
121. BEDLACK R: A pilot trial of levetiracetam for cramps, spasticity and neuroprotection in patients with motor neuron disease. *Northeast ALS Consortium Meeting, Boston USA* (2006).
122. SMITH K: The effects of levetiracetam on the sod-1 mutant mouse model of amyotrophic lateral sclerosis/motor neuron disease. *Unpublished data on file at UCB Pharma.*
123. YOSHINO H, KIMURA A: Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* (2006) **7**:241-245.
124. POMPL P, HO L, BIANCHI M *et al.*: A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J.* (2003) **17**:725-727.
125. CUDKOWICZ ME, ANDRES PI, SCHOENFELD D *et al.*: Safety and dose escalating study of oral sodium phenylbutyrate in subjects with ALS. *Amyotroph. Lateral Scler.* (2006) **7**(S1):9.
126. DANZEISEN R, SCHWALENSTOECKER B, GILLARDON F *et al.*: Targeted antioxidative and neuroprotective properties of the dopamine agonist pramipexole and its non-dopaminergic enantiomer SND919CL2x. *J. Pharm. Exp. Therap.* (2006) **316**:189-199.
127. LORENZL S, NARR S, ANGELE B *et al.*: The matrix metalloproteinase inhibitor Ro 26-2853 extends survival in transgenic ALS mice. *Exp. Neurol.* (2006) **200**:166-171.
128. XU L, YAN J, CHEN D *et al.*: Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation* (2006) **82**:865-875.
129. KIAEI M, PETRI S, KIPIANI K *et al.*: Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. *J. Neurosci.* (2006) **26**:2467-2473.
130. ABITBOL J, CUVIER V, BORDET T, DROUOT C, BERNA P, PRUSS R: Safety and pharmacokinetics of repeated doses of TRO-19622, a drug candidate for the treatment of amyotrophic lateral sclerosis and spinal muscular atrophy. *Amyotroph. Lateral Scler.* (2006) **7**(S1):9.
131. SUGAI F, YAMAMOTO Y, MIYAGUCHI K *et al.*: Benefit of valproic acid in suppressing disease progression of ALS model mice. *Eur. J. Neurosci.* (2004) **20**:3179-3183.
132. BENATAR M, POLAK M, KAPLAN S, GLASS J: Preventing familial amyotrophic lateral sclerosis: is a clinical trial feasible? *J. Neurol. Sci.* (2004) **251**:3-9.
133. PASINETTI G, UNGAR L, LANGE J *et al.*: Identification of potential CSF biomarkers in ALS. *Neurology* (2006) **66**:1218-1222.
- **Implicates biomarkers that may facilitate diagnosis and facilitate more efficient trial designs.**
134. CHEUNG Y, GORDON P, LEVIN B: Selecting promising ALS therapies in clinical trials. *Neurology* (2006) **67**:1748-1751.
135. ROSEN DR, SIDDIQUE T, PATTERSON D *et al.*: Mutations in CuZn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* (1993) **362**:59-62.
136. HAND CK, KHORIS J, SALACHAS F *et al.*: A novel locus for familial amyotrophic lateral sclerosis on chromosome 18q. *Am. J. Hum. Genet.* (2002) **70**:251-256.
137. CHANCE P, RABIN B, RYAN S *et al.*: Linkage of the gene for an autosomal dominant form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. *Am. J. Hum. Genet.* (2002) **62**:633-640.
138. ABALKHAIL H, MITCHELL J, HABGOOD J, ORRELL R, DE BELLEROCHE J: A new familial amyotrophic lateral sclerosis locus on chromosome 16q12.1-16q12.2. *Am. J. Hum. Genet.* (2003) **73**:383-389.
139. SAPP P, HOSLER B, MCKENNA-YASEK D *et al.*: Identification of two novel loci for dominantly inherited familial amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* (2003) **73**:397-403.
140. NISHIMURA A, MITNE-NETO M, SILVA H *et al.*: A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* (2004) **75**:822-831.
141. GREENWAY MJ, ALEXANDER MD, ENNIS S *et al.*: A novel candidate region for ALS on chromosome 14q11.2. *Neurology* (2004) **63**:1936-1938.
142. GREENWAY M, ANDERSEN P, RUSS C *et al.*: ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. *Nat. Genet.* (2006) **38**:411-413.
143. MOMENI P, SCHYMIK J, JAIN S *et al.*: Analysis of IFT74 as a candidate gene for chromosome 9p-linked ALS-FTD. *BMC Neurol.* (2006) **6**:44.
144. MORITA M, AL-CHALABI A, ANDERSEN P *et al.*: A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology* (2006) **66**:839-844.
145. HUTTON M, LENDON C, RIZZU P *et al.*: Association of

missense and 5' splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* (1998) **393**:702-705.

146. WOOD JD, BEAUJEUUX TP, SHAW PJ: Protein aggregation in motor neurone disorders. *Neuropathol. Appl. Neurobiol.* (2003) **29**:529-545.
147. ROSS C, POIRIER M: Protein aggregation and neurodegenerative disease. *Nat. Med.* (2004) **10**(s):S10-S17.
148. STRONG M, KESAVAPANY S, PANT H: The pathobiology of amyotrophic lateral sclerosis: a proteinopathy? *J. Neuropathol. Exp. Neurol.* (2005) **64**:649-664.
- **Recent comprehensive review of protein aggregation hypothesis.**

Websites

201. <http://www.neuro.wustl.edu/neuromuscular/synmot.html>
Neuromuscular Disease Center of Washington University (2006).
202. <http://www.drugstore.com/pharmacy/prices/drugprice.asp?ndc=00075770060&trx=1Z5006>
Drug Store website (2006).
203. <http://www.aeoluspharma.com/AEOL10150dev.php>
AEOLUS Pharmaceuticals website (2006).
204. <http://www.clinicaltrials.gov/ct/show/NCT00355576?order=21>
The National Institute of Health Clinical Trials website, Combination Therapy Selection Trial (2006).
205. <http://www.alsconsortium.org/index.html>
Northeast ALS Consortium website (2006).

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