Clinical trials in amyotrophic lateral sclerosis: why so many negative trials and how can trials be improved?

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Amyotrophic lateral sclerosis (ALS) is one of the most rapidly progressive neurodegenerative diseases of unknown cause. Riluzole is the only drug that slows disease progression. More than 50 randomised controlled trials (RCTs) of proposed disease-modifying drugs have failed to show positive results in the past half-century. In the past decade, at least 18 drugs have been tested in large phase 2 or 3 RCTs, including lithium, which was tested in several RCTs. Potential reasons for the negative results can be classified into three categories: first, issues regarding trial rationale and preclinical study results; second, pharmacological issues; and third, clinical trial design and methodology issues. Clinical trials for stem cell therapy and RCTs targeting pharmacological or non-pharmacological symptomatic treatment in ALS are examples of areas that need novel design strategies. Only through critical analyses of the failed trials can new and important suggestions be identified for the future success of clinical trials in ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and potentially rapidly terminal disease. Riluzole, approved for use in 1995, is the only available treatment for ALS. However, despite a slight positive effect on survival and function in some patients, riluzole use is limited by its overall poor benefits. For the past 20 years, results from most clinical trials of other drugs have been disappointing, which has reinforced the need to find new and effective treatments. Both the US Food and Drug Administration (FDA)[1] and the European Medicine Agency (EMA)[2] in 2013 spearheaded initiatives to address necessary changes in ALS clinical trials. There has been a surge of publications of ALS in the past decade, suggesting that a major breakthrough for the treatment of ALS might inevitably be realised. In this Review we assess past ALS clinical trials in an effort to glean insights that might pave the way for future successes.

Modern ALS clinical trials began in the 1980s and initially investigated whether treatment of a presumed poliovirus infection would be effective, because latent polio was suspected as the main cause of ALS at the time. The appendix summarises the general process of drug development, from the initial stages of basic science to the execution of clinical trials, and illustrates the specific phases of randomised controlled trials (RCTs). The standard diagnostic criteria for ALS (El Escorial Criteria; appendix)[3,4] were developed in 1994 and revised in 1998; further revisions are in process. Present clinical trial guidelines were established in 1999 by the World Federation of Neurology (WFN) Research Group on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis.[5,6] Implementation of these guidelines has led to progress in the field of ALS and clinical outcome measures have been widely validated. However, although advances in the care of patients with ALS in multidisciplinary clinics and the wider deployment of advanced respiratory and nutritional treatments have led to improved overall survival, no successes have been achieved in the pharmacological treatment of ALS.

Disease-modifying treatments are developed and tested to target specific hypothetical pathogenic mechanisms; drugs for ALS are no exception. Many drugs with different modes of action have been tested in RCTs.[7–18] However, because the precise, possibly heterogeneous, pathogenesis of ALS remains mostly unknown, development of treatments that are effective across the spectrum of sporadic and familial ALS has not been achieved. Disease-modifying therapies, stem cell therapy, and advanced symptomatic treatments (either pharmacological or non-pharmacological) might help to reduce a patient's symptoms, such as sialorrhoea, pseudobulbar affect, dyspnoea, muscle cramps, and spasticity.

Why are there so many negative trials?

RCTs of disease-modifying treatments

Nearly 50 RCTs for disease-modifying treatments have been undertaken in the past half-century (table 1). The FDA approval rate of investigational compounds from the time they first entered clinical trials is about 16% for trials initiated by pharmaceutical companies.[19] With around 50 RCTs undertaken, riluzole is the only FDA and EMA approved drug, which emphasises the need for a crucial reassessment of methods used in ALS drug development at the level of clinical trial design and at implementation.

Our poor knowledge about the prime mechanism of motor neuron degeneration in ALS is a barrier to drug development (figure). Hypotheses of ALS pathogenesis have been derived from studies of patients, ex-vivo tissues, genetics, a combination of human disease and animal models, or solely from animal studies. Because of the rapidly fatal and so far intractable nature of this disease, the ALS community tends to welcome new ideas and hypotheses. Because we have several hypotheses but the definitive pathogenic mechanisms are unknown, when a potential drug does become available, the general feeling is that it should be tested expeditiously. Hopefully, the focus will be put on justifying the need for vigorous investigations to elucidate and target disease mechanisms.[20,21]

