Emerging targets and treatments in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease that is currently untreatable. Many compounds have been tested in laboratory-based models and in patients with ALS, but so far only one drug, riluzole, has shown efficacy, yet it only slightly slows disease progression. Several new insights into the causes of motor neuron death have led to the identification of some important novel targets for intervention. At no time have studies involved such a wide range of innovations and such advanced technologies. Many promising studies are underway to test potential targets that will hopefully translate into meaningful therapeutics for patients with ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rare and devastating disease characterised by the progressive degeneration of motor neurons in the primary motor cortex, brainstem, and spinal cord, which results in muscle atrophy, paralysis, and death. The incidence is around two to three cases per 100 000 general population annually, and the prevalence is around four to six per 100 000.[1,2] Although ALS was first described more than 140 years ago by Charcot, most major advances in our understanding of the disease have been made in the past 10–15 years (figure 1). Notable advances include discovery of several causal gene mutations, development of in-vitro and in-vivo models, formation of national and international ALS consortia, and experience in the design and execution of clinical trials. The creation of multidisciplinary ALS teams has also greatly improved clinical care in the past few decades and improved patients' survival and quality of life. Emphasis is placed on the maintenance of adequate nutrition, and the use of non-invasive ventilatory support for respiratory symptoms and computerised communication devices.

Although disease pathogenesis is not fully understood, advances in genetics and molecular biology have led to identification of some important upstream mechanisms that might contribute to the death of motor neurons. Many compounds directed at these potential targets are being developed (figure 2) and human studies are underway or pending. Because of the continuing uncertainty about mechanisms underlying disease pathophysiology, we aim in this Review to describe the most promising putative mechanisms and discuss some of the therapeutics that have emerged (table 1), rather than to provide a comprehensive overview of all potential contributors.

Glutamate targets

Glutamate-mediated excitotoxic effects have long been postulated to have an important role in motor neuron degeneration in ALS. Additionally, despite many clinical trials testing promising compounds, the inhibitor of presynaptic glutamate release, riluzole (figure 2), is currently the only drug that has shown efficacy in a phase 3 study.[29,30] The drug was tolerated well, but survival was extended by only 2–3 months compared with placebo. Other medications targeting glutamate pathways in neurons—talampanel, memantine, topiramate, lamotrigine, gabapentin, and ONO-2506—have been studied, but all trials have been negative.[31–36]

Inactivation of synaptic glutamate is a key function of the EAAT2 (formerly GLT1) glutamate transporter on astroctyes in the protection of motor neurons from toxic effects. Ceftriaxone, a β-lactam antibiotic, increased astrocyte-mediated glutamate transport by stimulating expression of EAAT2 (figure 2) in an in-vitro blind screening study of 1040 medications approved by the US Food and Drug Administration.[37] In an animal model of ALS, use of this compound was associated with prolonged survival and upregulated transcription of messenger RNA for EAAT2.[37] A novel phase 1–3 trial with an adaptive design to test intravenous ceftriaxone against placebo is in its final phase and patients with ALS are actively being recruited across the USA and Canada (ClinicalTrials.gov, number NCT00349622).[3]

Protein misfolding and accumulation

5–10% of patients have familial ALS, which is clinically indistinguishable from sporadic ALS. The familial forms are generally autosomal dominant disorders with high penetrance. Around 20% of patients with familial ALS have a mutation in the gene encoding superoxide dismutase (SOD1)[38] that results in protein misfolding and an apparent gain in toxic function.[39] Abnormal protein aggregates are also seen in brain and spinal cord samples from patients with sporadic ALS, which suggests that protein misfolding and aggregation contribute to the pathogenesis of the disease, although a causative role remains controversial.[40–42]

Abnormal cytoplasmic accumulation of the nuclear protein TAR DNA binding protein 43 (TDP-43) is observed in most patients with sporadic ALS.[43] Inclusions of this protein are found in neurons and glial cells in the primary motor cortex, motor nuclei of the brainstem, spinal cord, and the associated white-matter tracts. TDP-43 is a DNA/RNA binding protein and is believed to play an important part in transcription and splicing regulation.[44] Although the exact role in ALS pathogenesis is unknown, TDP-43 inclusions are associated with

