Full title: Advances in the development of disease-modifying treatments for amyotrophic lateral sclerosis

Short title: Drug targets and animal models for the treatment of ALS

Diane Moujalled[1] & Anthony R. White[1]

[1]Department of Pathology, and Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Victoria, Australia.

Address correspondence to:

Diane Moujalled

Department of Pathology

The University of Melbourne

Victoria

Australia 3010

Ph: +61 (3) 9035 6041

Fax: + 61 (3) 8344 4004

Email: diane.moujalled@unimelb.edu.au

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive adult onset, neurodegenerative disease characterized by the degeneration of upper and lower motor neurons. Over recent years, numerous genes have been identified that promote disease pathology including *SOD1, TARDBP,* and the expanded hexanucleotide repeat (GGGGCC) within *C9ORF72.* However, despite these major advances in identifying genes contributing to ALS pathogenesis, there remains only one currently approved therapeutic; the glutamate antagonist, Riluzole. Seminal breakthroughs in the pathomechansims and genetic factors associated with ALS have heavily relied on the use of rodent models that recapitulate the ALS phenotype, however, while many therapeutics have proved to be significant in animal models by prolonging life and rescuing motor deficits, they have failed in human clinical trials. This may be due to fundamental differences between rodent models and human disease, the fact that animal models are based on overexpression of mutated genes, and confounding issues including difficulties mimicking the dosing schedules and regimen implemented in mouse models to humans. Here, we review the major pathways associated with the pathology of ALS, the rodent models engineered to test efficacy of candidate drugs, the advancements being made in stem cell therapy for ALS, and what strategies may be important in order to circumvent the lack of successful translational studies in the clinic.

Key points

- The pathogenesis of amyotrophic lateral sclerosis (ALS) is mediated by various cellular pathways such as oxidative stress, mitochondrial dysfunction, excitotoxicity and neuroinflammation.
- The failure of anti-neurodegenerative therapeutics to have positive translatable outcomes in the clinic may be associated with poor preclinical study design in mouse models, and lack of drug candidates being tested in different genetic backgrounds.
- Mouse models engineered to express C9ORF72 repeats may prove most beneficial for testing potential drug targets as C9ORF72 mouse models display characteristics which closely recapitulate the ALS phenotype, including RNA foci, TDP-43 inclusions, cortical neuron loss and display behavior and motor deficits with no signs of early lethality.

1 Introduction

Neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's diseases

and Motor Neuron Disease (MND) make up a group of pathologies characterized by the degeneration and/or death of neurons predominantly in the central nervous system (CNS). While these disorders have separate etiology with distinct morphological and pathophysiological features, they share common neuropathological hallmarks such as impaired protein dynamics with defective protein degradation and aggregation, oxidative stress and free radical formation, impaired bioenergetics and mitochondrial dysfunction and compromised neuroinflammatory processes [1]. Amyotrophic lateral sclerosis (ALS) is a chronic adult-onset MND that causes death of lower motor neurons in the brainstem and spinal cord, and of upper motor neurons in the motor cortex. The resulting motor neuron loss leads to muscle atrophy and weakness, fasciculations, spasticity and typically death from respiratory failure [2]. Neurons in the prefrontal and temporal cortex are also affected in a proportion of patients with ALS in which degeneration of neurons results in frontal executive dysfunction and concurrent frontotemporal lobar degeneration (FTLD) in approximately 15% of patients. This is known as ALS with FTLD. ALS affects approximately two individuals per 100,000 per year. While the disease course may vary drastically among patients, once afflicted, the average survival is approximately 3–5 years from symptom onset [1].

ALS can be classified into two subtypes; familial ALS (fALS) or sporadic ALS (sALS), which clinically overlap. The disease is most commonly sporadic, with no clear genetic component, however the association of environmental factors such as heavy metals, pesticides, and excitotoxins including β-N -methylamino-L -alanine (BMAA) [3, 4] or epigenetic modifications have been reported. However, 5–10% of ALS cases are familial, whereby a single genomic DNA mutation results in disease, with most cases having an autosomal dominant pattern of inheritance; x-linked and recessive fALS cases are rare [5]. FALS is associated with mutations in a heterogeneous group of genes. Of the 5–10% of fALS cases, 20% have a mutation in the copper–zinc superoxide dismutase 1 (*SOD1)* gene [6]. Discovered over 20 years ago, this was the first causative gene identified to harbor mutations linked to ALS [7]. Since the discovery, over 155 mutations in *SOD1* have been described. This was followed by the discovery of transactivation response DNA-binding protein 43 *(TARDBP)* involvement in ALS in 2008 [8]. Over the years, there have been seminal discoveries and breakthroughs including recently identified expanded hexanucleotide repeat (GGGGCC) within the chromosome 9 open reading frame 72 *(C9ORF72)* gene, which contributes to the most common cause of fALS [9-11].

The identification of multiple RNA binding proteins frequently mutated in ALS has placed them at a pivotal point in disease etiopathology and has led to ALS being categorized as an RNA misprocessing disease. However, despite major recent breakthroughs in identifying genes contributing to ALS pathogenesis driven by a new generation of genomic sequencing technologies (refer to table 1), there remains only one currently approved therapeutic; the glutamate antagonist, Riluzole (brand name *Rilutek*™). Riluzole only extends survival by ~2−3 months, and usually with considerable adverse side effects [12]. Thus, while there remains a major push to identify the remaining genetic factors that underlie ALS, there is an urgent need to convert the genetic understanding we already have into effective disease-modifying treatments for this chronic, debilitating and lethal disease.

Much of our current understanding of ALS stems from transgenic mouse models that emulate the fALS cases. However, since the development of the first transgenic ALS (SOD1) mouse more than 20 years ago, a cure, or even robust treatment for ALS (or even for cases of ALS with mutant SOD1) has yet to be achieved. There are significant issues facing researchers today in the development of a therapeutic agent to treat ALS, the reasons being; the etiology of ALS is highly multifactorial being

associated with impairment of different molecular processes such as oxidative stress, excitotoxicity and mitochondrial dysfunction. Additionally, it is heterogeneous with misprocessing of a myriad of RNA binding proteins implicated in the pathogenesis of ALS and further to this, primary targets of mutant proteins identified to cause ALS are not well understood, which makes it increasingly challenging to decipher a mechanism of disease pathogenesis. Other challenging issues reflect the disparate nature of the disease, with disease course varying greatly between patients.

In this review, we summarize some of the known cellular pathways contributing to the pathology of ALS and the pharmacological compounds currently being developed to target these pathways (refer to figure 1). We will also examine the available transgenic mouse models and interrogate their advantages and drawbacks for shedding insight on ALS pathology. We question if the mouse models are essentially helping us or is overexpression of a protein driven by an artificial promoter producing misleading outcomes that do not translate well through clinical trials? What are the alternatives, if any? We also analyze some of the ongoing drug developments and clinical trials and examine how these are progressing against a backdrop of broad failure of anti-neurodegenerative disease therapeutics. We also discuss the potential for stem cell therapy to harness disease modeling, drug discovery, and autologous cell replacement therapies and finally, we discuss how we can move forward in the development of therapeutics in light of this knowledge to try and make some real advances in the treatment of ALS by the end of the decade.

2 Cellular mechanisms of ALS pathogenesis and drug targets

2.1 Excitotoxicity and anti-excitotoxic agents

Glutamate is the primary excitatory amino acid neurotransmitter in the central nervous system (CNS), produced throughout the brain, spinal cord, primarily in astrocytes, and also in neurons and oligodendrocytes. It is indispensible for normal brain functions including motor function, cognition, memory and learning [13, 14]. Glutamatergic neuron stimulation causes the release of glutamate into the synapse where it activates ionotropic and metabotropic glutamate receptors. The excitatory signal is terminated by the clearance of glutamate from the synaptic cleft by glutamate reuptake transporters, the most abundant in the CNS of which is the excitatory amino acid transporter 2 (EAAT2, also known as GLT1). Glutamate-induced excitotoxicity resulting in motor neuron injury is one pathogenic mechanism of ALS [15]. This may result from increased levels of synaptic glutamate which causes excessive stimulation of glutamate receptors, or by increased sensitivity of the postsynaptic neuron to glutamate, resulting from modifications in neuronal energy homeostasis or glutamate receptor expression [16, 17].

GLT1 is responsible for the clearance of synaptic glutamate, and its knockout in transgenic mice results in neuronal death [18]. A large body of evidence has demonstrated a potential role for glutamate-induced toxicity in ALS. Despite the proliferation of astrocytes, the expression of GLT1 was down-regulated in $SOD1^{G93A}$ mice with disease progression [19]. Examination of human post-mortem ALS brain (motor cortex) and spinal cord tissue also demonstrated profound loss of GLT1 expression compared to controls [20]. As mentioned earlier, Riluzole the only US FDA approved treatment for ALS, is thought to offer protection against motor neuron injury through excitotoxicity by interrupting glutamatergic transmission and attenuates glutamate concentrations by affecting NMDA or a-amino-3-hydroxy- 5-methyl-4-isoxazolepropionic acid receptors, exerting an anti-excitotoxicity effect. Another potential mode of action for Riluzole is to block inactivated voltage-gated sodium channels [12]. However, this drug has limited therapeutic value, as it only

prolongs the ALS patients' life by 3 months [21]. Ceftriaxone, a β-lactam antibiotic, has been shown to protect neurons from apoptosis and increase the gene expression of glutamate transporters, making it a tantalizing candidate to intercept excitotoxicity in ALS [22]. *In vivo,* in a $SOD1^{G93A}$ ALS mouse model, this drug displayed efficacy in preventing glutamate neurotoxicity, and when administered at disease onset, delayed the loss of muscle strength and body weight and prolonged survival [22].
In translational studies, an open trial of 108 ALS patients showed that Ceftriaxone did not substantially improve muscle strength and disability scores [23]. However, Ceftriaxone was still pursued for clinical trials and was reported to be a safe and tolerable drug for humans in clinical stages I and II. Unfortunately, this drug failed in phase III clinical trials due to lack of efficacy in increasing length of patient survival or preventing a decline in motor function [13, 24].
This is a classical example of an increasing number of recent studies in mice failing to translate well in humans and raises the issue of inadequate preclinical study design in ALS mouse models. More rigorous therapeutic assessments of these drugs in ALS mouse models need to be implemented and indeed many journals and funding bodies now require evidence of careful assessment to show that studies are adequately powered. A related issue is that the majority of drug studies are only performed in animal models on a single inbred genetic background (e.g. C57BL6) and drug efficacy does not always translate to mixed genetic backgrounds [25].