	Drugs tested	Presumed mechanisms	General comments
Viral infection	Transfer factor, tilorone, indinavir	Antiviral	Weak rationale, side-effects
Excitotoxicity	Branched-chain aminoacids, threonine, lamotrigine, riluzole, gabapentin, nimodipine, dextromethorphane, topiramate, memantine, talampanel*, ceftriaxone*	Reduces glutamate release, calcium channel blocker, reduces glutamate, NMDA receptor blocker, GABA-analog, glutamate AMPA receptor blocker antagonists, increases astrocytic glutamate transporter activity[7–9]	NMDA receptors are not critical on motor neurons; AMPA receptor antagonist might cause adverse effects[10]*
Growth factors	Cholinesterase inhibitors, octacosanor, gangliosides, thyrotropine releasing hormone, growth hormone, erythropoietin*	Myotrophic effects, systemic trophic factors, ergotropic effects[11]	Weak rationale, short plasma half-life for thyrotropine releasing hormone[11]*
Neurotrophic factors	CNFT, IGF-1*, BDNF, GDNF, xaliprodene*, GCSF	Pleotropic neurotropic receptors, retrograde transport from the muscle axon terminals, serotonin ($5HT_{1A}$) agonist[12]	Systemic injections of neurotrophic factors do not cross blood–brain barrier[12]*
Neuroinflammation	Plasma exchange, cyclosporine, total lymphoid irradiation, glatiramer acetate*, cerecoxib*, minocycline*, NP001	Humoral factors, T-cell, microglial suppressor, general anti-inflammatory, vaccination theory, T-helper cells[13,14]	*
Oxidative stress	Acetylcysteine, glutathione, selegiline, vitamin E*, CoQ10*, edaravone	Increases anti-oxidative property, free radical scavenger[15,16]	Access to nervous system is uncertain in most drugs tested
Apoptosis	Pentoxyfilline*, TCH346*, minocycline*	TNFα linked apoptosis, GAPDH-linked apoptosis	*
Mitochondrial dysfunction	Creatine*, acetyl-L-carnitine*, dexpramipexole*, olesoxime*	Mitochondrial membrane permeability stabilising effects	*
Genetic defects	Phenylbutirate, valproic acid, antisense oligonucleotide treatment	Histone deacetylase inhibitor, blocks production of some proteins	Early phase 1 and 2 studies with more studies ongoing
Autophagic vesicles and peroxisome	Lithium carbonate*, pioglitazone*	Facilitates degradation of protein aggregates[17]	*
Astrocytes	Ono-2506	Blocks gliosis	A negative study, but the results were not published
Proteinopathy	Arimoclomol	Facilitates degradation of protein aggregates[18]	Being tested in patients with SOD1 mutations

Drugs tested in RCTs are listed in chronological order for every pathogenic category. CNFT=ciliary neurotrophic factor. IGF-1=insulin-like growth factor 1. GCSF=granulocyte colony-stimulating factor. RCT=randomised controlled trial. CoQ10=coenzyme Q 10. TNFα=tumour necrosis factor α. SOD1=superoxide dismutase 1. *Disease-modifying therapies discussed in more detail in table 2. The overall review is available in reference 19.

Table 1: **Hypothetical pathogenic targets and disease-modifying treatments tested during the past 50 years**

23 RCTs (of 18 different drugs) completed in the past decade[22–47] were progressively better organised than previous trials, but had recurrent and similar issues (table 2). Eight different hypothetical, pathogenic targets underlie the rationale for these RCTs, which were undertaken on the basis of the hypothetical therapeutic targets and evidence derived from superoxide dismutase (SOD) 1 transgenic mouse models, previous studies in human beings, or both. Opinions, voiced by the principal investigators, varied on the study failures (panel 1). There was no single reason why results were negative, instead, many possibilities were noted and thus could have interacted to contribute to the outcomes. These reasons for failure can be classified into three categories. First, with respect to the rationale that precedes RCTs, investigators showed that two-thirds of negative studies were potentially misled by positive studies in SOD1 mice: 14 (78%) of 18 RCTS were based on previously positive SOD1 preclinical studies; however, SOD1 transgenic rodent models do not recapitulate sporadic disease in man and minimally replicate mutant *SOD1* familial ALS.[48] Additionally, early animal studies had serious methodological flaws. In most studies treatment began at the presymptomatic stages, leading to possible neuroprotective outcomes. Such results have reduced clinically applicability because, by the time treatment begins for patients with ALS, the disease is well established and has already substantially progressed. Failure after results from an early, positive human trial, suggested that small phase 2 studies can also be potentially misleading (panel 1).[45]

Second, in most studies, investigators expressed concerns about pharmacological analyses, including issues of doses being too low, U-shaped effectiveness curves, CNS access, or the absence of pharmacokinetic and pharmacodynamic analyses (panel 1). The most frequent concern was the potential interaction between the study drug and riluzole. Almost all European investigations of new drugs were add-on studies because patients are already taking riluzole, whereas in North American studies, a proportion of patients (around 20–40%) do not take riluzole, mainly because of cost and an absence of insurance coverage. Pharmacological considerations of study drugs and effects of riluzole have not been fully studied during many RCTs, which raises a serious concern that drug effects might have been eluded.

Third, potential problems in clinical trial design and methodology issues can lead to negative results in RCTs. One of the simplest problems is the expected treatment