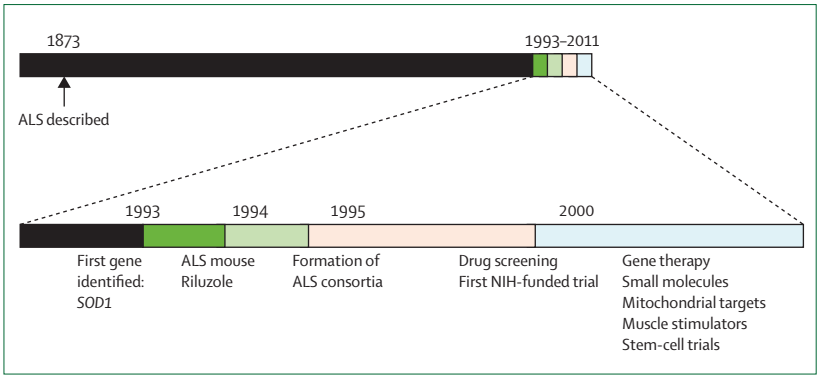

Figure 1: **The increasing pace of advances in ALS**
ALS=amyotrophic lateral sclerosis. NIH=National Institutes of Health.

abnormal nuclear staining, and pathological forms of the protein show evidence of abnormal processing.[45]

Heat shock proteins (HSPs) are important for continued cellular function under conditions of stress. They function as molecular chaperones that aid in the folding of normal proteins and degradation of abnormal proteins. Abnormalities in HSPs are purported to lead to motor neuron degeneration in ALS, and upregulation of HSP expression prolonged motor neuron survival in a *SOD1* transgenic mouse cell line.[46]

Arimoclomol is an oral compound that amplifies expression of *HSPA4* and *HSP90AA1*, which encode HSP70 and HSP90 proteins, respectively, leading to induction of endogenous cellular cytoprotective mechanisms that might prevent motor neuron degeneration under conditions of stress.[47,48] Arimoclomol delayed disease progression and extended survival by 22% in the *SOD1* transgenic ALS mouse model.[49] Placebo-controlled studies have shown that the drug is safe in human beings and that it penetrates the CSF (figure 2).[5] Efficacy studies in patients with familial ALS are underway and are actively recruiting participants (NCT00706147).[4]

The development of vaccine or passive infusion delivery of immunoglobulins to remove misfolded protein in ALS is a novel therapeutic strategy under investigation. Vaccination with SOD1 mutant protein in the ALS *SOD1* transgenic mouse model delayed disease onset and significantly extended survival.[6,7] Additionally, passive immunisation with antibodies against SOD1, via infusion through an intraventricular pump, also prolonged survival in this model.[6,8] Further preclinical safety data in animal models are required before studies are started in human beings, as an Alzheimer's disease study was stopped early after 6% of patients immunised with amyloid-β peptide developed meningoencephalitis.[50]

RNA targets

Advances in gene therapy have provided new targets for intervention in patients with autosomal dominant forms of familial ALS in whom the mutation is identified. Genetic testing of relatives of patients with familial ALS could also lead to intervention in family members who test positive but are presymptomatic or in the early symptomatic phase. The use of antisense oligonucleotides and small inhibitory RNA molecules to lower concentrations of mutant messenger RNA slowed disease progression and increased survival in the *SOD1* transgenic mouse model.[10–12] A phase 1 human trial of safety, tolerability, and pharmacokinetics of antisense SOD1 oligonucleotides administered intrathecally to patients with *SOD1* familial ALS is underway (NCT01041222).[9]

Although great potential exists in these novel interventions, RNA interference techniques are unlikely to benefit most patients with sporadic ALS or familial ALS caused by unknown mutations. To provide continuous downregulation of mutant RNA, compounds would probably need to be administered frequently and directly to the CNS through an intrathecal pump, from a reservoir, or as a bolus.

Abnormalities in RNA processing and metabolism might have pathogenic roles in sporadic and familial ALS. TDP-43[43] and fused in sarcoma protein (FUS)[51,52] have both been identified in inclusions in patients with ALS, and both function as DNA-binding and RNA-binding proteins. Growing understanding of the genetic and pathogenic post-translational modifications of these proteins has yielded new insights into disease pathogenesis and could lead to biomarker assays being developed for early diagnosis. Additionally, the development of novel transgenic animal models will improve the testing of promising compounds.[53,54]

Mitochondrial targets

Mitochondrial dysfunction might have an important early role in the development of ALS. In patients with sporadic disease, spinal cord samples taken at autopsy have demonstrated mitochondrial abnormalities in the anterior horn.[55,56] Abnormal aggregates of mitochondria were also found in intramuscular nerves and skeletal muscle.[57–61] Whether these features are causes or consequences of sporadic ALS remains uncertain. Although limitations exist when extrapolating findings from rodents to human beings, ALS animal models can help to provide clues about the most upstream pathological events. Israelson and colleagues[62] demonstrated a direct link between misfolded SOD1 and mitochondrial dysfunction. They found that the mutant protein binds directly to a key mitochondrial membrane protein, which interferes with normal function.