2.2 Oxidative stress and anti-oxidative agents

Oxidative stress results from an imbalance between the generation of reactive oxygen species (ROS) and their removal, and/or the ability of the biological system to remove or repair ROS-induced damage [26]. This cellular stress response pathway has gained considerable attention as the potential underlying mechanism of neurodegeneration in ALS because mutations in SOD1, an antioxidant enzyme can cause fALS.
With thorough investigations of oxidative stress in ALS, it has become apparent that oxidative stress interacts with and potentially impairs other pathophysiological processes contributing to motor neuron injury, including excitotoxicity, mitochondrial dysfunction, protein aggregation, endoplasmic reticulum stress and changes in cellular signaling in astrocytes and microglia [26]. Analyses of cerebro-spinal fluid (CSF) and human postmortem CNS tissue have shown the presence of biochemical changes as a result of free radical damage or abnormal free radical metabolism, with these changes more robust in ALS/MND cases than in controls [27-29]. Additionally, elevated levels of oxidative damage to proteins, lipids and DNA have been found in postmortem tissue from sALS and SOD1 linked fALS cases [27, 30]. Further to this, using human stem cell derived motor neurons combined with RNA sequencing, Kiskinis and colleagues demonstrated a transcriptional signature of heightened oxidative stress in mutant SOD1 linked ALS [31]. Aberrant RNA metabolism is a key feature of ALS pathogenesis. With this, it is not surprising that oxidation of RNA species has been well documented. In mutant SOD1 mice, increased mRNA oxidation was observed in spinal cord motor neurons and oligodendrocytes at early pre-symptomatic stages. RNA species encoding proteins involved in mitochondrial electron transport and protein biosynthesis, folding, and degradation were also highly oxidized. While treatment with the antioxidant vitamin E did not alter survival of $SOD1^{G93A}$ mice, it did reduce mRNA oxidation, delay disease onset and improve motor performance during course of the disease [32].
Nuclear factor erythroid-2-related factor 2 (Nrf2) is the chief regulator of the antioxidant response and responds to oxidative stress by binding to and upregulating antioxidant response element (ARE) genes [26]. Alterations in Nrf2 expression and dysregulation of the Nrf2 signaling cascade could contribute to the chronic motor

neuron degeneration in ALS and other neurodegenerative diseases. Indeed, Nrf2 signaling may be impaired in models of SOD1-related ALS and in the CNS of ALS patients [33], therefore, Nrf2 appears as a key neuroprotective molecule in ALS. Recent studies strongly support that the Nrf2 signaling pathway is an important mediator of neuroprotection and therefore exemplifies a promising target for development of innovative therapies against ALS [34, 35]. An antioxidant, N-acetyl-L-cysteine (NAC), improves survival and delays the onset of motor impairment in $SOD1^{G93A}$ mice [36]. However, a clinical trial for N-acetyl-L-cysteine, administered at 50 mg/kg per day subcutaneously for 12 months (compared to placebo) failed to produce significant differences in survival or change in decline of motor symptoms after 12 months [37, 38].

Manganese porphyrin (AEOL10150), an antioxidant and a free radical scavenger has been proven to extend the survival of $SOD1^{G93A}$ mice when administered at symptom onset, improving motor neuron structure and attenuating astrogliosis [39]. While antioxidant therapies have proven beneficial in mouse models of ALS, they have failed in preclinical trials. Generally, antioxidant trials have been of suboptimal methodological design and quality, and to date unpromising [40]. There have been numerous small studies for antioxidant therapies in clinical trials implementing agents such as creatine (5-10g) and selegiline, a selective monoamine oxidase B inhibitor which has some therapeutic benefit in Parkinson's disease (10mg daily), however there treatment did not benefit patients, with no significance in survival evident [41, 42]. With conflicting data from animal models of ALS and poor translational outcomes, this could be attributed to age, or strain differences used in studies and indeed need to be scrutinized closely in future studies. Nevertheless, meta-analysis of drug treatment trials in SOD1 transgenic mouse model of ALS concluded that antioxidants have been the most effective at extending survival among animals treated at symptom onset [43, 44]. Recently, a number of potentially novel antioxidant therapies have been identified, however these must be pursued with thorough attention to pre-clinical and clinical trial design, pharmacology and pharmacokinetics [45-47]. Therefore, ongoing antioxidant clinical trials are warranted, and indeed offer some hope for the future.

2.2.1 Mitochondrial dysfunction and mitochondrial protective agents

Mitochondria are the energy producers for the cell, and regulate critical cellular pathways such as apoptosis, intracellular calcium homeostasis and the generation of intracellular free radicals. There is a large body of evidence indicating impaired mitochondrial function in ALS. This is evident by the high occurrence of mitochondrial DNA mutations in motor cortex tissue from sporadic ALS cases and diminished mitochondrial DNA in muscle and spinal cord in sALS [48, 49].

Aggregation of mutant SOD1 (mSOD1) in mitochondria is a common feature in ALS. Expression of mSOD1 proteins in motor-neuronal cell lines demonstrated the propensity to associate with the mitochondria and form cross-linked oligomers. It

was reasoned that their presence causes a shift in the redox state of these organelles and results in impairment of respiratory complexes [50]. The accumulation of mSOD1 at the site of mitochondria may also disrupt the association of cytochrome *c* with the inner mitochondrial membrane [51]. This suggests a strong link between defective mitochondria due to the toxic function of mutant SOD1 and the pathogenesis of ALS, instigating the search for neuroprotective therapies targeting mitochondria [52]. Impaired mitochondria in ALS is not exclusive to mSOD1 cases, reports have emerged of mutations in *TARDBP*, the principle component of ubiquitinated inclusions in ALS to alter mitochondrial dynamics; affecting mitochondrial length and density in neurites from primary motor neurons [53]. Additionally, juxtanuclear aggregates of mitochondria were observed, along with increased levels of fission 1 (Fis1) and phosphorylated dynamin-like protein (DLP1), key components of the mitochondrial fission machinery in TDP-43 transgenic mice [54].

Candidate mitochondrial modulators have included Olesoxime, which promotes survival of mutant motor neurons *in vitro*, conceivably by targeting the Voltage-Dependent Anion Channel (VDAC) and the benzodiazepine receptor [55]. Olesoxime was used in a double-blinded, randomised, placebo-controlled trial over the course of 18 months in combination with riluzole. However, olesoxime though was tolerated well with patients, did not show a significant beneficial synergistic effect in ALS patients concomitantly treated with riluzole, with no significance in survival [56]. Dexpramipexole, noted to function as a mitochondrial membrane stabilizer showed promise in animal models of ALS by increasing the life span of human $SOD1^{G93A}$ (hSOD1) mice by 7 days, and in double blinded phase II trials was safe and well tolerated in ALS patients [24], however in human phase III trials proved negative because it failed to meet its primary efficacy end point [57]. Despite the failures, there is still promise with ongoing candidates being investigated in animal models of ALS. One promising candidate is the synthetic antioxidant [10-(4,5-dimethoxy-2-methyl-3,6-dioxo-1,4-cyclohexadien-1-yl)decyl]triphenylphosphonium methane sulfonate analogous to the natural antioxidant coenzyme Q10, and is known as MitoQ. In a $SOD1^{G93A}$ mouse model of ALS, administered at 500 μM in the drinking water of mice from early symptom onset, it significantly prolonged the survival of mice and slowed the progression of ALS symptoms and attenuated nitroxidative damage in the nervous system [46]. These studies support the role of mitochondrial dysfunction in the progression of ALS and indeed mitochondria-targeted antioxidants may be of pharmacological use for ALS treatment and MitoQ holds hope as a treatment strategy for ALS.

2.2.2 Neuroinflammation and anti-inflammatory therapies

ALS is characterized by the death of motor neurons in the brain and spinal cord, and is accompanied by a neuroinflammatory response that is denoted by microglial activation and T-cell infiltrates localized at affected regions in the CNS. Therefore, inflammatory mechanisms and immune reactivity are hypothesized to play a role in the pathogenesis of ALS. Significant levels of microgliosis have been observed in the spinal cord of ALS patients at autopsy, with T-cell infiltrates found in close proximity in affected brain regions including the primary motor cortex and motor nuclei of the brain stem [58]. Conspicuously, patients suffering from sALS have been reported to have increased levels of circulating inflammatory (CD16+) monocytes in peripheral blood, that positively correlated with increased levels of lipopolysaccharide (LPS), an inducer of M1 activation in macrophages in plasma [59, 60]. Although

neuroinflammation is often considered a secondary effect rather than a cause of neurodegeneration in ALS patients and in ALS mouse models, numerous studies have demonstrated that regulation of the inflammatory response in mSOD1 mice alters disease progression [61, 62]. In ALS mice and human patients, there is significant elevation in tumor necrosis factor α(TNF α) and Fas ligand (FASL) immunoreactivity, hallmarks of proinflammatory responses [63]. This body of evidence supports the strong contention that neuroinflammation is a key feature of ALS and plays an important role in ALS pathogenesis. Anti-inflammatory therapies to date have been discouraging. The same notion exists for anti-inflammatory agents as for anti-oxidant agents, in that they produce adequate outcomes in animal models of ALS; for example delaying motor neuron degeneration and prolonging survival [64], however fail in human pre-clinical trials.