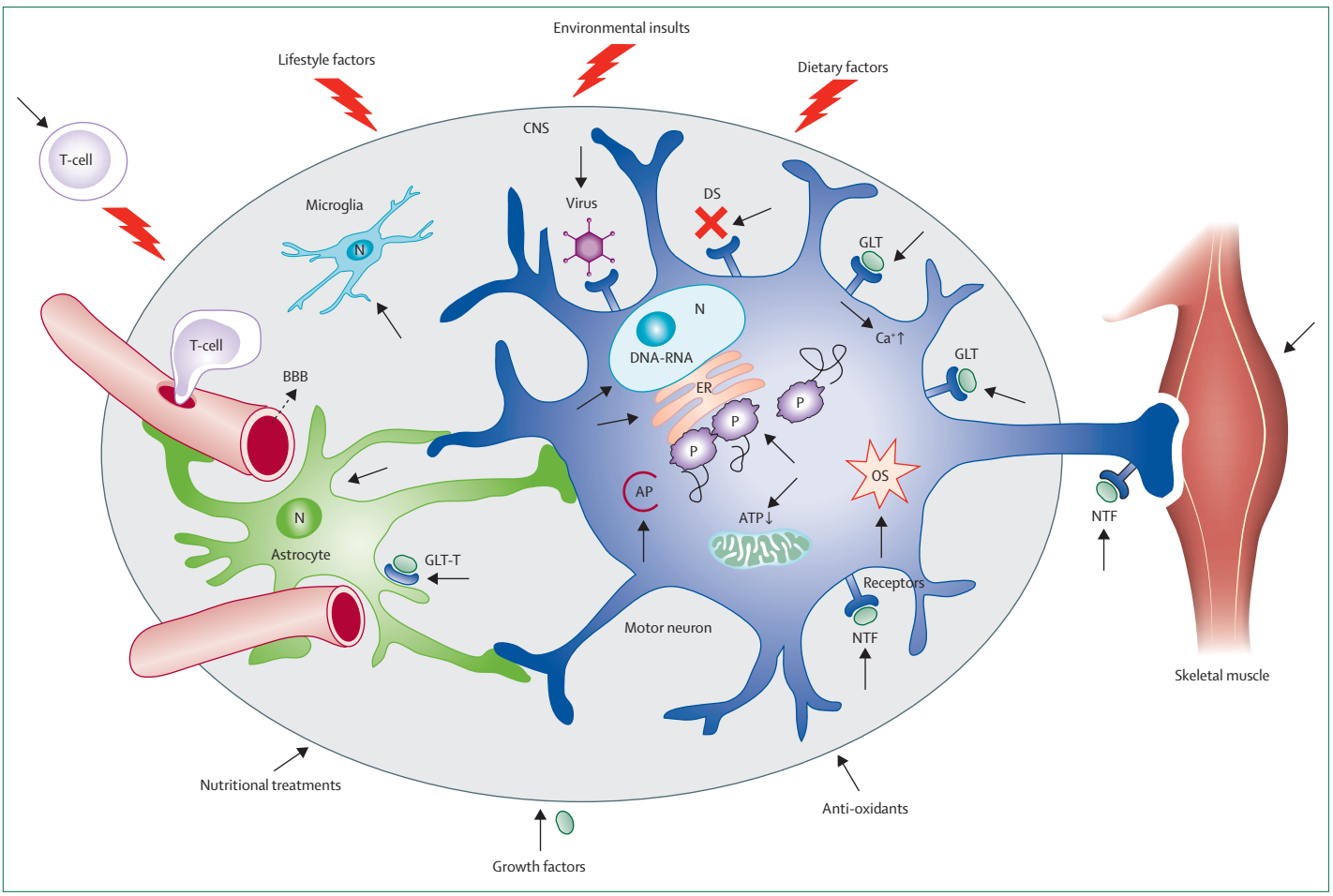

Figure: **Hypothetical pathogenic mechanisms and treatment interventions**
Presumed drug targets are shown that act on presumed pathogenic sites compiled on the basis of the best available evidence in motor neurons, astrocytes, microglia, and T-cell lymphocytes in the CNS. Black arrows show drug targets that have been investigated in randomised controlled trials. Red lightning bolts show other insults to the CNS and neuronal system for which no specific treatments are available. AP=autophagy. BBB=blood–brain barrier (in association with neurovascular impairment). Ca^{+}=calcium ion. DS=death signal or apoptosis. ER=endoplasmic reticulum. GLT=glutamate. GLT-T=glutamate transporter. N=nucleus. NTF=neurotrophic factors. OS=oxidative stress. P=proteins.

effects reported in studies (50% or 40% difference from placebo), which might be unrealistic. At times, budget constraints drive investigators to set high treatment effects. Therefore, small positive effects could have been missed. Investigators raised other diverse and highly concerning problems, including disease variability, patients of an advanced stage of disease progression at enrolment, and the study duration being too short.

Across most of these trials, a glaring deficiency is that most studies did not attempt to test whether the drug affected the disease pathogenic target in patients with ALS and did not test objective biomarkers. With the present level of knowledge in ALS clinical science, no methods exist to test such targets in patients. Therefore, present RCTs in ALS can only establish whether the drug has any clinical effects. Because the cause and pathogenesis of ALS are unknown, investigations of whether the drugs tested have any biological effects on the target are essential.

Several positive reports either in patients with ALS or animal models triggered many RCTs. Thyrotropin-releasing hormone, which exerted substantial transient improvement of muscle strength, led to several RCTs.[49] Gabapentin[50] and creatine[51] showed benefits in SOD1 models, which again prompted several RCTs with these drugs. After a study in 2008[17] described positive results of lithium, both in a preclinical study with SOD1 mice and patients with ALS, understandably, many patients with ALS desperately wanted lithium. Yet, many investigators were uneasy about this unusual report of positive results, which presented preclinical animal and patient data.[52] In the preclinical animal study of lithium, only male mice in the presymptomatic stages were tested. Further studies were advised.[53] A year after the original report, a study of female mice and a separate sex-balanced study in two different SOD1 models failed to reproduce earlier results.[54,55] The clinical trial of the original report consisted of a small, single-blind,