Some compounds proposed to improve mitochondrial function, such as minocycline and creatine (figure 2), have had beneficial effects in the ALS *SOD1* transgenic mouse model,[63–67] but have proved disappointing in human trials.[66,68,69] Although many possible reasons have been proposed for this discordance and the validity of animal models has been questioned, uncertainty about adequate bioavailability remains in the absence of a

reliable biomarker in ALS. Thus, future studies testing minocycline and creatine at different doses might prove efficacious. Atassi and colleagues[70] tested several doses of creatine in patients with ALS to assess effects on brain metabolites. They used magnetic resonance spectroscopy and found that the highest dose of 30 mg daily was associated with increased brain creatine concentrations and reduced glutamate concentrations. A phase 2 selection trial of creatine at this dose and two doses of tamoxifen is planned for 2011.

Other agents targeting mitochondrial function have been identified. Olesoxime (previously TRO19622) is a mitochondrial pore modulator that was discovered after screening about 40 000 compounds in an in-vitro motor neuron cell death assay.[14] A benefit was seen in the *SOD1* transgenic mouse model, with disease onset being delayed and survival being extended.[14] The neuroprotective effect of olesoxime is purported to be secondary to its direct binding to two components of the mitochondrial permeability transition pore. A phase 2/3 study of olesoxime is underway in Europe (NCT00868166).[13]

Pramipexole is a dopamine agonist used in the treatment of Parkinson's disease. It lowers oxidative stress, maintains mitochondrial function, and has neuroprotective effects independent of dopamine-receptor agonism.[71,72] Dexpramipexole, previously R+ pramipexole, is the optical enantiomer, which has less dopaminergic activity and can be tolerated at much higher doses. This drug prolonged survival of ALS *SOD1* transgenic mice,[73] and in a phase 2 study of 102 patients with ALS it was found to be safe and well tolerated. Motor decline seemed to lessen with increasing doses.[15] A large, international, phase 3 study of dexpramipexole is in progress.

Growth factors

A deficiency of growth factor support could provoke motor neuron death in patients with ALS. Several growth factors have shown efficacy in animal models but not in human trials. Clinical trials of brain-derived neurotrophic factor, ciliary neurotrophic factor, and insulin-like growth factor demonstrated no significant survival benefits.[74–80] With no reliable marker of biological activity in ALS, whether the compound was ineffective or whether physiological CNS concentrations were subtherapeutic remains uncertain. Additionally, neutralising antibodies and binding proteins that lower a compound's bioavailability can be produced when it is administered peripherally. Although the divergence between ALS animal and human studies might result from inherent mechanistic differences in disease pathophysiology, without a biomarker it remains unknown whether different doses or routes of administration would be effective.

Vascular endothelial growth factor (VEGF) is an endogenous protein that functions in the development of the nervous and vascular systems. A link between VEGF and ALS was first demonstrated in 2001, when