Minocycline, a second-generation tetracycline that has anti-inflammatory properties, was shown to delay motor neuron degeneration and increase survival in different mSOD1 mouse models of ALS [64, 65]. Minocycline had an effect directly on motor neurons by decreasing apoptosis through a decrease in the release of cytochrome *c* and also decreasing the level of microglial activation and proliferation in mSOD1 mouse models of ALS [65]. In a randomized placebo-controlled phase III trial for minocycline, 412 patients were assigned to receive placebo or minocycline in doses up to 400 mg/day for 9 months. Measurement of drug efficacy included rate of change in the revised ALS functional rating scale (ALSFRS-R), manual muscle testing (MMT), quality of life, survival and safety. Patients administered with minocycline displayed a faster decline in MMT score and greater mortality during the treatment period compared to patients on placebo, with minocycline alarmingly having adverse and harmful effects on patients with ALS [66]. With contrasting and adverse effects from mouse to humans, reflections from this study have further prompted the questioning of the ALS mouse model, questioning if it resembles the ALS patients clinical profile and whether it is suitable as a model to base clinical trials on. Other factors to be considered are dosage and dosing schedules, with varying reports of minocycline being neurotoxic or neuroprotective depending on the dose [67]. Along with cytokines and other inflammatory factors, Cyclooxygenase-2 (COX-2) is involved in the inflammatory process in the CNS, and highly expressed in astrocytes and neurons. Inhibition of COX-2 was shown to have beneficial properties in animal models of ALS. The administration of the COX-2 inhibitor and anti-inflammatory agent celecoxib significantly delayed the onset of weakness and weight loss, prolonged survival of ALS mice and significantly attenuated astrogliosis and microglial activation [68]. Celecoxib was safe and well tolerated in a double-blinded, placebo-controlled clinical trial, amongst 300 ALS patients that received celecoxib (800 mg/day) or placebo for 12 months. However, celecoxib did not slow the decline in muscle strength, vital capacity, motor unit number estimates, ALSFRS-R, or affect survival, therefore having no beneficial effect to patients [69]. Table 2 summarizes the drug trials discussed, and clinical trial outcome.

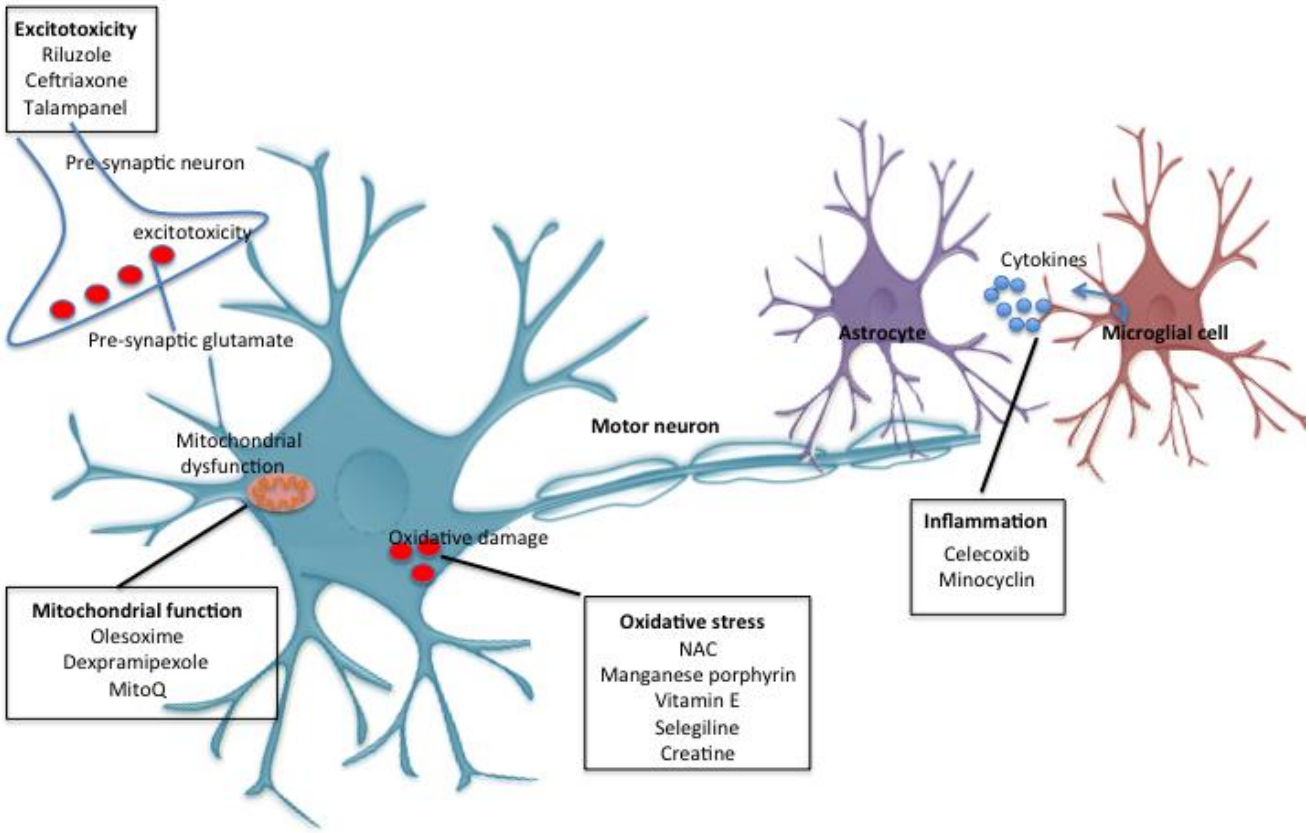

Figure 1: Molecular pathways affected in ALS and candidate drugs tested for intervention. ALS, a multifactorial disease, is associated with the deregulation of many signaling pathways. A subset of affected pathways include oxidative stress, mitochondrial dysfunction, excitotoxicity, and neuroinflammation. Candidate drugs listed have entered clinical trials or will enter clinical trials soon, such as MitoQ. Abbreviations: NAC, N-acetyl-L-cysteine.

Table 1: List of known genes mutated in ALS and related cellular pathways

Genes mutated	Potential related cellular pathway(s)	Refs
SOD1	Oxidative stress	[44, 6]
TARDBP, FUS, C9ORF72, SETX	mRNA and transcriptional defects	[70-75]
SQSTM1, UBQLN2, VCP	ER stress and protein accumulation	[76-78]

TARDP43, SOD1	Mitochondrial defects	[79, 53]
SOD1	Glutamate excitotoxicity	[19, 22]
SQSTM1, OPTN	Autophagy	[80, 81]
PFN1	Defects in cytoskeletal dynamics	[82]
VCP, VAPB, ALS2	Vesicle trafficking	[83, 84, 81]
ANG	Angiogenesis	[85]

Abbreviations: *SOD1*, Superoxide Dismutase 1; *TARDBP*, TAR DNA-binding protein 43; *FUS*, Fused In Sarcoma; *C9ORF72*, Chromosome 9 Open Reading Frame 72; *SETX*, Senataxin; *SQSTM1*, Sequestosome 1; *UBQLN2*, Ubiquilin 2; *VCP*, Valosin Containing Protein; *OPTN*, Optineurin; *PFN1*, Profilin 1; *VAPB*, Vesicle-Associated Membrane Protein-Associated Protein B/C; *ALS2*, Amyotrophic Lateral Sclerosis 2; *ANG*, Angiogenin.

Table 2: Summary of drug trials in ALS and relevant mouse models*

Drug	*In vivo* Model	Mechanism of action	Mouse trial outcome	Clinical stage/ trial outcome	Refs
Anti-excitotoxic drugs					
Riluzole	G93A	Glutamate antagonist that inhibits voltage-gated Ca^{2+} and Na^{+} channels	Prolongs life by 13 days, however Riluzole did not delay disease onset	FDA approved, prolongs patients life span by 2-3 months	**[12, 86]**
Ceftriaxone	G93A	Protect neurons from apoptosis and increases the gene expression of glutamate transporters	When administered at disease onset, delayed the loss of muscle strength and body weight and prolonged survival	Failed in phase III clinical trials	**[13, 24, 22]**
Talampanel	G93A	A noncompetitive AMPA antagonist	Mice showed a significant delay of the decline in muscle strength and prolonged survival; a delay in mortality of 13.4 days	In a phase II study it was well tolerated, however, no statistical significance in efficacy measure including ALSFRS, rate of decline in respiratory function and isometric leg strength	**[87, 88]**
Anti-oxidative agents					
NAC	G93A	Anti-oxidant precursor of glutathione, modulates glutamatergic pathways	Improves survival and delays the onset of motor impairment	Failed to produce significant differences in survival or change in decline of motor symptoms after 12 months	**[36-38]**
Manganese porphyrin	G93A	Anti-oxidant, free radical scavenger	Extended the survival of mice when administered at symptom onset, improving motor neuron structure and attenuating astrogliosis	-	[39]
Vitamin E	G93A	Anti-oxidant	Oxidation of mRNA was reduced, delayed disease onset and improved motor performance during course of the disease	No significance in patient survival	**[32, 42]**
Creatine	G93A	Stabilizes the mitochondrial transition pore and is important in mitochondrial ATP production	A dose-dependent improvement in motor performance was evident and survival was extended by 13 days with 1% creatine and by 26 days with 2% creatine	Early phase-At doses ranging from 5 to 10 g per day did not have a statistically significant effect on patient survival, ALSFRS, or FVC compared to placebo	**[41, 89]**
Mitochondrial protective agents					
Olesoxime	G93A	Inhibition of	Treatment	Phase II/III, was tolerated	**[55, 56]**

		mitochondrial permeability pore (mPTP), inhibition of apoptosis	improved motor performance in mice, delayed the onset of the clinical disease as measured by loss in motor function, and extended survival	well with patients, did not show a significant beneficial synergistic effect in ALS patients treated with Riluzole, with no significance in survival compared to placebo	
Dexpramipexole	G93A	Mitochondrial membrane stabilizer	Increased the life span of hSOD1 mice by 7 days, preserved motor function	In double blinded phase II trials, it was safe and well tolerated in ALS patients, however in human phase III trials it failed to meet its primary efficacy end point	**[24, 57]**
MitoQ	G93A	Mitochondrial anti-oxidant	Significantly prolonged the life span of mice and slowed the progression of ALS symptoms. A reduction in nitroxidative damage in the nervous system was evident	Expected to enter clinical trials soon	**[46]**
Anti-inflammatory agents					
Minocycline	G93A G37R	Anti-inflammatory properties, inhibition of apoptosis	Onset of motor neuron degeneration and muscle strength decline was delayed. It increased the longevity of SOD1(G37R) mice by approximately 5 weeks	Phase III trial, adverse and harmful effects on patients with ALS	**[64, 65, 90]**
Celecoxib	G93A	COX-2 inhibitor and anti-inflammatory	Significantly delayed the onset of weakness and weight loss, prolonged survival of mice by 28 days and significantly attenuated astrogliosis and microglial activation	Early phase trial- safe and well tolerated. Did not slow the decline in muscle strength, vital capacity, motor unit number estimates, ALSFRS-R, or affect survival, therefore having no beneficial effect to patients	**[68, 69]**

- This table summarizes the clinical trials discussed in this review. For further information on completed and current trials, visit ClinicalTrials.gov.

