

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:<https://orca.cardiff.ac.uk/id/eprint/134969/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Kukharsky, Michail S., Skvortsova, Veronika I., Bachurin, Sergey O. and Buchman, Vladimir L. 2021. In a search for efficient treatment for amyotrophic lateral sclerosis: Old drugs for new approaches. *Medicinal Research Reviews* 41 (5) , pp. 2804-2822. 10.1002/med.21725

Publishers page: <http://dx.doi.org/10.1002/med.21725>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



In a search for efficient treatment for amyotrophic lateral sclerosis: old drugs for new approaches

Running title: Clinical trials of “old” drugs for ALS

Michail S. Kukharsky^{1,2*}, Veronika I. Skvortsova¹, Sergey O. Bachurin², Vladimir L. Buchman^{2,3}

¹Pirogov Russian National Research Medical University, Ostrovitianov Str., 1, Moscow, 117997, Russian Federation

²Institute of Physiologically Active Compounds of the Russian Academy of Sciences, 1 Severniy proezd, Chernogolovka, 142432, Moscow Region, Russian Federation

³School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff, CF10 3AX, United Kingdom

* To whom correspondence should be sent at e-mail: kukharskym@gmail.com

Abstract

Recent progress in understanding pathological changes in the nervous system and in certain other body systems (e.g. immune system) that lead to the development and progression of amyotrophic lateral sclerosis (ALS) revealed a number of molecular and cellular processes that can potentially be used as therapeutic targets. Many of these processes are compromised not only in ALS but also in other diseases and a repertoire of drugs able to restore, at least partially, their functionality has been developed. In this review, we briefly describe current approaches to the repurposing of such “old” drugs for treatment of patients with ALS.

Keywords

Amyotrophic lateral sclerosis, clinical trials, drugs, therapeutic strategies.

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease, a condition characterised by selective damage to lower and upper motor neurons¹. Death of lower motor neurons in the spinal cord and brainstem nuclei causes progressive muscle weakness, pareses, paralyses and ultimately, death of ALS patients typically 3-5 years after manifestation of first symptoms of the disease^{2,3}. The incidence of ALS in Europe and North America is quite stable at the rate of approximately 1.5 to 3 new cases per 100 000 persons per year^{4,5}.

Degeneration of lower motor neurons is commonly accompanied by degeneration of upper motor neurons in the primary motor cortex and profound neuroinflammatory reaction in all affected regions of the nervous system³. Another histopathological hallmark of ALS is the presence in degenerating neurons of ubiquitinated inclusions that often contain aggregated TDP-43 protein or its fragments⁶.

Although most of ALS cases are sporadic (sALS), there are between 5 and 10 per cent of familial cases (fALS) caused by various, mainly autosomal dominant mutations⁷. The fALS-linked genes encode proteins involved in many important cellular functions, including but not limited to RNA metabolism, proteostasis and regulation of cytoskeleton dynamics^{1,7-9}. This suggests that motor neurons are intrinsically vulnerable cells whose homeostasis can be easily disrupted with dramatic consequences for their physiology and that such disruptions can be caused by malfunction of diverse intracellular processes. Consequently, molecular targets for the disease modifying therapy might be different not only for various forms of fALS but also for different cases of sALS¹⁰⁻¹². We still do not understand well enough molecular and cellular processes involved in ALS pathogenesis and therefore, how therapeutic targets should be chosen. Not surprisingly, a disease-modifying therapy for ALS remains only a dream and even

therapies that can significantly prolong survival of patients or slow down the disease progression are not yet available. Only two drugs, **riluzole** (Rilutek, Teglutik) and **edaravone** (Radicava, Radicut), are currently approved for the treatment of ALS.