	Rationale to proceed to RCT			Initiated by	Primary outcome	Study duration	Percentage of effect size	Number of patients	Riluzole use of all patients enrolled	Pharmacological assessment	Presumed biological target analysed	Discussion of negative results	Comments
	Hypothesis	SOD1 transgenic model	Early patient studies										
Xaliproden (2004)[22]	Neurotrophic factor	Others	Positive phase 2	Ind	Survival VC <50%	18 months	38–34%	867 (study 1); 1210 (study 2)	none (study 1); 100% (study 2)	Insufficient	None	Yes, detailed	Large numbers of patients needed for positive results; drugs might affect survival and function differently
Creatinine (2004, 2008)[23,24]	Mitochondria	Yes	No	Inv	MVIC Slope	6 months; 9 months	50%; 15%	104; 107	No data	Only urine levels	None	Yes, detailed	Different phase 2 studies are needed
Vitamin E (2005)[25]	Oxidative stress	Yes	No*	Inv	Survival	18 months	50%	160	100%	Insufficient	None	Yes, detailed	More patients and longer duration studies are needed
Celecoxib (2006)[26]	Inflammation	Yes	No	Inv	MVIC slope	12 months	35%	200	69%	Yes	Yes, with CSF; PGE2	Yes, detailed	Detailed discussion on the rationale for the clinical trial
Pentoxifylline (2006)[27]	PDE4B-inhibitors and TNF-inhibitors	No animal tests	Off-label	Ind	Survival	18 months	N/A	400	100%	Insufficient	None	Yes, detailed	Survival worsened; drugs might affect survival and function differently
Minocycline (2007)[28]	Inflammation, apoptosis	Yes	Phase 2	Inv	ALSFRS-R slope	4 months lead-in; 9 months	18%	412	67%; 66%	None	None	Yes, detailed	No interaction with riluzole, but another study suggested adverse effects with riluzole[29]
TCH346 (2007)[30]	Apoptosis	Others	Phase 2	Ind	ALSFRS-R slope	16 week lead-in; 24 weeks	25%	591	86%	None	None	Yes, detailed	Several doses showed more deaths at higher doses
IGF-I (2008)[31]	NTF	Others	2 RCTs	Inv	MMT	24 months	25%	330	70%	None	None	None	Trial done to settle previous conflicting results
CoQ10 (2009)[32]	Oxidative stress	Yes	Yes	Inv	Decreased on ALSFRS-R	9 months	20%	185	76%	Plasma levels	Planned but not pursued	None	Study showed futility to progress to a phase 3 study
Erythropoietin (2009)[33]	Neuroprotective	Yes†	Positive phase 2	Inv	Survival, Tracheostomy, or 23h-NIV	18 months; 12 months	N/A	208	100%	None	None	N/A	Phase 3 results available only as abstract
Glatiramer (2009)[34]	Inflammation	Yes, various	Phase 2	Ind	ALSFRS-R slope	>52 weeks	30%	366	100%	Discussed but not done	Mentioned but not pursued	None	Early immunological studies were done by others[35]
Lithium (2010–13)[36–40]	Autophagy	Yes‡	Yes‡	Inv	TTE; survival/LOA; ALSFRS-R; survival; survival	6 months; 15 months; 13 months; 16 months; 18 months	40%; 30%; 30%; 15%; 17·5%	88; 171; 107; 133; 214	100%; 100%; 65%; 100%; 100%	Plasma levels only	None	Reached futility; stopped; no placebo; reached endpoint; a standard full study	All studies done to confirm previously reported results[17]
Talampanel (2010)[41]	Excitotoxicity	Yes	Phase 2	Ind	ALSFRS-R change	12 months	20%	559	83%	Insufficient	None	None	Increased adverse effects; phase 3 results, available only as abstract

(Table 2 continues on next page)

	Rationale to proceed to RCT			Initiated by	Primary outcome	Study duration	Percentage of effect size	Number of patients	Riluzole use of all patients enrolled	Pharmacological assessment	Presumed biological target analysed	Discussion of negative results	Comments
	Hypothesis	SOD1 transgenic model	Early patient studies										
(Continued from previous page)													
Pioglitazone (2012)[42]	Peroxisome	Yes	No	Inv	Survival	18 months	18%	219	100%	None	None	Yes, detailed	None
Ceftriaxone (2013)[43]	Excitotoxicity	Yes; cell-based	Positive phase 2	Inv	Survival	>52 weeks	50%	513	50%	Yes	None	N/A	Phase 3 results; available only as abstract
Acetyl-L-Carnitine (2013)[44]	Mitochondria	Yes	No	Inv	Loss of self-sufficiency	12 months	30%	82	100%	None	None	Positive results for primary endpoint	Novel endpoint
Dexpramipexole (2013)[45]	Mitochondria	Yes	Other human; positive phase 2	Ind	Survival and ALSFRS-R§	12 months	37%	943	76%	PK levels; CSF	None	Yes, detailed	Challenges that concern interpretation of phase 2 study results; separate post-hoc analysis was done[46]
Olesoxime (2014)[47]	Mitochondria	Yes; cell-based	Phase 2	Ind	Survival	18 months	N/A	512	100%	Yes	None	Yes, detailed	Additional small phase 2 studies are needed

ALSFRS-R=amyotrophic lateral sclerosis functional rating scale-revised. CoQ10=coenzyme Q10. IGF-1=insulin-like growth factor 1. Ind=industry. Inv=investigators. LOA=loss of autonomy. MMT=manual muscle testing. MVIC=maximum voluntary isometric (muscle) contraction. NIV=noninvasive ventilation. N/A=not applicable. NTF=neurotrophic factors. Others=used other models (not SOD1). PDE4B=phosphodiesterase 4B. PGE2=prostaglandin E2. PK=pharmacokinetic. RCT=randomised controlled trial. SOD1=superoxide dismutase-1. TTE=time-to-event. TNF=tumour necrosis factor. VC=vital capacity. *No formal, previous human study, but there was an anecdotical report of a patient that vitamin E stabilised the disease. †Beneficial effects were only found in female mice. ‡Data source: Fornai and colleagues.[17] §Combined assessment of function and survival used.