3 Transgenic mouse models of ALS and their impact on drug development

The use of animal models allows researchers to administer therapeutics before, during and after symptom onset in order to assess the efficacy of potential treatments for ALS. Undeniably, mouse models have shed considerable light on the cell pathways involved in the pathogenesis of ALS, recapitulate the key histopathological and

biochemical features of ALS and have identified targets that are promising candidates to intercept in ALS pathogenesis [91]. Unfortunately, while mice show behavioral and motor changes that are loosely reminiscent of ALS, these changes are also seen in most mouse models of neurodegeneration. Due to the limited brain structure, the mouse is restricted in how it can express neuronal impairment compared to the complex neurodegenerative disease phenotypes in humans. In such cases are we really seeing ALS symptoms or simply symptoms of a 'sick' mouse? In addition, it is evident that it is difficult to mimic the dosing schedules and regimen implemented in mouse models to humans as patients do not have the prospect to be treated for symptoms before they arise, therefore perplexing therapeutic regimen in clinical trials.

Next, we discuss the available transgenic mouse models used in testing efficacy of ALS therapeutics, their advantages and disadvantages and evidently which model/s are appropriate to continue using in the thorough investigations for a therapeutic treatment for ALS. No single animal model seamlessly reflects the clinical and pathological profile of ALS patients; however continuing studies from the most relevant models will provide a better understanding of the pathogenesis of ALS.

3.1 SOD1

Since the discovery of SOD1 mutations in fALS, the $SOD1^{G93A}$ transgenic mouse model was developed, expressing approximately 20–24 copies of human mutant SOD1, driven by the human SOD1 promoter [92]. This was followed by the development of over 20 SOD1 transgenic models engineered to recapitulate the ALS phenotype. SOD1 transgenic mouse lines display characteristic ALS-like motor neuron degeneration, with hind limb paralysis, muscle atrophy, loss of spinal motor neurons, damage to astrocytes, attenuated GLT-1 expression, and axonal and mitochondrial dysfunction [93, 79, 92]. The most widely used SOD1 animal model is the $SOD1^{G93A}$ transgenic model; these mice display disease onset and survival correlated with transgene expression level. Additional factors that contribute to development of ALS symptoms in these mice include genetic background, and gender [94, 95]. Other models have been developed which have given further insight into ALS pathology such as $SOD1^{G37R}$ and $SOD1^{G85R}$.

The $SOD1^{G37R}$ transgenic mice, which retain full SOD1 activity, accumulate to 3–12 times the endogenous levels in the spinal cord, and the levels of the mutant protein influence the age of onset. Low expression of $SOD1^{G37R}$ is restricted to lower motor neurons, however high copy-number causes severe abnormalities with the presence of membrane bound vacuoles which may be a result of degenerating mitochondria [79].

The $SOD1^{G85R}$ mouse model has been reported to cause MND at low expression levels, this appeared to be mediated by direct damage of astrocytes and attenuated GLT-1 expression [93]. Analysis of various SOD1 models have identified cell pathways implicated in the pathology of ALS, including excitotoxicity, mitochondrial dysfunction, oxidative stress and neuroinflammation, refer to figure 1.

Numerous drug targets found to be effective in the $SOD1^{G93A}$ transgenic model failed in clinical trials in humans including Ceftriaxone, Vitamin E, NAC, Manganese porphyrin, Dexpramipexole and MitoQ. Another classical example of poor translational outcomes was testing minocycline in two SOD1 mutant models (G93A and G37R). Three independent studies tested the efficacy of this drug with positive outcomes in the two models, however subsequent clinical trials were negative [65, 90, 64].

As the majority of pre-clinical studies have heavily relied on the $SOD1^{G93A}$ transgenic model, confounding issues have been identified. Firstly, this model has substantial over-expression of mutant SOD1 to recapitulate the ALS phenotype, in addition, it

has rapid disease onset. An alternative model to consider is the $SOD1^{G93Adl}$, this model has reduced copy number and onset of disease-like symptoms is delayed and shows slower progression [96]. However, it is imperative to consider the underlying issue, that mutations in SOD1 only account for a small proportion of total ALS cases (approximately 2%), therefore therapeutics which demonstrate encouraging results in SOD1 models may not be appropriate for the majority of fALS or sALS cases [97]. Despite the poor translational outcomes, transgenic mouse models overexpressing mutant SOD1 have been pivotal to our progress and major advances in identifying molecular pathways implicated in ALS such as oxidative stress, mitochondrial dysfunction and glutamate excitotoxicity [44, 19], and have formed the basis of much of our knowledge of the underlying pathophysiology. These models have formed the backbone for the development of new treatments and remain a good model to unravel the complexity of ALS and develop therapeutic interventions for the treatment of ALS.

3.2 TDP-43

The discovery of TDP-43 as the chief protein constituent of cytoplasmic inclusions in motor neurons led to a prominent role for TDP-43 in ALS pathogenesis. TDP-43 is post-translationally modified, being heavily ubiquitinated in cytoplasmic inclusions in motor neurons of affected ALS patient brain and spinal cord [98, 99]. The redistribution of TDP-43 in the cytoplasm is associated with concomitant loss of normal diffuse nuclear TDP-43 staining in inclusion-bearing cells. This reinforces a likely nuclear loss-of-function model of TDP-43, promoting toxicity in ALS.

Numerous studies have identified missense mutations in the C-terminal region in fALS and sALS cases, and, to date, over 40 mutations in *TARDBP* have been identified. The identification of *TARDBP* mutations has led to the development of a number of transgenic mouse models that express either wild-type (WT) or mutant TDP-43. These models recapitulate ALS pathology with protein fragmentation and aggregation, reactive gliosis, neuronal loss, as well as reduced survival.

3.2.1 mPrp, mThy1.2 and CaMKII promoters

Akin to SOD1 transgenic mouse models, development of disease phenotypes in TDP-43-ALS transgenic mouse models is related to the promoter used and the level of transgene expression. The first published transgenic TDP-43 mouse model was the A315T transgenic mouse with expression of the transgene driven under control of the mouse prion promoter (mPrp), with inherent toxic properties. At 3-fold above endogenous protein levels, mutant TDP-43 caused loss of upper motor neurons and concomitant axonal degeneration, and paralysis and death by 22 weeks of age [100].
Further analysis of these mice revealed that they succumb to gut obstruction, which explains their early mortality [101]. This symptom is caused by transgene expression in the myenteric plexus of the gut, possibly due to activity of the promoter outside the CNS, and is therefore, not an ideal model for the study of potential therapeutic treatments [102].
The mPrp has also been used to drive transgene expression of WT or Q331K or M337V mutant TDP-43 [71]. This study demonstrated that the mutant but not WT expressing mice developed age dependent progressive motor axon degeneration, motor neuron death and muscle atrophy. These changes occurred without the loss of TDP-43 in nuclei and accumulation of TDP-43 aggregates. These studies suggest that overexpression of mutant TDP-43 increases its toxicity *in vivo*. Indeed, over-expression of WT or mutant TDP-43; for example TDP-43^{M337V}, under the control of the mPrp often induces early lethality in transgenic mice [103]. Therefore, consideration of TDP-43 transgene expression regulated by human TDP-43 endogenous promoter may be more feasible to elicit age-related neurodegeneration and motor dysfunction mimicking ALS patients. As such, endogenous human TDP-43 promoters expressing mutant TDP-43 (A315T or G348C) have been developed and display TDP-43 aggregation, cytoplasmic TDP-43 over expression and truncated TDP-43 expression in the CNS of transgenic mice [104, 97]. It also manifests with cognitive and motor dysfunction during aging, which recapitulates the course observed in ALS patients [105].
Some groups have also developed transgenic mice expressing mutant or WT TDP-43 driven under the murine Thy-1.2 promoter (mThy1.2). This promoter can drive expression of TDP-43 exclusively in neurons of the CNS. Analysis of the neuronal populations shows that postnatal TDP-43 overexpression in all neurons results in the formation of neuronal intranuclear inclusions (NII) and neuronal cytoplasmic inclusions (NCI) in layer V of the anterior cortex, and truncation of TDP-43 [105-107]. Overexpression of TDP-43 under the Calmodulin-dependent protein kinase II (CaMKII) promoter triggers WT or mutant TDP-43 overexpression in the forebrain. This transgenic model elicits similar TDP-43 proteinopathy to FTLD and ALS patients, exhibiting cognitive decline from 8 weeks of age, and deficits in long-term potentiation of hippocampal neurons with concomitant cytoplasmic TDP-43 inclusion formation and loss of nuclear TDP-43 [108].
The latter models expressing TDP-43 transgene under the mPrp, mThy1.2 and CaMKII promoters may be ideal to study TDP-43 biology and pathomechanisms, and provide evidence for the toxic functions and mislocalization of TDP-43 in ALS. It is evident that there is variation in the phenotypes exhibited between models according

to the promoter and expression levels of transgenes. No TDP-43 model is flawless, however, from the models engineered, we have gained a thorough understanding of TDP-43 biology in ALS, which is significant in terms of drug design and therapeutic intervention.