Riluzole does prolong patients' survival but only slightly, while edaravone slows down the disease progression but this effect is marginal. Importantly, there is no clear understanding of why these drugs provide observed though small, clinical benefits, in other words, what molecular mechanisms are responsible for the effects of riluzole and edaravone. A number of reviews summarise experimental and clinical data, and discuss various hypotheses regarding possible molecular targets and mechanisms of action of both drugs (for recent examples see references¹³⁻¹⁷). Therefore, this review will not touch on these subjects any further but will focus on several other therapeutic approaches that have been suggested and tested for treatments of ALS patients, specifically on those that employ repurposing of drugs already used to treat other diseases (Supplementary table 1). Because ALS is a multifactorial disease¹⁸, a number of diverse targets and therapeutic approaches were suggested and tested in clinical trials. According to ClinicalTrials.gov, more than 530 clinical trials have been registered so far. Approximately half of these trials were dealing with potentially disease-modifying therapies: 199 (37%) trials of drugs and 39 (7%) trials of other types of biomedical therapeutic approaches, for example, antibodies and stem cells. The rest of trials examined the efficiency of new medical equipment, procedures, diagnostic methods, early disease biomarkers, etc.

An active search for new disease-modifying therapies started at the beginning of the century as reflected by initially gradually increasing and lately stably high number of clinical trials for drugs and other therapeutic approaches registered each year (Figure 1). At present, 63 clinical trials of drugs are still active, most of them (52%) are in the Phase II and 18% in the Phase III (Figure 2A). Majority

of clinical trials for biomedical therapeutic approaches are still in the Phase I (67%) but several have already reached Phases II and III (Figure 2B).

Drugs used for treatment of psychiatric disorders

These groups of drugs are actively used for treatment of patients with psychiatric disorders but a growing body of evidence linking ALS with these disorders suggest certain common underlying mechanisms and therefore, potentially disease-modifying effects of neuroleptics and antidepressants for at least some groups of ALS patients.

Psychiatric problems are quite common in ALS patients and in some cases occurs before the development of motor symptoms. This is not restricted to the well-known overlap of clinical patterns and pathomechanism of ALS and fronto-temporal lobar degeneration (FTLD)¹⁹⁻²¹. Patients diagnosed with schizophrenia-like psychosis, bipolar disorder, depression and anxiety have a higher risk of developing ALS within 1-5 years after manifestation of psychiatric symptoms²². Relatives of ALS patients more often suffer from schizophrenia, obsessive-compulsive disorder, autism, suicide and alcoholism^{23,24}. Genome-wide association studies (GWAS) revealed genetic correlation between ALS and schizophrenia (14.3%)²⁵. The highest association was demonstrated for *C9orf72* locus whose mutation is the most common cause of both fALS and sALS²⁶⁻³¹.

Between other genes that have been found associated with both diseases are several previously known ALS risk genes and several new, including those associated not only with schizophrenia but also with other neurodevelopmental disorders, for example autism-linked *CNTN6* gene, encoding contactin 6, a cell adhesion protein³².

Results of several clinical trials indirectly confirm a notion about an overlap of molecular mechanisms involved in ALS and certain psychiatric disorders and consequently, common therapeutic targets. For instance, a complementation of an antipsychotic **risperidone** treatment with riluzole alleviated negative

symptoms in chronic schizophrenia patients³³. All 13 compounds selected from a set of 3850 repurposing drugs due to their ability to prevent paralysis in two models of TDP-43-ALS (*C. elegans* expressing TARDBP^{A315T} and *D. rerio* expressing TARDBP^{G348C}) appeared to be known neuroleptics³⁴. Further studies revealed **pimozide** as a neuroleptic with the most profound effect in the TARDBP^{G348C} zebrafish system, which was also evident in two other fALS models, *D. rerio* expressing FUS^{R521H} and SOD1^{G93A}. Results of studies in *C. elegans* and zebrafish models led to the suggestion that pimozide improves synaptic transmission in neuromuscular junctions (NMJ), which was confirmed in experiments on neuromuscular preparations of extensor digitorum longus of SOD1^{G37R} mice, where pimozide normalised electrophysiological parameters of neuromuscular synaptic transmission³⁴. As a neuroleptic, pimozide specifically targets dopamine D2 receptors but its effect on NMJs can be explained by an ability to block T-type of Ca²⁺ channels³⁴⁻³⁷. In a pilot 6-week randomized controlled trial of sporadic ALS patients pimozide showed an ability to preserve decremental responses that worsened in some muscle groups (e.g. compound motor action potential for right *abductor pollicis brevis* and MRC sum score for muscle strength), which suggested protection against decline of impaired NMJ transmission³⁴. These encouraging results stimulated a new clinical trial to assess effects of chronic pimozide administration on safety, tolerability and clinical outcome measures in 100 ALS patients that started in the autumn of 2017 and should be finished in 2020 (NCT03272503). Publication of results and conclusions about the feasibility of pimozide use for ALS treatment is expected soon. However, expectations are low due to a drawback that occurred already after the start of this trial. In 2018 the same group published results of experiments with chronic pimozide administration in two mouse models expressing either TDP-43^{A315T} or SOD1^{G93A}. Unexpectedly, pimozide aggravated pathology in both models. Significant increase of pathogenic protein aggregate accumulation in the nervous system, worsening of the neuromuscular connectivity and consecutively,