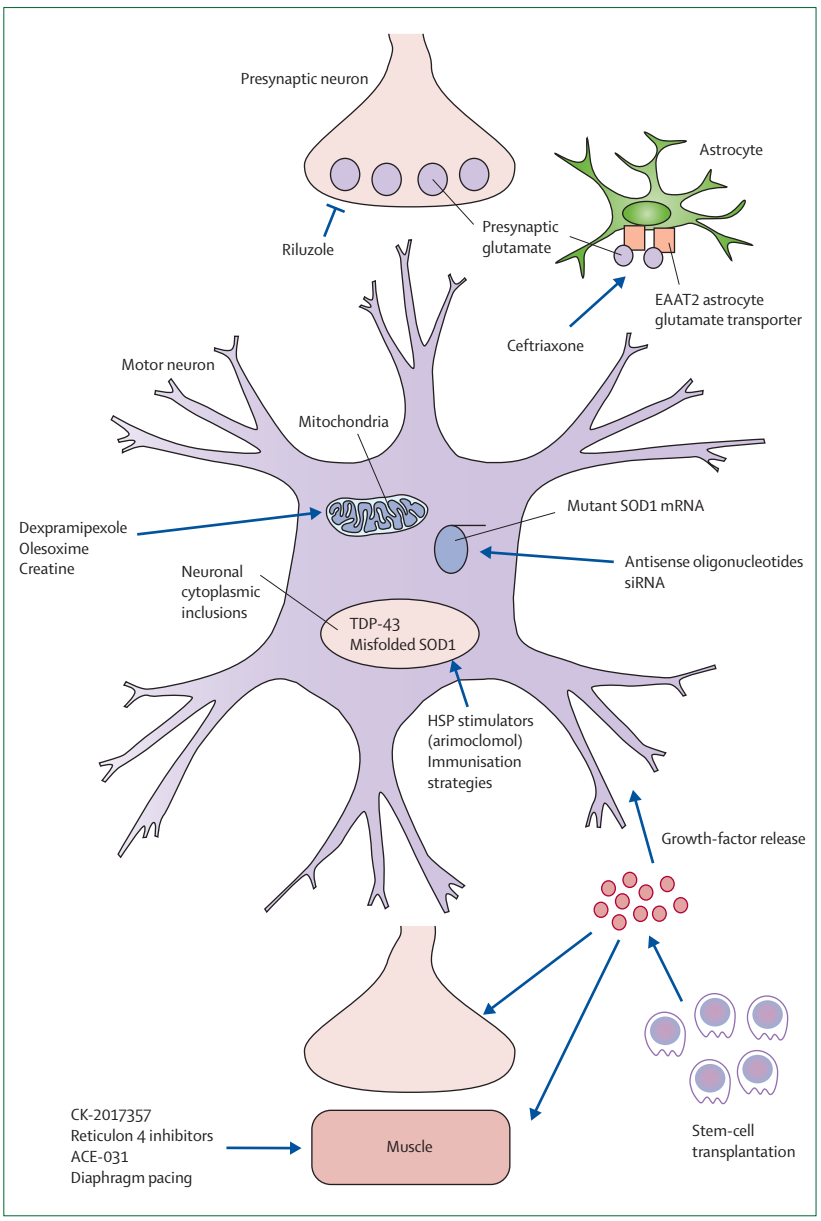

***Figure 2*: Novel therapeutic targets in ALS**
ALS disease pathogenesis remains unclear but a diverse range of targets offer promise for treatment. Glutamate-mediated excitotoxicity is a potential underlying disease mechanism, and presynaptic glutamate release is inhibited by riluzole, the only compound that currently shows efficacy in ALS. Ceftriaxone increases astrocyte-mediated glutamate transport by stimulating EAAT2 (formerly GLT1) expression, which inactivates synaptic glutamate, and is being assessed in a phase 3 study. For patients with familial ALS with a known mutation, antisense oligonucleotide infusion and administration of siRNA molecules are associated with reduced concentrations of mutant mRNA and slowed disease progression in animal ALS models. These treatments might prove effective in symptomatic and presymptomatic stages. Protein misfolding and accumulation can be neurotoxic and immunisation with HSP stimulators (arimoclomol) might lessen the formation and propagation of inclusions and prevent motor neuron degeneration under conditions of stress. Mitochondrial impairment could be a key upstream pathogenic mechanism. Olesoxime, dexpramipexole, and creatine, which target mitochondrial function, are under investigation. Although ALS is a motor neuron disease, interventions directed towards improving muscle function might improve quality of life. These include the insertion of diaphragm pacers to improve respiratory function, skeletal muscle troponin activators (CK-2017357), GDF-8 (myostatin) inhibitors (ACE-031), and reticulon 4 (Nogo-A) inhibitors (GSK1223249). The most effective outcomes might result from combinations of compounds targeting multiple mechanisms simultaneously. Growth factor infusion and stem-cell transplantation into the CNS might serve to support motor neurons and delay degeneration. ALS=amyotrophic lateral sclerosis. mRNA=messenger RNA. siRNA=small interfering RNA. HSP=heat shock protein.

	Proposed mechanism	Stage of development	Preliminary results and comments
Glutamate targets			
Ceftriaxone[3]	Decreases synaptic glutamate	Phase 3 study	Criteria for tolerability met; study in stage 3 and more than two-thirds of patients recruited
Protein misfolding			
Arimoclomol[4]	Amplifies HSP gene expression	Phase 2/3 study in FALS	Human placebo-controlled study showed safety and CSF penetration[5]
Immunisation	Removes misfolded SOD1	Preclinical studies	Promising preclinical data in *SOD1* transgenic mouse model[6–8]
RNA targets			
Antisense *SOD1* oligonucleotides (ISIS 333611)[9]	Lowers concentrations of mutant SOD1	Phase 1 study in FALS	Concentrations of mutant messenger RNA reduced with antisense oligonucleotides and small inhibitory RNA molecules, leading to slowed disease progression in the mutant *SOD1* transgenic mouse model[10–12]
Mitochondrial targets			
Olesoxime (TRO19622)[13]	Mitochondrial pore modulation	Phase2/3 study	Preclinical studies showed in-vitro and in-vivo efficacy[14]
Dexpramipexole[15]	Increases mitochondrial function	Phase 3 study	Phase 2 study of 102 patients with ALS showed safety and tolerability, and motor decline lessened and survival improved in a dose-response manner
Growth factors			
VEGF (sNN0029)[16]	Angiogenesis and neuroprotection	Phase 1/2 study of intracerebroventricular administration	Preclinical animal data showed efficacy[17,18]
Stem-cell therapy			
Bone marrow or embryonic stem cells into CNS[19–22]	Neuroprotection	Phase 1 study	For both strategies, additional preclinical and safety data required Optimum cell-type, dose, cofactor requirements, and location of transplantation unknown
iPS cells in SC	Neuroprotection	Phase 1 study pending	
Muscle targets			
Diaphragm pacing[23]	Diaphragm contraction	Phase 1 study	Safety and efficacy reported[24] FDA approval for humanitarian designation exemption pending
Skeletal muscle troponin activator (CK-2017357)[25]	Increases muscle force	Phase 2 study completed	Fatigue, strength, and pulmonary function improved in a dose-response manner[26]
GDF-8 (myostatin) inhibitor (ACE-031)[27]	Promotes muscle growth	Phase 1 study in postmenopausal women	Future studies in ALS expected
Reticulon 4 (Nogo-A) inhibitor (GSK1223249)[28]	Promotes neurite growth	Phase 1 study	Study to be completed in 2011