3.3 FUS

Since the identification of *TARDBP* mutations in ALS, this sparked the identification of mutations in the *FUS* gene from ALS patients [8, 72]. FUS belongs to the FET (FUS/TLS, EWSR1 and TAF-15) family of DNA/RNA-binding proteins, is a multifunctional, predominantly nuclear protein and is ubiquitously expressed. Analysis of large ALS cohorts has established that mutations in *FUS* account for approximately 4% of fALS cases and 1% of sALS cases [109]. Transgenic mice engineered to over express FUS include HA-tagged human WT FUS driven under control of the mPrp [110], mice expressing mutant FUS harboring R521C mutation and lacking a nuclear localization signal (NLS) [111], rats which conditionally express human WT or mutant FUS transgene under a tetracycline response element (TRE) [112], and transgenic rats which express mutant FUS under the CaMKII promoter plus a TRE [113].

Transgenic mice overexpressing HA-tagged WT human FUS have been shown to develop an aggressive phenotype with an early onset tremor. Homozygous transgenic mice also displayed progressive hind limb paralysis and death by 12 weeks [110]. Biological changes in FUS were evident with motor neurons in the spinal cord displaying robust cytoplasmic expression of FUS, with globular and skein-like FUS-positive and ubiquitin-negative inclusions. Cytoplasmic FUS inclusions were also evident in cortical neurons of these mice, in the absence of neuronal loss and gliosis [110, 97].

Somatic brain transgenic (SBT) mice expressing adeno-associated virus human WT or mutant FUS (R521C or lacking a NLS; Δ14) postnatally in neuronal cells of the mouse brain were engineered to investigate the role of FUS in the pathogenesis of ALS. Mutant but not WT FUS had aberrant subcellular redistribution, which correlated with disease mutation and clinical severity of the mutations in patients. While no neuronal death or motor defects were evident, neuronal cytoplasmic inclusions analogous to those seen in post mortem ALS tissue were observed in cortical neurons [111]. This model recapitulates the neuropathology of FUS in FUS linked ALS, and supports the contention that mutations in *FUS* promote its accumulation in the cytoplasm and is ideal to investigate the pathomechanisms of FUS in ALS. Transgenic rats engineered to express WT human or mutant (R521C) FUS under stringent control by Doxycycline (Dox) was developed by Huang and colleagues [112]. Mutant FUS transgenic rats developed progressive paralysis akin to ALS, requiring euthanasia at 10 weeks, which was not evident in transgenics expressing WT FUS. Degeneration of motor axons and a significant loss of neurons in the cortex and hippocampus also occurred with concomitant ubiquitin aggregation and glial reaction in mutant FUS transgenics, however a minimal loss of spinal motor neurons was evident. While transgenic rats that overexpressed the wild-type human FUS had no motor phenotype at young ages, they developed deficits in spatial learning and memory, significant loss of cortical and hippocampal neurons and ubiquitin aggregation and gliosis at one year of age [112, 97]. This model suggests that expression of WT FUS can cause neuronal cell death, however mutant FUS is more toxic to neurons. Expression of mutant FUS in rats driven by the CaMKII promoter and using TRE from 30 days of age resulted in impaired spatial learning and memory by 5 weeks of age. By 8-9 weeks, there was loss of neurons in the dentate gyrus and frontal cortex. This simultaneously occurred with abnormal neuritic

branching, altered dendritic spine density and progressive Golgi fragmentation and mitochondrion aggregation [113]. Pathologically, FUS and ubiquitin-positive aggregates were observed in the cytoplasm of neurons, interestingly with the absence of endogenous TDP-43. Additionally, there was no co-localization of ubiquitinated aggregates with fragmented Golgi or aggregated mitochondria. This model is ideal to investigate mechanistic study of cortical dementia in FTLD [113].

Clearly there are differences in the phenotypes presented by the transgenic FUS rodent models. Problems with early lethality in transgenic rats expressing mutant FUS (R521C) regulated by Dox that require euthanasia at 10 weeks of age and display incomplete loss of motor neurons makes it difficult to emulate the ALS phenotype in these mice. In addition mice developed by Verbeeck and colleagues [111], did not show signs of motor defects or succumb to disease, with the confounding issue of viral delivery of mutant FUS and inadequate expression compared to transgenic models. Overall, these rodent models are essentially worthy to investigate the pathomechanisms of FUS in ALS, however, it may be time to develop a FUS transgenic model which recapitulates expression of endogenous FUS in humans. This may be by a knock-in mouse model, whereby endogenous mouse *Fus* is replaced by mutant *Fus*, this may replicate the ALS phenotype in humans and may circumvent the issues we face today with the current FUS models.

3.4 C9orf72

In 2011, two groups independently identified the largest genetic cause of ALS and FTLD as a repeat expansion of the hexanucleotide sequence GGGGCC (G_4C_2) in the *C9ORF72* gene [9, 10]. With thorough investigations of *C9ORF72* in various patient cohorts, it has come to light that expansions in the gene contribute up to ~40% to 50% of fALS and ~10% of sALS cases [73]. hnRNP A1, hnRNPA2/B1 and hnRNP A3 proteins have also been recently described as interacting factors of C9ORF72 hexanucleotide RNA [11], which may impede the nuclear role of these proteins contributing to the pathology associated with C9ORF72 expansions. There are multiple hypotheses describing the underlying mechanisms by which the repeat expansion leads to neuropathology, including loss-of-function caused by haploinsufficiency of the endogenous C9ORF72 protein product or gain-of-function induced by the formation of RNA foci in neuronal nuclei that sequester important RNA-binding proteins. This mechanism is also supported by the production of toxic

dipeptide repeat (DPR) proteins by repeat-associated non-AUG translation (RAN) of the repeat [11].
The confounding technical issue associated with mouse models of C9ORF72 repeats is that in human cases, there are >1000 repeats [114], however in mouse models, they express at most 80 repeats [115]. Nevertheless, as discussed below, the development of these mice still prove useful in order to investigate the pathomechanisms associated with C9ORF72 or for testing potential therapeutics targeting G_4C_2 expansions.
A gain-of-function mouse model of *C9orf72* was developed by Hukema and colleagues in 2014 [115]. A spatially and temporally inducible transgenic mouse model expressing 80 G_4C_2 repeats was engineered. This transgene is regulated by the TRE promoter and inducible in response to Dox. Expression of 80 G_4C_2 repeats lead to the formation of ubiquitin-positive inclusions in the striatum and the cuneate nucleus of mouse brain, however no TDP-43 positive inclusions were evident after twelve weeks of Dox treatment. The mice did not develop any obvious behavioral phenotype and showed no cell loss or dipeptide protein repeat (DPR) pathology, which has been postulated as a toxic product from translation of the repeat [11]. However, this model may be suitable for investigating the effects of the repeats without toxicity elicited by DPRs.
Most recently, Chew and colleagues report on a *C9orf72* transgenic mouse model, which to date, closely recapitulates the phenotype of C9ORF72 associated ALS/FTD [74]. Adeno-associated viral vector was employed for the expression of either 2 (a number found in healthy people) or 66 G_4C_2 repeats, lacking an ATG start codon in the CNS of mice [74]. RNA fluorescence in situ hybridisation revealed that in transgenic mice expressing 66 G_4C_2 repeats, RNA foci were detected in nuclei containing transcribed repeats, indicative of those observed in C9ORF72 ALS/FTD patients, which was not evident in control counterparts. Brains (frontal cortex and hippocampus) of 6 month old mice contained inclusions of poly(Gly-Pro), poly(Gly-Ala), and poly(Gly-Arg) DPRs, and positive for phospho TDP-43 immunoreactivity. At 6 months of age $(G_4C_2)_{66}$ mice developed cortical neuron loss and exhibited behavioral and motor deficits [74]. Indeed, G_4C_{266} mice should prove useful in deciphering pathomechanisms associated with the C9ORF72 repeat expansion [74]. This model is valuable for testing potential therapeutics targeting G_4C_2 expansions such as the use of anti-sense oligonucleotide technology. These molecules are short oligo-nucleotide sequences that are able to interfere with RNA processing. This is a promising strategy for treating ALS associated with G_4C_2 expansions as they have been shown to attenuate intranuclear RNA foci and mitigate the sequestration of important RNA binding proteins, which are known to promote neuronal toxicity [116].

4 Stem cell therapies for ALS; neural stem cells (NSCs) mesenchymal stem cells (MSCs) and Induced pluripotent stem cells (IPSCs)

The development of cell-based models for ALS has shed light on the molecular pathways associated with the disease and has provided the much needed alternative to rodent models of ALS for potentially high-throughput drug screenings. Preclinical studies have been carried out transplanting different types of cells in mouse models of ALS, such as neural stem cells (NSCs) and mesenchymal stem cells (MSCs).
MSCs are bone marrow (BM) cells that can be expanded *ex vivo* to produce large numbers of cells and can readily differentiate into mesodermal cell derivatives [117]. Their potential therapeutic mode of action is thought to be associated with the release of protective factors, including trophic factors, anti-inflammatory cytokines, and immunomodulatory chemokines released from transplanted cells even distal to the site of injection. Following intravenous injection into $SOD1^{G93A}$ ALS mice, the mice

showed a significant delay in disease onset (14 days), increased lifespan (18 days), and delayed disease progression compared to untreated mice [118]. Phase I clinical trials for direct intraparenchymal transplantation of MSCs into ALS patients reinforced the safety of MSC transplantation into the spinal cords of ALS patients, however disease progression in the majority of patients did not appear to be slowed by the MSC transplant [119, 120].

NSCs are multipotent self-renewing stem cells that have the ability to differentiate into neurons, astrocytes, and oligodendrocytes. Results from a dual grafting paradigm of NSCs into cervical and lumbar spinal cord of $SOD1^{G93A}$ rats demonstrated that rats with live NSCs grafted at two spinal levels lived 17 days longer and disease onset in dually grafted animals was delayed by 10 days compared to control counterparts that received dead NSC grafts [121]. Results from a phase I trial with the same NSI-566RSC cells concluded that transplantation of these human NSCs into the lumbar and even cervical spinal cord of ALS patients is feasible, safe and well-tolerated, and disease progression did not accelerate in any patient [122]. These results have supported future trial phases to examine therapeutic dosing and efficacy.