motor functions and reduced lifespan were observed for pimozide-treated compared to vehicle-treated animals³⁸. Reasons for a damaging effect of chronic pimozide treatment on mouse models of ALS pathology are not clear but these latest observations cast doubts on the future of this drug for ALS treatment.

Mood stabilisers is another group of drugs that are commonly used for treatment of psychiatric diseases and considered as potential disease-modifying treatments for ALS. In particular, **lithium**, an inhibitor of glycogen synthase kinase 3 (GSK-3), and **valproic acid**, an inhibitor of histone deacetylases (HDACs), have been tested in animal models and ALS clinical trials. The rationale for testing these drugs was their neuroprotective potential, that has been linked to their ability to interfere with many pathways and processes involved in neuronal death, including excitotoxicity³⁹, expression of glutamatergic postsynaptic density proteins of Homer family^{40,41}, aberrant Notch signalling⁴², apoptosis⁴³, endoplasmic reticulum stress (ERS) and autophagy^{39,44}. Moreover, a synergistic effect of these two drugs has been demonstrated^{45,46}. Marked neuroprotection observed for therapeutically-relevant doses of lithium in SOD1^{G93A} mouse model⁴⁷ triggered several clinical trials in patients with ALS. In some of these trials, lithium slowed down the disease progression⁴⁷⁻⁴⁹, but no improvement was observed in other trials⁵⁰⁻⁵⁵. Further studies revealed that only patients homozygous for C allele of *UNC13A* locus, which is known to be genetically linked to ALS and FTL^D⁵⁶⁻⁵⁸, response to lithium therapy⁵⁹. Cotreatment with lithium and valproic acid has also been tested in animal models^{40,60} and used in a small size clinical trial in patients with sALS that revealed increased survival and signs of neuroprotection although doses used were too high and caused side-effects that forced termination of the trial⁶¹. Results of another clinical trial of lithium and valproic acid cotreatment (NCT03204500) are not yet published. Chemical structures of drugs described in this section are shown in Supplementary Figure S1.

Drugs targeting endoplasmic reticulum stress

Protein misfolding, formation of unfolded protein oligomers, higher order aggregates and finally, large intracellular inclusions are biochemical and histopathological hallmarks of ALS^{28,62,63}. Accumulation of unfolded proteins and products of their aggregation causes various intracellular stress reactions, including endoplasmic reticulum stress (ERS) and triggers unfolded protein response (UPR), a mechanism that enables cells to restore its protein homeostasis and survive for some time under conditions of mild to moderate stress. This is achieved by activation of intracellular processes controlling protein folding and degradation^{64,65}. However, in conditions of chronic and strong stress UPR initiates apoptotic death of affected cells^{66,67}.

ERS is believed to be one of important mechanisms regulating sensitivity of motor neurons to pathological changes associated with ALS^{68,69}. Evidence for ERS in neurons affected by ALS pathology was obtained by analysis of post mortem samples of fALS and sALS patients and in various cell and animal models of the disease⁷⁰⁻⁷³. Moreover, mutations in genes *PDIA1* and *PDIA3*, whose protein products are ER-residing molecular chaperones that prevent the formation of protein aggregates, have been associated with ALS^{74,75}.