ALS=amyotrophic lateral sclerosis. HSP=heat shock protein. FALS=familial ALS. VEGF=vascular endothelial growth factor. iPS=induced pluripotent stem. SC=spinal cord. FDA=US Food and Drug Administration.

***Table 1*: Summary of ALS therapeutic targets being tested in clinical trials**

transgenic mice with a homozygous deletion in the VEGF promoter region developed motor neuron disease with pathological features similar to those of *SOD1* transgenic mice.[81] Motor neuron death was postulated to be secondary to inadequate neurotrophic support by VEGF, to insufficient vascular supply resulting in chronic ischaemia, or both. Delayed disease progression and improvement in survival was seen after intraperitoneal injection of VEGF in *SOD1* transgenic mice,[17] and after intracerebroventricular injection of VEGF in *SOD1* transgenic rats.[18] In human beings, an increased risk of sporadic ALS was initially associated with three VEGF promoter region haplotypes, and concentrations of VEGF in serum were lower than in unaffected spouses.[82] Several studies have subsequently contradicted these findings, and a large meta-analysis did not support the association.[83] In view of the observed benefits of VEGF in ALS animal models, a phase 1/2 study is underway to test intracerebroventricular administration of VEGF in patients with ALS (NCT00800501).[16]

Penetration of large peptides, such as growth factors, into the CNS is limited when they are administered peripherally. In addition to intrathecal injections and pumps, novel modes of delivery of growth factors and large molecules will probably emerge. Stem cells can be modified to serve as a reservoir for growth factor release, and the safety of injection directly into the CNS is being assessed. A novel, non-invasive technique currently being developed is the use of focused ultrasound to transiently and safely disrupt the blood–brain barrier and enable passage of large molecules. This technique has potential in the treatment of various CNS diseases, and is actively being tested in animal models.[84]

Stem-cell therapy

Cell-based therapies for the treatment of neurodegenerative diseases continue to be a growing source of attention and hope worldwide. The possibility that transplanted stem cells could replace dead motor neurons or protect surviving neurons (figure 2) is an exciting prospect for patients with ALS and the research community. The creation of induced pluripotent stem cells from skin fibroblasts has circumvented ethical issues related to use of embryonic stem cell tissue. Additionally, transplanted induced pluripotent stem cells derived from an individual's own fibroblasts would lower the risk of rejection and the need for immunosuppressive treatments. However, despite the existence and advertisement of many for-profit commercial stem-cell facilities keen to treat patients with various diseases, no appropriately designed study has yet shown efficacy.

Although stem cells can differentiate into motor neurons, the ability to replace dead motor neurons and make meaningful connections with previously denervated muscle currently seems doubtful.[85] Stem-cell-derived motor neurons implanted in the spinal cord in animals have extended axons towards muscles, which has resulted in improved limb function.[86] The functional relevance in patients with ALS is, however, questionable in view of the pace of disease progression and the length of axon growth that would be required compared with that in rodents.