Induced pluripotent stem cells (IPSCs), which possess unique properties such as self-renewal and differentiation into multiple neural cell subtypes including neurons, motor neurons, astrocytes, and oligodendrocytes among others, can be used to harness disease modeling, drug discovery, and autologous cell replacement therapies. Motor neurons generated from IPSCs derived from patient skin fibroblasts with familial or sporadic forms of ALS have provided a highly innovative technique to model the disease [123]. Importantly, this technique allows researchers the opportunity to develop cell subtypes from ALS patients with known genotypes and phenotypes. However, they do have their limitations such as variability and undergoing epigenetic modifications during reprogramming [124]. IPSCs carrying the *TDP-43* M337V mutation have been shown to reproduce several key aspects of TDP-43-related proteinopathies, including reduced cell survival, and features of TDP-43 accumulation [125]. IPSCs may be ideal for the study of disease mechanisms and for drug screening. The availability of ALS disease-specific cells will allow researchers the unprecedented opportunity to investigate the mechanism and cause of disease in a human model system [126]. There remains hope that stem cell therapy will facilitate high throughput drug screening, and stem cells may eventually express enough complexity (i.e. 'organ on a chip') to dispel the need for animal studies altogether.

5 Conclusion: How can we move forward to achieve positive translatable outcomes for the treatment of ALS?

Immense efforts have gone into engineering transgenic mice to dissect the molecular basis of ALS disease pathology and to trial candidate therapeutics for treatment. However, researchers today are still faced with the complexity and heterogeneity of ALS, which makes it all the more difficult to target the underlying pathology of the disease. We have seen that transgenic mouse models that recapitulate the ALS phenotype have no doubt been beneficial in determining the pathomechanisms associated with ALS and have verified the role of candidate proteins that have been identified by *in vitro* studies. While candidate drugs have proved effective in mice, such as prolonging life and rescuing motor deficits, they have not proven to succeed in human patients [56, 69]. This conundrum may be addressed by researchers investing their research efforts in mouse models that tick many boxes when it comes to paralleling the ALS phenotype in humans. Indeed, the recent C9ORF72 mouse model ticks most and perhaps all the boxes; displaying RNA foci, DRPs, TDP-43

inclusions, cortical neuron loss and exhibit behavioral and motor deficits with no signs of early lethality [74]. Furthermore, the high prevalence of *C9ORF72* mutations in fALS, suggests that mouse models replicating this phenotype will be the most beneficial for identifying drug targets for disease intervention. Additionally, the development of sophisticated *in vitro* models will provide crucial information about the intracellular modulations of disease relevant proteins and hence a better understanding of the disease. However, with these sophisticated models come limitations that hamper their novelty and unique capabilities. While several *in vitro* models capture some key features of ALS, given the relative simplicity of *in vitro* models, the significance of results may not reflect the complex human phenotype. Therefore, to counteract these inherent limitations, results generated from *in vivo* and *in vitro* models should be combined to better understand and characterise the ALS phenotype. In future, researchers should also focus on testing potential drugs in multiple animal models, preferably in multi-center trials, as is becoming the norm for human clinical trials, and to also ensure the trials are adequately powered. Combination therapy of drugs acting in synergy in the appropriately powered studies should also be considered, in order to circumvent the lack of efficacy of the drugs administered alone in humans. Therefore, not ruling out drugs that showed promise in mice however failing in human clinical trials, and may consequently provide new avenues for treatment strategies. In conjunction, drug candidates should be tested in different genetic backgrounds in order to determine the effects of genetic variability on therapeutic efficacy [127]. Differing mouse genetic backgrounds have their advantages and disadvantages, therefore researchers need to thoroughly consider their choices based on the experimental paradigm and objective [128]. It is becoming increasingly imperative to develop effective therapies for sufferers of ALS, and with the right tools and collaborations, the disappointing failures may come to an end in the not to distant future.

Compliance with Ethical Standards

Funding

D.M is supported by an Alzheimer's Australia Dementia Research fellowship (AADRF) and funding from the Motor Neuron Disease Research Institute of Australia (MNDRIA). A.R.W is supported by an Australian Research Council Future Fellowship and The National Health and Medical Research Council of Australia.

Disclosure of potential conflicts of interest

Patent protection has previously been sought by the University of Melbourne for the use of bis(thiosemicarbazones) for treatment of diseases. AR White is a co-inventor on the patent application PCT/AU2007/001792 that is the subject of a commercialization contract between the University and a private company. The company has not funded nor contributed to research described in this manuscript. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

References

1. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci. 2013;14(4):248-64. doi:Doi 10.1038/Nrn3430.
2. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O et al. Amyotrophic lateral sclerosis. Lancet. 2011;377(9769):942-55. doi:10.1016/S0140-6736(10)61156-7.
3. Wood LK, Langford SJ. Motor neuron disease: a chemical perspective. Journal of medicinal chemistry. 2014;57(15):6316-31. doi:10.1021/jm5001584.
4. Joyce PI, Fratta P, Fisher EM, Acevedo-Arozena A. SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mammalian genome : official journal of the International Mammalian Genome Society. 2011;22(7-8):420-48. doi:10.1007/s00335-011-9339-1.
5. Andersen PM, Al-Chalabi A. Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nature reviews Neurology. 2011;7(11):603-15. doi:10.1038/nrneurol.2011.150.
6. Rosen DR. Mutations in Cu/Zn Superoxide-Dismutase Gene Are Associated with Familial Amyotrophic-Lateral-Sclerosis (Vol 362, Pg 59, 1993). Nature. 1993;364(6435):362-.

7. Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY et al. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. Science. 1993;261(5124):1047-51.
8. Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science. 2008;319(5870):1668-72. doi:Doi 10.1126/Science.1154584.
9. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. Neuron. 2011;72(2):245-56. doi:Doi 10.1016/J.Neuron.2011.09.011.
10. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. Neuron. 2011;72(2):257-68. doi:Doi 10.1016/J.Neuron.2011.09.010.
11. Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E et al. The C9orf72 GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTLD/ALS. Science. 2013;339(6125):1335-8. doi:10.1126/science.1232927.
12. Bryson HM, Fulton B, Benfield P. Riluzole. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in amyotrophic lateral sclerosis. Drugs. 1996;52(4):549-63.
13. Fontana AC. Current approaches to enhance glutamate transporter function and expression. Journal of neurochemistry. 2015. doi:10.1111/jnc.13200.
14. Peng S, Zhang Y, Zhang J, Wang H, Ren B. Glutamate receptors and signal transduction in learning and memory. Molecular biology reports. 2011;38(1):453-60. doi:10.1007/s11033-010-0128-9.
15. Soni N, Reddy BV, Kumar P. GLT-1 transporter: an effective pharmacological target for various neurological disorders. Pharmacology, biochemistry, and behavior. 2014;127:70-81. doi:10.1016/j.pbb.2014.10.001.
16. Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. Nature reviews Neurology. 2011;7(11):616-30. doi:10.1038/nrneurol.2011.152.
17. Van Damme P, Dewil M, Robberecht W, Van Den Bosch L. Excitotoxicity and amyotrophic lateral sclerosis. Neuro-degenerative diseases. 2005;2(3-4):147-59. doi:10.1159/000089620.
18. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996;16(3):675-86.
19. Guo Y, Duan W, Li Z, Huang J, Yin Y, Zhang K et al. Decreased GLT-1 and increased SOD1 and HO-1 expression in astrocytes contribute to lumbar spinal cord vulnerability of SOD1-G93A transgenic mice. FEBS letters. 2010;584(8):1615-22. doi:10.1016/j.febslet.2010.03.025.
20. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Annals of neurology. 1995;38(1):73-84. doi:10.1002/ana.410380114.
21. Miller RG, Mitchell JD, Lyon M, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Amyotroph Lateral Sc. 2003;4(3):191-206.
22. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005;433(7021):73-7. doi:10.1038/nature03180.
23. Beghi E, Bendotti C, Mennini T. New ideas for therapy in ALS: critical considerations. Amyotrophic lateral sclerosis : official publication of the World

Federation of Neurology Research Group on Motor Neuron Diseases. 2006;7(2):126-7; discussion 7. doi:10.1080/14660820510012040.
24. Cudkowicz M, Bozik ME, Ingersoll EW, Miller R, Mitsumoto H, Shefner J et al. The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. Nat Med. 2011;17(12):1652-U169. doi:Doi 10.1038/Nm.2579.
25. Scott S, Kranz JE, Cole J, Lincecum JM, Thompson K, Kelly N et al. Design, power, and interpretation of studies in the standard murine model of ALS. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2008;9(1):4-15. doi:10.1080/17482960701856300.
26. Turner MR, Bowser R, Bruijn L, Dupuis L, Ludolph A, McGrath M et al. Mechanisms, models and biomarkers in amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis & frontotemporal degeneration. 2013;14 Suppl 1:19-32. doi:10.3109/21678421.2013.778554.
27. Shaw PJ, Ince PG, Falkous G, Mantle D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. Annals of neurology. 1995;38(4):691-5. doi:10.1002/ana.410380424.
28. Ferrante RJ, Browne SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U et al. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. Journal of neurochemistry. 1997;69(5):2064-74.
29. Smith RG, Henry YK, Mattson MP, Appel SH. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. Annals of neurology. 1998;44(4):696-9. doi:10.1002/ana.410440419.
30. Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A et al. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. Brain research. 2001;917(1):97-104.
31. Kiskinis E, Sandoe J, Williams LA, Boulting GL, Moccia R, Wainger BJ et al. Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. Cell stem cell. 2014;14(6):781-95. doi:10.1016/j.stem.2014.03.004.
32. Chang Y, Kong Q, Shan X, Tian G, Ilieva H, Cleveland DW et al. Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. PloS one. 2008;3(8):e2849. doi:10.1371/journal.pone.0002849.
33. Sarlette A, Krampfl K, Grothe C, Neuhoff N, Dengler R, Petri S. Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. Journal of neuropathology and experimental neurology. 2008;67(11):1055-62. doi:10.1097/NEN.0b013e31818b4906.
34. Petri S, Korner S, Kiaei M. Nrf2/ARE Signaling Pathway: Key Mediator in Oxidative Stress and Potential Therapeutic Target in ALS. Neurology research international. 2012;2012:878030. doi:10.1155/2012/878030.
35. Neymotin A, Calingasan NY, Wille E, Naseri N, Petri S, Damiano M et al. Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. Free radical biology & medicine. 2011;51(1):88-96. doi:10.1016/j.freeradbiomed.2011.03.027.
36. Andreassen OA, Dedeoglu A, Klivenyi P, Beal MF, Bush AI. N-acetyl-L-cysteine improves survival and preserves motor performance in an animal model of familial amyotrophic lateral sclerosis. Neuroreport. 2000;11(11):2491-3.
37. Orrell RW, Lane RJM, Ross M. A systematic review of antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Amyotrophic Lateral Sclerosis. 2008;9(4):195-211. doi:Doi 10.1080/17482960801900032.