***Table 2*: Large, multicentre, randomised clinical trials (RCTs) for disease-modifying drugs between 2004 and 2014**

randomised phase 2 study.[17] After the original report, five clinical trials were independently done in five countries (table 2).[36–40] No study confirmed the original results, and it took more than 4 years to finally settle this so-called lithium fever. These clinical trials recruited 713 patients in total. A patient advocacy group, PatientsLikeMe, did a web-based, voluntary clinical trial with lithium and independently reported negative results.[56] This lithium episode was an unfortunate, but perhaps unavoidable, event, especially considering the paucity of available therapies. Retrospectively, an international authority group, such as the WFN Research Group on MND/ALS, could have provided advice on how to handle this type of situation. However, a major result of this event is that effective clinical trial methods, which were developed during this period, are now available to rapidly test potential drug candidates[36,38,39] and hopefully better handle a similar future event.

Stem cell therapy

Stem cell therapy for ALS has been highly anticipated and publicised but remains to be thoroughly tested and approved. However, because of the widespread publicity, several so-called stem cell therapies have been offered at a high cost, both financially and in terms of health, to patients.[57] For example, patients have travelled to China to receive olfactory sheath stem cells, but cases of serious side-effects have been reported.[58,59]

The rationale for stem cell therapy in ALS is based on preclinical evidence that led to two different postulated mechanisms of action: neuroprotection or replacement of degenerating motor neurons. A neuroprotective trial with ten patients showed that the injection into the thoracic spinal cord of autologous bone marrow-derived mesenchymal stem cells was safe.[60] One non-RCT phase 1 study[61] and an ongoing phase 2 study (Clinical trials number: NCT01051882) have high investigational standards and goals of neuroprotection or neuronal restoration, respectively. The first entails stereotactic injection of fetal spinal cord-derived neuronal stem cells into the cervical or lumbar spinal ventral horn.[62] The other study tests autologous bone-marrow-derived neuronal progenitor cells, which are engineered to secrete BDNF and GDNF. These trials are not randomised or controlled, and results are not available yet. Additional innovative stem cell treatments are in progress by use of induced pluripotent stem cells derived from motor neurons or glial cells.[63] Appropriately designed RCTs are imperative to assess the true benefits and potential adverse effects of stem cell therapies

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Panel 1: **Potential reasons for negative results from amyotrophic lateral sclerosis randomised controlled trials***

1 Rationale
- Relevance of SOD1 models[22,24,26,28,33,42,47]
- Interpretation of phase 2 results[45]
- Overall rationale itself flawed[26,28]

2 Pharmacology
- Interaction with riluzole[22,25,27,30,42]
- Dose too low[23,26,33]
- Broad pharmacological issues[22,26,30,34]
- Pharmacokinetic issues[42,47]
- Pharmacodynamics not pursued[34]
- Poor CSF penetration or bioavailability[25,26]
- Biomarker relevance[26]

3 Design and methodological issues
- Expected effect sizes were too high[24–26]
- Disease at enrolment was already too advanced[30]
- Two primary endpoints caused confounding[22]
- Study period was too short[30]
- Disease diversity or heterogeneity[23]
- Imbalance of enrolled patients[27]
- Need for different phase 2 study[23,25]
- Patient population differed from phase 2 study[45]
- Patient diagnostic changed during enrolment[46]
- Off-label drugs are easily available to anyone (placebo)[24]

SOD1=superoxide dismutase-1. * Information is derived from the past decade of clinical trials (2004–14).

because their clinical benefits have been highly anticipated, particularly by patients with ALS.

How can ALS trials be improved?

Study planning and design

Because clinical trials test the efficacy of a treatment of interest in patients, most RCTs have not attempt to study the underlying disease mechanisms of ALS or test whether the drug target reported in animal models is valid in patients (table 2). RCTs provide one of the best opportunities to investigate underlying disease mechanisms and drug targets in patients with ALS. An analysis of oxidative stress biomarkers was planned for the coenzyme Q10 clinical trials but not pursued because the study[32] reported negative results.[32,64] A search for evidence of a retrovirus infection was initially included in the indinavir studies, but this was not pursued because there was no compelling evidence of viral infection, among other reasons.[65] A small RCT[35] with glatiramer acetate included an investigation of T-helper cell cytokine concentrations, which increased after treatment.[35] An RCT of rasagiline, which has just begun, will investigate mitochondrial function, oxidative stress biomarkers, and glutathione magnetic resonance spectroscopy in selected sites (IND 104360). Creatinine might be used as a biomarker of survival for ALS clinical trials.[46,66] Thus, every trial could measure creatinine concentrations as a secondary or exploratory outcome. We believe that, as clinician-scientists, it is our responsibility to incorporate such additional analyses into trials to learn more about the disease and its biomarkers. We advocate that future RCTs need to ensure that these additional components are incorporated in the trial design.