The strategy of using stem cells to protect damaged motor neurons seems more feasible than replacement. Neighbouring astrocytes and microglia are important contributors to motor neuron survival and disease progression in ALS.[87–90] Stem cells can be directed to differentiate into non-neuronal cells and protect surviving motor neurons through the release of specific growth factors or the expression of enzymes or transporters to detoxify the local environment. This strategy is supported by studies in transgenic rat models of ALS that showed neuroprotective effects after stem-cell transplantation without any meaningful motor neuron growth or muscle reinnervation.[91]

Intrathecal and intravenous transfer of autologous mesenchymal stem cells in patients with ALS seems safe,[92] but it is generally accepted that the optimum strategic approach would include implantation of stem cells directly into the brain and spinal cord. Mazzini and co-workers[20] reported on an open-label pilot study in which mesenchymal stem cells were injected into the thoracic spinal cord of nine patients with ALS. No serious adverse events were seen, and in four patients the linear decline of forced vital capacity and changes in the ALS functional rating scale scores were significantly slowed. In another non-randomised study of 10 patients with ALS, autologous CD133+ bone marrow stem cells were transplanted into the frontal motor cortex. The procedure was safe and well tolerated and survival improved in treated patients compared with that in non-treated patients.[21] Deda and colleagues[22] harvested autologous bone-marrow stem cells and transplanted them into the upper cervical cord in 13 patients with bulbar ALS. Nine patients improved notably after administration and had no adverse effects.

In view of the small sample sizes, heterogeneity of disease severity within the cohorts, and lack of adequate control groups in these pilot studies, the results must be interpreted with caution. Assessment of the integrity and survival of the grafted cells at autopsy would be useful, and long-term safety must be better ascertained. Although several studies of stem-cell transplantation are underway, the optimum cell type, dose, dosing frequency, and location of administration remain unknown. Whether systemic trophic factors and immunosuppressant drugs are required also needs to be clarified. Preclinical and safety studies are still required to improve our understanding of stem-cell interventions and to better ascertain the potential risks and benefits. Clinical trial designs need to be debated owing to the importance of ethical challenges in including sham control groups to asses the efficacy of this invasive therapy.[93] The distinction between stem-cell trials approved by academic research ethics boards and the commercial delivery of stem cells for payment is crucial for patients.

Muscle targets

The major cause of mortality and an important source of morbidity in patients with ALS is respiratory failure resulting from progressive weakening of the diaphragm. Class I evidence supports the initiation of non-invasive ventilation to improve survival and quality of life.[94] Although the sample size was small, the median survival benefit of bi-level positive airway pressure in patients with ALS without severe bulbar impairment was 205 days, which was better than that achieved with riluzole.

In view of the importance of diaphragm function in improving quality of life and survival, various devices have been assessed. Preliminary results from a trial of diaphragm pacing with laparoscopically placed electrodes suggest that use is safe and slows respiratory decline in patients with ALS (NCT00420719).[23,24] If proven effective, diaphragm pacing could lengthen the time until assisted ventilation is required. For now, the device remains experimental and should be used only as part of a clinical trial. In the USA, Food and Drug Administration approval for humanitarian designation exemption is pending, which, if granted, would increase access for patients with ALS.

Although ALS is a motor neuron disease, compounds that strengthen the diaphragm and other muscles might have clinical benefits. ACE-031 is an investigational protein therapeutic that inhibits GDF-8 (myostatin) and other factors that act as negative regulators of muscle growth (figure 2). Notable growth of lean muscle mass and increased strength has been seen with ACE-031 treatment in animal models.[95,96] Preliminary results from a phase 1b

	Possible solutions
Patient factors	
Poor recruitment owing to low disease incidence and prevalence	Improve education and information about study rationale and design Advertise trials in clinics, ALS societies, and patient-run internet forums Use video recruitment tools to share information about treatments being studied Broaden inclusion criteria—eg, inclusion of patients with possible ALS
Resistance to receiving placebo because of easy access to medication outside trial	Increase amount of information on trial design and use of placebos Develop surrogate markers to shorten study duration and decrease sample size requirements Use time-to-event study designs that enable crossover to active treatment Use 2:1 randomisation to active compound groups
Disease heterogeneity	Identify biomarkers and imaging disease markers
Study design factors	
High costs, large sample size requirements, and resource limitations	Develop sensitive and surrogate outcomes (to replace survival) for early phase trials Do multidrug phase 2 selection studies Develop adaptive study designs Make survival a coprimary or secondary outcome Develop sequential trial designs Do non-superiority (futility) analyses
Retention of participants and missing data	Replace some clinic visits with home assessments Remotely collect outcome data by telephone or computer
Drug factors	
Development and screening of promising compounds	Develop novel transgenic animal models that better represent human disease Use high-throughput screening
Pharmacokinetics and bioavailability	Do dose-ranging and pharmacodynamic phase 2 studies Measure drug concentrations in CSF and use intrathecal pumps Identify biomarkers

Table 2: **Issues in ALS clinical trials and potential solutions**

study in 60 healthy, postmenopausal women suggest that subcutaneous treatment is well tolerated and increases lean muscle mass and volume.[27] A phase 2 study in patients with Duchenne muscular dystrophy is expected in the future, and this agent might have uses in ALS.