38. Louwerse ES, Weverling GJ, Bossuyt PMM, Meyjes FEP, Dejong JMBV. Randomized, Double-Blind, Controlled Trial of Acetylcysteine in Amyotrophic-Lateral-Sclerosis. Arch Neurol-Chicago. 1995;52(6):559-64.
39. Crow JP, Calingasan NY, Chen JY, Hill JL, Beal MF. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. Annals of neurology. 2005;58(2):258-65. doi:Doi 10.1002/Ana.20552.
40. Orrell RW, Lane RJ, Ross M. Antioxidant treatment for amyotrophic lateral sclerosis / motor neuron disease. The Cochrane database of systematic reviews. 2007(1):CD002829. doi:10.1002/14651858.CD002829.pub4.
41. Pastula DM, Moore DH, Bedlack RS. Creatine for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Db Syst Rev. 2010(6). doi:Artn Cd005225 Doi 10.1002/14651858.Cd005225.Pub2.
42. Kwiecinski H, Janik P, Jamrozik Z, Opuchlik A. [The effect of selegiline and vitamin E in the treatment of ALS: an open randomized clinical trials]. Neurologia i neurochirurgia polska. 2001;35(1 Suppl):101-6.
43. Benatar M. Lost in translation: treatment trials in the SOD1 mouse and in human ALS. Neurobiology of disease. 2007;26(1):1-13. doi:10.1016/j.nbd.2006.12.015.
44. Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. Free radical biology & medicine. 2010;48(5):629-41. doi:10.1016/j.freeradbiomed.2009.11.018.
45. Barber SC, Mead RJ, Shaw PJ. Oxidative stress in ALS: A mechanism of neurodegeneration and a therapeutic target. Bba-Mol Basis Dis. 2006;1762(11-12):1051-67. doi:Doi 10.1016/J.Bbadis.2006.03.008.
46. Miquel E, Cassina A, Martinez-Palma L, Souza JM, Bolatto C, Rodriguez-Bottero S et al. Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. Free Radical Bio Med. 2014;70:204-13. doi:Doi 10.1016/J.Freeradbiomed.2014.02.019.
47. Nanou A, Higginbottom A, Valori CF, Wyles M, Ning K, Shaw P et al. Viral Delivery of Antioxidant Genes as a Therapeutic Strategy in Experimental Models of Amyotrophic Lateral Sclerosis. Mol Ther. 2013;21(8):1486-96. doi:Doi 10.1038/Mt.2013.115.
48. Wiedemann FR, Manfredi G, Mawrin C, Beal MF, Schon EA. Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients. Journal of neurochemistry. 2002;80(4):616-25.
49. Dhaliwal GK, Grewal RP. Mitochondrial DNA deletion mutation levels are elevated in ALS brains. Neuroreport. 2000;11(11):2507-9.
50. Ferri A, Cozzolino M, Crosio C, Nencini M, Casciati A, Gralla EB et al. Familial ALS-superoxide dismutases associate with mitochondria and shift their redox potentials. P Natl Acad Sci USA. 2006;103(37):13860-5. doi:Doi 10.1073/Pnas.0605814103.
51. Kirkinezos IG, Bacman SR, Hernandez D, Cossio JO, Arias LJ, Perez-Pinzon MA et al. Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. J Neurosci. 2005;25(1):164-72. doi:Doi 10.1523/Jneurosci.3829-04.2005.
52. Pandya RS, Zhu HN, Li W, Bowser R, Friedlander RM, Wang X. Therapeutic neuroprotective agents for amyotrophic lateral sclerosis. Cell Mol Life Sci. 2013;70(24):4729-45. doi:Doi 10.1007/S00018-013-1415-0.
53. Wang WZ, Li L, Lin WL, Dickson DW, Petrucelli L, Zhang T et al. The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. Hum Mol Genet. 2013;22(23):4706-19. doi:Doi 10.1093/Hmg/Ddt319.

54. Xu YF, Gendron TF, Zhang YJ, Lin WL, D'Alton S, Sheng H et al. Wild-Type Human TDP-43 Expression Causes TDP-43 Phosphorylation, Mitochondrial Aggregation, Motor Deficits, and Early Mortality in Transgenic Mice. J Neurosci. 2010;30(32):10851-9. doi:Doi 10.1523/Jneurosci.1630-10.2010.
55. Bordet T, Buisson B, Michaud M, Drouot C, Galea P, Delaage P et al. Identification and characterization of cholest-4-en-3-one, oxime (TRO19622), a novel drug candidate for amyotrophic lateral sclerosis. J Pharmacol Exp Ther. 2007;322(2):709-20. doi:Doi 10.1124/Jpet.107.123000.
56. Lenglet T, Lacomblez L, Abitbol JL, Ludolph A, Mora JS, Robberecht W et al. A phase II-III trial of olesoxime in subjects with amyotrophic lateral sclerosis. Eur J Neurol. 2014;21(3):529-36. doi:Doi 10.1111/Ene.12344.
57. Genc B, Ozdinler PH. Moving forward in clinical trials for ALS: motor neurons lead the way please. Drug discovery today. 2014;19(4):441-9. doi:10.1016/j.drudis.2013.10.014.
58. Kawamata T, Akiyama H, Yamada T, McGeer PL. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. The American journal of pathology. 1992;140(3):691-707.
59. Zhang RZ, Gascon R, Miller RG, Gelinas DF, Mass J, Lancero M et al. MCP-1 chemokine receptor CCR2 is decreased on circulating monocytes in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol. 2006;179(1-2):87-93. doi:Doi 10.1016/J.Jneuroim.2006.06.008.
60. Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol. 2009;206(1-2):121-4. doi:Doi 10.1016/J.Jneuroim.2008.09.017.
61. Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK et al. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc Natl Acad Sci U S A. 2008;105(46):17913-8. doi:10.1073/pnas.0804610105.
62. Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci U S A. 2008;105(40):15558-63. doi:10.1073/pnas.0807419105.
63. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms Underlying Inflammation in Neurodegeneration. Cell. 2010;140(6):918-34. doi:Doi 10.1016/J.Cell.2010.02.016.
64. Kriz J, Nguyen MD, Julien JP. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. Neurobiology of disease. 2002;10(3):268-78.
65. Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature. 2002;417(6884):74-8. doi:10.1038/417074a.
66. Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C et al. Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. The Lancet Neurology. 2007;6(12):1045-53. doi:10.1016/S1474-4422(07)70270-3.
67. Gamez J. [Minocycline for the treatment of amyotrophic lateral sclerosis: neuroprotector or neurotoxin? Reflections on another failure of translational medicine]. Neurologia. 2008;23(8):484-93.
68. Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, Przedborski S et al. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. Annals of neurology. 2002;52(6):771-8. doi:10.1002/ana.10374.

69. Cudkowicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD et al. Trial of celecoxib in amyotrophic lateral sclerosis. Annals of neurology. 2006;60(1):22-31. doi:10.1002/ana.20903.
70. Koza P. [RNA processing TDP-43 protein has a main pathological role in FTLD and ALS]. Postepy biochemii. 2015;61(2):159-67.
71. Arnold ES, Ling SC, Huelga SC, Lagier-Tourenne C, Polymenidou M, Ditsworth D et al. ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. Proc Natl Acad Sci U S A. 2013;110(8):E736-45. doi:10.1073/pnas.1222809110.
72. Kwiatkowski TJ, Jr., Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science. 2009;323(5918):1205-8. doi:10.1126/science.1166066.
73. Philips T, Rothstein JD. Rodent Models of Amyotrophic Lateral Sclerosis. Curr Protoc Pharmacol. 2015;69:5 67 1-5 21. doi:10.1002/0471141755.ph0567s69.
74. Chew J, Gendron TF, Prudencio M, Sasaguri H, Zhang YJ, Castanedes-Casey M et al. Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. Science. 2015;348(6239):1151-4. doi:10.1126/science.aaa9344.
75. Hirano M, Quinzii CM, Mitsumoto H, Hays AP, Roberts JK, Richard P et al. Senataxin mutations and amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2011;12(3):223-7. doi:10.3109/17482968.2010.545952.
76. Fecto F, Yan J, Vemula SP, Liu E, Yang Y, Chen W et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol. 2011;68(11):1440-6. doi:10.1001/archneurol.2011.250.
77. Matus S, Valenzuela V, Medinas DB, Hetz C. ER Dysfunction and Protein Folding Stress in ALS. International journal of cell biology. 2013;2013:674751. doi:10.1155/2013/674751.
78. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature. 2011;477(7363):211-5. doi:10.1038/nature10353.
79. Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA et al. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. Neuron. 1995;14(6):1105-16.
80. Majcher V, Goode A, James V, Layfield R. Autophagy receptor defects and ALS-FTLD. Molecular and cellular neurosciences. 2015;66(Pt A):43-52. doi:10.1016/j.mcn.2015.01.002.
81. Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. Molecular neurodegeneration. 2013;8:28. doi:10.1186/1750-1326-8-28.
82. Wu CH, Fallini C, Ticozzi N, Keagle PJ, Sapp PC, Piotrowska K et al. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature. 2012;488(7412):499-503. doi:10.1038/nature11280.
83. Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron. 2010;68(5):857-64. doi:10.1016/j.neuron.2010.11.036.
84. Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D et al. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. American journal of human genetics. 2004;75(5):822-31. doi:10.1086/425287.