Assessment of a new drug candidate alone and with riluzole as an add-on treatment is a prerequisite that should be included in preclinical animal studies, including SOD1 transgenic models. All preclinical animal studies need to adhere to the latest consensus guidelines as a minimum requirement.[67] Positive results from preclinical studies strongly affect decision making for subsequent clinical trials (panel 1), and therefore these studies should ideally use human RCT methodology, including a preliminary study for power calculation to assess treatment effect sizes; randomised and blinded design for drug administration, outcome measurement, and analyses; confirmation of drug access to the nervous system; and pharmacological assessments. Although these specific precautions might decrease the chance for type 1 and type 2 errors,[68] preclinical studies are needed. Current studies with arimoclomol[69] and antisense oligonucleotide treatment[70] are therefore highly important to determine whether SOD1 transgenic mice can be used to predice results of human clinical trials who have same SOD1 mutations. In addition to SOD1 rodent models, we strongly hope translational scientists identify additional animal models for other genetic forms and sporadic ALS.

Many issues can affect pharmacological investigations and assessments of RCT results (panel 1). Negative results can arise from study design and methodological issues, which include phenotypic variability in participants, widespread electrophysiological disease at enrolment, and too-short study duration. However, additional confounding factors and other reasons could have also contributed.

Phenotypic variability of patients with ALS

ALS has been characterised by different sites of onset and evolution of disease burden, topographically and over time.[71] Large ALS registries have identified that these phenotypic forms might have different rates of progression to death. Controversy remains as to whether phenotypes can be linked to one cause, several potential causes, or the presence of modifying genes that affect the expression of ALS in a particular patient. If we adopt the hypothesis that there could be different phenotypes of ALS caused by a single unifying pathogenic mechanism, then clinical trials need to be stratified to account for these different phenotypes. Patients with fast progressing phenotypes of ALS, such severe weight loss[72] or those with El Escorial Criteria clinically definite ALS,[46,73] would need a treatment that is randomly assigned among patients with that phenotype. The same applies for patients with possible phenotypes with a slow progression rate. Other changes in trial design could also be implemented.[74] ALS clinical

trials have previously excluded ALS-frontotemporal degeneration, but identification of additional, less severe cognitive changes that might affect disease progression would mean that these clinical characteristics and genetic testing, such as for *C9orf72*, would need to be included in any future stratification frameworks.[75,76]

Widespread electrophysiological disease at enrolment

By the time muscle weakness is clinically detected, many motor neurons might already be lost or dysfunctional.[77] Furthermore, to confirm a diagnosis of ALS takes at least 9 months after symptom onset.[78] Thus, when a patient enrols into an RCT, a long time has already elapsed, meaning that early motor neuron degeneration is widespread, although clinical weakness is apparently localised. Use of the Awaji electrodiagnostic criteria as a supplement to the El Escorial diagnostic criteria might allow identification of the electrophysiological signature of ALS before clinical weakness is fully developed.[79] Electrophysiological variables on electromyography, particularly the topographical distribution of acute denervation, might predict survival and the extent of disease progression in ALS.[80] The natural history of electrophysiological changes and clinical changes in ALS[81] needs to be studied to determine whether the quality and quantity of widespread electrophysiological changes suggest a different burden of disease, with patients possibly responding differently to treatment and necessitating stratification for future clinical trials.

Study duration

In RCTs for ALS, the time needed to investigate the effect of disease-modifying therapies on ALS function is generally set at 12 months, whereas 18 months is the standard duration to investigate the effect on survival. Clinical trials in patients with ALS have substantially changed during the past quarter of a century, and particularly in the past decade. Although the length of time between disease onset to entry into a clinical trial has remained similar, survival in the 18 months after randomisation has substantially improved from 40–50% to 70–80% in clinical trials undertaken with patients on riluzole and receiving treatment at multidisciplinary clinics.[78,82] For this reason, we advocate prolonging the duration of future clinical trials to reflect this change in the natural history of ALS. Positive effects of drugs might be missed in trials that are too short, particularly in add-on studies with riluzole.

Paillisse and colleagues[66] showed that low concentrations of creatinine and low leucocyte counts were associated with disease progression in retrospective analyses of several RCTs. Post-hoc analyses[46] of the dexpramipexole RCT also showed that a fast rate of creatinine loss was associated with fast progression of disease, and creatinine loss, as a marker of muscle mass, was slower in patients who were receiving dexpramipexole and had clinically definite ALS (based on the El Escorial Criteria). Post-hoc analyses of RCTs have also suggested possible responder groups. Although all these findings need to be validated, post-hoc analyses could offer a unique opportunity to identify potential biomarkers and responder groups that might have been overlooked in large RCTs and provide possible reasons for failures in clinical trials. Data mining to find potential beneficial effects always presents a risk in post-hoc analyses. When designing future RCTs, we should consider prespecified statistical designs to identify potential responder groups, even if the overall study has negative findings. Such