CK-2017357 is another drug that targets skeletal muscle (figure 2), and it is being tested in patients with ALS (NCT01089010).[25] It selectively activates fast skeletal muscle troponin complex by increasing sensitivity to calcium, which leads to increased muscle force. In a phase 2 study of patients with ALS given two doses of CK-2017357, treatment was well tolerated and fatigue, strength, and pulmonary function improved in a dose-dependent way, compared with placebo.[26]

Reticulon 4, also known as neurite outgrowth inhibitor A, is a protein found in skeletal muscle and neurons that functions as an inhibitor of nerve growth, sprouting, and regeneration.[97] Expression of this protein in the skeletal muscles of patients with ALS correlates with disease severity,[98] and genetic ablation of reticulon 4 in an ALS *SOD1* transgenic mouse model extended survival.[99] An international phase 1 trial is underway to investigate the safety and pharmacodynamics of a humanised monoclonal antibody against reticulon 4 (NCT00875446).[27]

Although muscle growth factors and augmenters and mechanical stimulation of the diaphragm might prove beneficial in patients with ALS, whether these interventions will affect the speed of motor neuron degeneration remains unclear. Nevertheless, further exploration of alternative strategies and targets is crucial owing to the severity of the disease and present lack of meaningful treatments.

Novel trial designs

As additional promising compounds are identified in animal and in-vitro studies, demand for resources and participants for phase 1–3 trials will increase. Several inherent challenges are faced by researchers in ALS studies, including the rarity of the disease, the long time between symptom onset and diagnosis, the heterogeneity of the disease, which necessitate large sample sizes, and the high drop-out rate in trials (table 2). Novel trial designs and endpoints can help to shorten the length and lower costs of studies, control for confounders, improve enrolment, and ensure that optimum therapeutic doses are chosen.

Various compounds that have yielded promising results in *SOD1* transgenic rodent models later showed no benefit in expensive human trials.[100] Several explanations have been proposed to account for the frequent inconsistencies between human and animal studies in ALS, including flawed trial design, but the lack of suitable biomarkers is of greatest importance. The pharmacokinetics of a drug in animals and human beings might differ substantially. Without a validated biomarker of disease progression, it is difficult to know whether a compound that failed in a clinical trial would have been efficacious if administered at a different dose or via a different route. Additionally, a reliable biomarker can

expedite the drug screening process, which could reduce the sample size, duration, and cost of a clinical trial. Biomarkers can also help to identify homogeneous cohorts of patients and those in the earliest phases of the disease, when therapeutics might be more effective. The former would be especially beneficial in view of the heterogeneity in ALS. The development and screening of compounds for treatment of multiple sclerosis has been helped substantially by the use of lesion burden on MRI as a marker of disease progression. The identification of protein-based biomarkers in the CSF and the development of sensitive imaging techniques will improve the efficiency of screening promising ALS treatments.

Phase 3 trials in ALS frequently use survival as the primary outcome and an experimental compound typically must yield a significant survival benefit before approval by regulatory agencies. Trials that use this endpoint, however, are expensive and require recruitment of a large number of patients over a long duration. Enrolment into survival studies can be difficult, as patients might be reluctant to commit to a long-term study in view of the risk of being assigned placebo for the entire study. The alternative approach of a trial in which all participants receive the active compound and historical controls are used for comparison might assist with enrolment, but confounders and co-interventions would limit interpretation. Drop-out rates in survival studies can be high if patients perceive no improvement in disease during the study, which presents major statistical challenges in intention-to-treat designs. These issues are compounded when a drug being tested is easily accessible through a family physician or can be ordered on the internet.

An alternative endpoint to survival is the use of the revised ALS functional rating scale (ALSFRS-R). This scale has shown high inter-rater and intrarater reliability.[101] The ALSFRS-R can be easily administered in person or remotely, which keeps to a minimum the number of clinic visits and data loss, is clinically relevant, and correlates well with survival.[102]

Change in the ALSFRS-R from baseline was used as a primary outcome in a study of lithium and riluzole therapy in ALS done by the Northeast and Canadian ALS Research consortia.[103] This approach enabled a time-to-event design to be used, whereby patients assigned to receive placebo were switched to the active compound once this endpoint was reached. This design enabled the study to be double-blind and placebo controlled while limiting the period of time patients were exposed to placebo. Other advantages were that recruitment was extremely rapid and that prespecified interim analyses were done, leading to the trial being terminated early for futility, which saved expense and resources.

The use of randomised sequential trials with multiple interim analyses might lead to a notable reduction in sample sizes without a loss in power compared with traditional phase 3 study designs. This type of design has been used effectively and the number of patients with ALS required to show that creatine[69] and valproic acid[104] were ineffective were kept to a minimum.