85. Wu D, Yu W, Kishikawa H, Folkerth RD, Iafrate AJ, Shen Y et al. Angiogenin loss-of-function mutations in amyotrophic lateral sclerosis. Annals of neurology. 2007;62(6):609-17. doi:10.1002/ana.21221.
86. Gurney ME, Cutting FB, Zhai P, Doble A, Taylor CP, Andrus PK et al. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. Annals of neurology. 1996;39(2):147-57. doi:10.1002/ana.410390203.
87. Van Damme P, Leyssen M, Callewaert G, Robberecht W, Van Den Bosch L. The AMPA receptor antagonist NBQX prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. Neuroscience letters. 2003;343(2):81-4.
88. Pascuzzi RM, Shefner J, Chappell AS, Bjerke JS, Tamura R, Chaudhry V et al. A phase II trial of talampanel in subjects with amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2010;11(3):266-71. doi:10.3109/17482960903307805.
89. Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA et al. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. Nat Med. 1999;5(3):347-50. doi:10.1038/6568.
90. Van Den Bosch L, Tilkin P, Lemmens G, Robberecht W. Minocycline delays disease onset and mortality in a transgenic model of ALS. Neuroreport. 2002;13(8):1067-70.
91. Ittner A, Bertz J, Suh LS, Stevens CH, Gotz J, Ittner LM. Tau-targeting passive immunization modulates aspects of pathology in tau transgenic mice. Journal of neurochemistry. 2015;132(1):135-45. doi:10.1111/jnc.12821.
92. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science. 1994;264(5166):1772-5.
93. Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron. 1997;18(2):327-38.
94. Heiman-Patterson TD, Sher RB, Blankenhorn EA, Alexander G, Deitch JS, Kunst CB et al. Effect of genetic background on phenotype variability in transgenic mouse models of amyotrophic lateral sclerosis: a window of opportunity in the search for genetic modifiers. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2011;12(2):79-86. doi:10.3109/17482968.2010.550626.
95. Mancuso R, Olivan S, Rando A, Casas C, Osta R, Navarro X. Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2012;9(4):814-26. doi:10.1007/s13311-012-0140-y.
96. Acevedo-Arozena A, Kalmar B, Essa S, Ricketts T, Joyce P, Kent R et al. A comprehensive assessment of the SOD1G93A low-copy transgenic mouse, which models human amyotrophic lateral sclerosis. Disease models & mechanisms. 2011;4(5):686-700. doi:10.1242/dmm.007237.
97. McGoldrick P, Joyce PI, Fisher EM, Greensmith L. Rodent models of amyotrophic lateral sclerosis. Biochimica et biophysica acta. 2013;1832(9):1421-36. doi:10.1016/j.bbadis.2013.03.012.
98. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochemical and biophysical research communications. 2006;351(3):602-11. doi:10.1016/j.bbrc.2006.10.093.

99. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006;314(5796):130-3. doi:10.1126/science.1134108.
100. Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A. 2009;106(44):18809-14. doi:10.1073/pnas.0908767106.
101. Guo Y, Wang Q, Zhang K, An T, Shi P, Li Z et al. HO-1 induction in motor cortex and intestinal dysfunction in TDP-43 A315T transgenic mice. Brain research. 2012;1460:88-95. doi:10.1016/j.brainres.2012.04.003.
102. Esmaeili MA, Panahi M, Yadav S, Hennings L, Kiaei M. Premature death of TDP-43 (A315T) transgenic mice due to gastrointestinal complications prior to development of full neurological symptoms of amyotrophic lateral sclerosis. International journal of experimental pathology. 2013;94(1):56-64. doi:10.1111/iep.12006.
103. Xu YF, Zhang YJ, Lin WL, Cao X, Stetler C, Dickson DW et al. Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. Molecular neurodegeneration. 2011;6:73. doi:10.1186/1750-1326-6-73.
104. Swarup V, Phaneuf D, Bareil C, Robertson J, Rouleau GA, Kriz J et al. Pathological hallmarks of amyotrophic lateral sclerosis/frontotemporal lobar degeneration in transgenic mice produced with TDP-43 genomic fragments. Brain : a journal of neurology. 2011;134(Pt 9):2610-26. doi:10.1093/brain/awr159.
105. Liu YC, Chiang PM, Tsai KJ. Disease animal models of TDP-43 proteinopathy and their pre-clinical applications. International journal of molecular sciences. 2013;14(10):20079-111. doi:10.3390/ijms141020079.
106. Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I et al. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci U S A. 2010;107(8):3858-63. doi:10.1073/pnas.0912417107.
107. Janssens J, Wils H, Kleinberger G, Joris G, Cuijt I, Ceuterick-de Groote C et al. Overexpression of ALS-associated p.M337V human TDP-43 in mice worsens disease features compared to wild-type human TDP-43 mice. Molecular neurobiology. 2013;48(1):22-35. doi:10.1007/s12035-013-8427-5.
108. Tsai KJ, Yang CH, Fang YH, Cho KH, Chien WL, Wang WT et al. Elevated expression of TDP-43 in the forebrain of mice is sufficient to cause neurological and pathological phenotypes mimicking FTLD-U. The Journal of experimental medicine. 2010;207(8):1661-73. doi:10.1084/jem.20092164.
109. Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. The Lancet Neurology. 2010;9(10):995-1007. doi:10.1016/S1474-4422(10)70195-2.
110. Mitchell JC, McGoldrick P, Vance C, Hortobagyi T, Sreedharan J, Rogelj B et al. Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. Acta neuropathologica. 2013;125(2):273-88. doi:10.1007/s00401-012-1043-z.
111. Verbeeck C, Deng Q, Dejesus-Hernandez M, Taylor G, Ceballos-Diaz C, Kocerha J et al. Expression of Fused in sarcoma mutations in mice recapitulates the neuropathology of FUS proteinopathies and provides insight into disease pathogenesis. Molecular neurodegeneration. 2012;7:53. doi:10.1186/1750-1326-7-53.
112. Huang C, Zhou H, Tong J, Chen H, Liu YJ, Wang D et al. FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. PLoS genetics. 2011;7(3):e1002011. doi:10.1371/journal.pgen.1002011.

113. Huang C, Tong J, Bi F, Wu Q, Huang B, Zhou H et al. Entorhinal cortical neurons are the primary targets of FUS mislocalization and ubiquitin aggregation in FUS transgenic rats. Hum Mol Genet. 2012;21(21):4602-14. doi:10.1093/hmg/dds299.
114. Almeida S, Gascon E, Tran H, Chou HJ, Gendron TF, Degroot S et al. Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons. Acta neuropathologica. 2013;126(3):385-99. doi:10.1007/s00401-013-1149-y.
115. Hukema RK, Riemslagh FW, Melhem S, van der Linde HC, Severijnen LA, Edbauer D et al. A new inducible transgenic mouse model for C9orf72-associated GGGGCC repeat expansion supports a gain-of-function mechanism in C9orf72-associated ALS and FTD. Acta neuropathologica communications. 2014;2:166. doi:10.1186/s40478-014-0166-y.
116. Donnelly CJ, Zhang PW, Pham JT, Haeusler AR, Mistry NA, Vidensky S et al. RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. Neuron. 2013;80(2):415-28. doi:10.1016/j.neuron.2013.10.015.
117. Thomsen GM, Gowing G, Svendsen S, Svendsen CN. The past, present and future of stem cell clinical trials for ALS. Experimental neurology. 2014;262 Pt B:127-37. doi:10.1016/j.expneurol.2014.02.021.
118. Zhao CP, Zhang C, Zhou SN, Xie YM, Wang YH, Huang H et al. Human mesenchymal stromal cells ameliorate the phenotype of SOD1-G93A ALS mice. Cytotherapy. 2007;9(5):414-26. doi:10.1080/14653240701376413.
119. Mazzini L, Ferrero I, Luparello V, Rustichelli D, Gunetti M, Mareschi K et al. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. Experimental neurology. 2010;223(1):229-37. doi:10.1016/j.expneurol.2009.08.007.
120. Mazzini L, Mareschi K, Ferrero I, Miglioretti M, Stecco A, Servo S et al. Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. Cytotherapy. 2012;14(1):56-60. doi:10.3109/14653249.2011.613929.
121. Xu L, Shen P, Hazel T, Johe K, Koliatsos VE. Dual transplantation of human neural stem cells into cervical and lumbar cord ameliorates motor neuron disease in SOD1 transgenic rats. Neuroscience letters. 2011;494(3):222-6. doi:10.1016/j.neulet.2011.03.017.
122. Feldman EL, Boulis NM, Hur J, Johe K, Rutkove SB, Federici T et al. Intraspinal neural stem cell transplantation in amyotrophic lateral sclerosis: phase 1 trial outcomes. Annals of neurology. 2014;75(3):363-73. doi:10.1002/ana.24113.
123. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science. 2008;321(5893):1218-21. doi:10.1126/science.1158799.
124. Ferraiuolo L. The non-cell-autonomous component of ALS: new in vitro models and future challenges. Biochemical Society transactions. 2014;42(5):1270-4. doi:10.1042/BST20140168.
125. Bilican B, Serio A, Barmada SJ, Nishimura AL, Sullivan GJ, Carrasco M et al. Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. Proc Natl Acad Sci U S A. 2012;109(15):5803-8. doi:10.1073/pnas.1202922109.
126. Saporta MA, Grskovic M, Dimos JT. Induced pluripotent stem cells in the study of neurological diseases. Stem cell research & therapy. 2011;2(5):37. doi:10.1186/scrt78.

127. Ittner LM, Halliday GM, Kril JJ, Gotz J, Hodges JR, Kiernan MC. FTD and ALS-translating mouse studies into clinical trials. Nature reviews Neurology. 2015;11(6):360-6. doi:10.1038/nrneurol.2015.65.
128. Lietner M, Menzies S, Cutz C. Working with ALS Mice: Guidelines for preclinical testing and colony management, in PRIZE4LIFE; The Jackson Laboratory.