	RCT target	Number of patients	Study duration	Results
Spasticity (1993)[91]	Effects of L-threonine	33 (all completed)	2 weeks (cross-over)	Effective; p=0·05 with Ashworth scale
Respiratory failure (2003)[92]	Effects of NIV	22 and 15 accepted NIV treatment (10 continued)	26 months	Improved QoL and survival; a prospective cohort study
Pseudobulbar affect (2004[93] and 2010[94])	Dextromethorphan/quinidine compound *vs* placebo	140 (129 completed)*	28 days	Palliates PBA and improved overall QoL
Bronchial secretion (2006)[95]	Benefits of HFCWO *vs* no treatment	46 (35 completed)	12 weeks	Less fatigue and breathlessness
Bone fractures (2006)[96]	Etidronate *vs* placebo	82 (all completed)	2 years	Significantly reduced fractures
Muscle weakness (2007)[97]	Resistance exercise *vs* standard stretch exercise	27 (18 completed)	6 months	Better with resistance exercise on ALSFRS-R, limb subscales, and QoL than with standard stretch exercise
Fatigue (2009)[98]	Modafinil *vs* placebo	32 (29 completed)	4 weeks	Indicates promising treatment
Sialorrhoea (2009)[99]	Botox B	20 (18 completed)	12 weeks	Global impression significantly improved
Sialorrhoea (2011)[100]	Botox A *vs* botox B in ALS and Parkinson's disease	27 (14 completed)	4 weeks and until benefits wore off	Botox B has shorter latency and is less expensive than botox A
Physical activity and coping mechanism (2011)[101]	Aerobic exercise with usual care *vs* behavioural therapy with usual care *vs* usual care alone	120 (enrolled)	16 weeks	Results pending†
Muscle weakness (2013)[102]	Tirasemtiv *vs* placebo	>300 (ongoing)	12 weeks	Interim results show benefits on SVC, but substantial adverse effects
Weight loss (2012)[103]	High calorie and high fat diet	26 (16 completed)	12 weeks	Weight was stabilised
Weight loss (2014)[104]	Control *vs* hyperalimentation with HC/HC *vs* HF/HC	24 (20 completed)	16 weeks	Slower progression with HC/HC than HF/HC on the basis of ALSFRS-R

ALSFRS-R=amyotrophic lateral sclerosis functional rating scale-revised. Botox=botulinum toxin. HC/HC=high calorie/high carbohydrate. HF/HC=high fat/high calorie diet. HFCWO=high-frequency chest wall oscillation. NIV=non-invasive ventilation. PBA=pseudobulbar affect. QoL=quality of life. RCT=randomised controlled trial. SVC=slow vital capacity. *Patient number is based on Brooks and colleagues;[93] these two studies by Brooks and colleagues[93,94] led to FDA approval of dextromethorphan/quinidine for the treatment of pseudobulbar affect. †Study is ongoing.

Table 3: **Randomised controlled trials for symptom management for patients with amyotrophic lateral sclerosis**

***Panel 2*: Considerations for future randomised controlled trials (RCTs)**

- Expand research to find the causes and pathogenesis of ALS
  - Investigate the drug target found in preclinical models in human studies
  - Translational scientists to identify drug targets
- Preclinical studies with animals
  - Follow guidelines published in 2010[67]
  - Ideally, incorporate human RCT methods
  - Confirm and validate studies at independent laboratories
  - Consider investigations of the candidate drug alone and with riluzole
  - True translation from basic science to clinical trials and vice versa
  - Evaluate the validity of preclinical studies by evidence-based medicine analyses and an advisory board of scientists
  - Develop new disease models for ALS
- Include other disciplines in RCTs
  - Clinical pharmacologist input
  - Clinical pharmacologists input for animal studies
  - Incorporate biomarker studies such as creatinine
- Improve the design and methodology of RCTs
  - Genetic and screening tests for cognitive impairment at enrolment
  - Consider a much longer study duration
  - Encourage the development and application of innovative phase 2 studies
  - Consider phase 2 studies before pivotal investigations
  - Consider prespecified post-hoc analyses to find potential responder groups and reasons for study failures
  - Additional studies on natural history and phenotypic variations
- Consider more RCTs for pharmacological and non-pharmacological symptomatic treatments
- Considerations of a meeting with international clinical trialists, experts in other disciplines, regulatory agencies, funding agencies, pharmaceutical companies, and patient advocacy groups
  - Discuss the future of RCTs in ALS

  and
  - Consider upgrading of the second Airlie House Workshop (held in 1994) product, which identified the Clinical Trial Guidelines[6]

RCT=randomised controlled trial. ALS=amyotrophic lateral sclerosis.

properly executed analyses are justified and could inspire new directions for ALS clinical trials.[83]

Combination drug treatment seems to be inevitable in ALS, because most complex diseases are treated with several drugs. Although concerns have been expressed about a so-called drug cocktail,[84] combination trials need to be investigated.[85,86] Furthermore, since approval of riluzole, combinations of riluzole and experimental drugs have been routinely tested. Clearly, combination treatment has risks, as is seen with many drugs (table 2; panel 1). With xaliproden,[22] pentoxifylline,[27] and minocycline,[29] patients' functional status worsened but riluzole combined with dexpramipexole seemed to be beneficial in some patients.[46] Therefore, the safety of the combination of riluzole with a second study drug is unpredictable, again justifying the need for input from clinical pharmacologists. In cancer, drug add-on and standard combination treatment with many drugs have become successful only through careful and deliberate formulation of treatment regimens.[87] In ALS, with careful preclinical and clinical safety precautions, innovative combination trials could also be feasible.