In view of the large number of promising therapeutics being developed, the limited number of patients, and the costs associated with phase 2 and 3 studies, the testing of more than one compound in a phase 2 trial would be advantageous. Multidrug phase 2 selection studies allow multiple compounds to be tested simultaneously to assess the best drug or optimum dose that can be investigated further against placebo. This approach would greatly expedite the search for effective therapies compared with the time that would be required to test each compound individually versus placebo.

A multistage adaptive design can also be used whereby the selection of the most promising compound or dose in a phase 2 study is immediately assessed in a larger, placebo-controlled, phase 3 study. This design has many advantages over traditional, independent, sequential phase 1, 2, and 3 studies, because separate trials take several years and incur high costs. Although multistage trials involve several statistical modifications that lead to a small loss in power, overall these strategies substantially shorten the period of time necessary to screen a large number of promising compounds.[105] This adaptive design strategy has been used successfully in a study of ceftriaxone, in which a high dose was chosen over low dose in the first stage of the trial.[106] The design is also being used in a phase 2 selection study of high-dose creatine plus two doses of tamoxifen.[106]

Conclusions

Although only one medication, riluzole, has so far been proven to slightly slow disease progression in ALS, and 16 years have passed without another success, there is a great sense of optimism and momentum among patients with ALS and researchers. Much has been learned from failed studies and emphasis has been placed on the importance of understanding disease pathogenesis. Although various pathological factors have been found in ALS animal models and human beings, the challenge is to elucidate the most upstream events that initiate and perpetuate motor neuron loss. ALS genetic models and the uncovering of novel mutations in cases of familial ALS have provided important clues to the understanding of these events.

We anticipate that targets identified through the study of genetic ALS models will also help in the understanding of the pathogenesis of sporadic disease, which accounts for most cases of ALS. A large proportion of patients diagnosed as having sporadic ALS might ultimately turn out to possess mutations acquired spontaneously or through non-Mendelian patterns of inheritance with incomplete penetrance. Blood samples should be collected from all patients with ALS and pooled and shared for genetic screening.

The identification of reliable markers of disease will be crucial to the development of effective therapeutics and

Search strategy and selection criteria

Full-text articles for this Review, published from January, 1993, to January, 2011, were identified in PubMed with a combination of the search terms "amyotrophic lateral sclerosis", "ALS", "motor neuron disease", "clinical trials", "glutamate", "mitochondria", "pathophysiology", "genetics", "heat shock proteins", "growth factors", "stem cells", and "biomarkers". Other journal articles were identified by manual searches of the reference lists of selected articles. Only English-language articles were reviewed. Papers were selected on the basis of relevance to the topic of this Review, favouring the most recent publications. We also accessed ClinicalTrials.gov to obtain information on current and future registered studies of ALS.

to clarify CNS bioavailability and the most efficacious doses for promising agents. A biomarker will enable development of therapeutics titrated towards specific targets and knowledge attained from negative studies will be much more valuable in helping to refocus future research. Protein or imaging disease markers will also help to identify patients in the earliest phase of the disease when treatment might be most effective.

ALS biomarkers might enable selection of more homogeneous cohorts of participants for studies, and several previously non-efficacious compounds might have reached efficacy if study groups had been less heterogeneous. At present, patients in studies are grossly dichotomised as having either bulbar or limb-onset ALS. A patient with bulbar disease and a weak, atrophic, and fasciculating tongue (lower motor neuron predominant pathology), however, might respond very differently to an intervention than a patient with a normal looking, slow-moving tongue and severely spastic speech (upper motor neuron predominant pathology). CSF samples should be collected for proteomic analysis, and advanced imaging techniques will greatly assist in reducing the variability of study cohorts and enable reductions in sample sizes.

At no time have studies involved such a wide range of innovations and such advanced technologies. Gene therapies, small molecules, mitochondrial modulators, muscle stimulators, and stem-cell studies are all underway. In the future, multiple pathways might be targeted within single studies or tested in trials with multistage adaptive designs to rapidly detect the most efficacious compounds for assessment in phase 3 trials. Such improvements in study design will lower costs and lead to the limited number of patients with ALS being studied in the most efficient ways. We are excited about a brighter future with effective therapeutics for patients with ALS.

Contributors

Both authors contributed equally to the literature searches and the writing and revision of the manuscript.

Conflicts of Interest

MC has received research grants from Neuralstem, ISIS, and Knopp Neuroscience, and has consulted for Trophos and Synapse, serving as chairperson of the data and safety monitoring board. LZ declares that he has no conflicts of interest.

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