The xaliproden study[22] had the best trial design to investigate of the candidate drug effect and riluzole as an add-on treatment. Yet, of all ALS RCTs so far, this trial needed the largest number of patients. In the past several years, new innovative designs have been introduced, including a drug-selection design that allows the better drug or drug combination to be identified.[86] To test dexpramipexole, a new design was developed that used the combined assessment of function and survival.[45] Opinions between the USA and Europe have sometimes differed about drug outcome selection but the use of the combined assessment of function and survival helps to resolve this difference. Other innovative designs for ALS trials include a sequential design to assess survival benefits with an already tested drug,[39,88] a dose-finding and futility design,[32,64] a time-to-event with futility design,[36] and an open-label study with historical controls.[38] These designs might be effective in reducing the number of patients needed in early phase 2 clinical trials, which directly influences costs and possibly reduces them by half by comparison with patient numbers in large phase 3 studies. These savings in cost could be used to fund more studies to test new drugs or to include studies of the drug target or disease mechanisms in phase 2 studies. Results of some ALS RCTs[24,25,46,47] concluded that more phase 2 studies were needed to test potential efficacy in different designs, with and without riluzole, before progression to large phase 3 studies (appendix). Further, analysis of the FDA and EMA processes for drug approval shows that highly innovative trial designs have been allowed for orphan diseases such as ALS.[89] We hope to see additional innovative and efficacious designs in this area. Finally, an international conference would be helpful to convene to discuss evidence-based and consensus methods to advance a more easy to follow set of clinical trials guidelines in ALS.

Other difficult problems surrounding ALS treatment

The lithium experience and the use of unapproved stem cell therapy are reminders of a difficult problem in the treatment of ALS. Some patients travel thousands of miles and spend an exorbitant amount of money to obtain an unproven wonder treatment, whereas other patients actively pursue their own, or an internet-recommended, treatment regimen. These patients decline to participate in RCTs, usually because the study includes a placebo arm. ALS clinicians regularly publish material on ALS web sites[90] where patients seek information to protect themselves from unproven treatments. We need to continue educating the public about the importance of RCTs in ALS.

RCTs for symptomatic treatments in ALS

Table 3 lists RCTs that specifically investigated drugs or medical devices to improve symptoms in ALS.[91–104] The 1999 and 2009 American Academy of Neurology (AAN) ALS treatment guidelines strongly encourage symptomatic treatment.[105,106] Identification of drugs that relieve particularly difficult symptoms or improve a specific function in ALS is a realistic goal and essential to improve quality of life for patients, in the absence of highly effective disease-modifying therapies. Clinical trials of symptomatic treatments are easier to design and complete because they need far fewer patients, have a shorter study period, and are less costly than investigations of ALS disease-modifying treatments. Nevertheless, controlled, but not randomised, clinical trials are generally small and would only provide class II evidence. For example, a controlled study of non-invasive ventilation that was not randomised and a prospective cohort study that did not have a placebo group[92] provided class II evidence according to the AAN ALS treatment guidelines. RCTs are difficult to undertake when an available treatment is already widely accepted as standard care. Therefore, it becomes even more essential to do RCTs in the best possible manner as soon as a new symptomatic treatment is introduced. Furthermore, RCTs for symptomatic treatments need additional improvements in design and methods, to ensure studies are executed effectively and yield accurate, useful results. Two RCTs to test whether a dextromethorphan/quinidine compound could improve pseudobulbar symptoms in ALS led to FDA approval, proving that RCTs for symptomatic treatments can lead to positive results and drug approval.[93,94] Another large, industry-initiated RCT of tirasemtiv, which might improve muscle strength through increasing sarcomere sensitivity to calcium,[102] showed benefits on slow vital capacity but was associated with substantial adverse effects, which will need clarification in future studies. It could still be some time before effective disease-modifying treatments that slow or reverse the disease process are reported. Therefore, in the meantime, we advocate RCTs that investigate drugs to improve the symptoms that are especially distressing for patients and would improve their quality of life. Such treatments include not only pharmacological but also non-pharmacological approaches for ALS, including non-invasive ventilation, mechanical respiratory preventive treatments, and nutritional enteral support.

Conclusions

Development of ALS treatments is at a pivotal point because most RCTs have reported negative results and no effective disease-modifying treatments have been introduced since riluzole. Critical analyses of all RCTs, particularly from the past decade, show potential reasons that account for these negative results. On the basis of the potential reasons for failure, we provide considerations for future trials (panel 2). These recommendations include collaboration of all stakeholders of the ALS community, including translational scientists, clinical trialists, and individuals from other disciplines, such as clinical pharmacologists. Now is the right time to identify a modern set of evidence-based guidelines for future ALS clinical trials. We believe that these considerations are essential to move ALS treatment forward. All investigators should be ready to undertake the most effective methods and study designs to test appropriate drugs with clear biological, therapeutic targets in ALS.

Search strategy and selection criteria

We searched PubMed between Jan 1, 1970, and Feb 28, 2014, and references from relevant articles. The search terms "Amyotrophic Lateral Sclerosis (ALS)", "Motor Neuron Disease (MND)", "Clinical trials", "Randomised controlled trials (RCTs)", and each drug or agent listed in this Review were used. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this Review.

Contributors

All authors exchanged ideas for the Review. HM reviewed the references and prepared the first draft including the reference list, figures, and tables. BRB and VS reviewed the first draft and provided comments on the references, figures, and tables. HM prepared the final draft. All authors approved the final manuscript.

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