A controllable switching of ERS-triggered UPR from its adaptive to apoptotic programme is regarded as a promising approach for treatment of ALS as well as other neurodegenerative conditions^{76,77}. Several intracellular pathways associated with ERS and UPR has been suggested as targets for ALS drugs.

A dual leucine zipper kinase (DLK) is a part of the intracellular signalling network that modulates cell response to ERS via c-Jun N-terminal kinases (JNK) and protein kinase R-like endoplasmic reticulum kinase (PERK). Upregulation of DLK in mammalian nervous system causes axonal degeneration and death of neurons, whereas its pharmacological inhibition or knockout of the encoding gene have neuroprotective effect in several models of neurodegeneration, including SOD1^{G93A} mouse model⁷⁸⁻⁸¹. Therefore, a number of DLK inhibitors

are currently tested as potential drugs for various neurodegenerative diseases and there is an ongoing Phase I clinical trial for one of them, **GDC-0134** (NCT02655614).

Tauroursodeoxycholic acid (TUDCA), a minor component of bile used in ancient Asian pharmacopoeias, possesses a chemical chaperone activity that can reduce ERS and alleviate cell death by stimulation UPR without activation of a proapoptotic branch of the PERK-eIF2 α -ATF4-CHOP pathway⁸²⁻⁸⁵. In an initial clinical trial significantly higher Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) parameters and slower disease progression has been demonstrated for a group of ALS patients treated with a combination of TUDCA and riluzole than for control riluzole and placebo group^{86,87}. Some effect of another bile component and molecular chaperone, **ursodeoxycholic acid** (UDCA) was observed in a separate clinical trial^{86,87}. However, the group sizes in both were too small to make strong conclusions about the usefulness of these molecular chaperones for treatment of ALS patients⁸⁸. **Sodium phenylbutyrate** (Buphenyl, Ammonaps, triButyrate), a compound used to treat urea cycle disorders, is another molecular chaperone, that also acts as an inhibitor of HDAC⁸⁹. It has successfully passed efficiency testing in SOD1^{G93A} mouse model as well as safety and tolerability tests in patients⁹⁰⁻⁹⁴. A combination of tauroursodeoxycholic acid and sodium phenylbutyrate, AMX0035, is now tested in a large CENTAUR Phase II clinical trial (NCT03127514). Although full results of this trial are yet to be published, according to the recent announcement AMX0035 statistically significantly slowed ALS disease progression as measured by the ALSFRS-R.

Arimoclomol, originally developed as a candidate drug for treatment of insulin resistance and diabetic complications has later been suggested for use in various lysosomal storage diseases and protein aggregation diseases, including ALS. Such diversity is due to the mechanism of arimoclomol action as a coinducer of heat shock proteins (HSPs). Following encouraging results obtained in various

cell and animal models, including SOD1^{G93A} mice⁹⁵, and a suggestion from the first clinical trial on SOD1-ALS patients that arimoclomol treatment might have a therapeutic benefit⁹⁶, a Phase III randomised, placebo-controlled trial of Arimoclomol in ALS (NCT03491462) started in July 2018.

A potential problem with using coinducers of HSPs as ALS drugs is that motor neurons have a high intrinsic threshold of stress-induced HSPs upregulation^{97,98} and are relatively resistant to arimoclomol and other HSP coinducers⁹⁹. It has been suggested that the combination of coinducers of HSPs and HDAC inhibitors might have a strong synergistic effect and should be considered in future clinical trials¹⁰⁰. Chemical structures of drugs described in this section are shown in Supplementary Figure S3.

Drugs targeting excitotoxicity

A common feature of virtually all ALS cases is altered excitability, particularly cortical hyperexcitability, that believed to be a crucial element in pathomechanism of the disease leading to excitotoxicity within affected neuronal circuits and ultimate death of motor neurons^{101,102}. Consequently, compounds affecting neuronal excitability and/or neuronal synaptic transmission are credible candidates for drugs capable to prevent motor neuron death in ALS.

Ezogabine (Potiga) or **retigabine** (Trobalt), an anticonvulsant used for the treatment of epilepsy, decreases neuronal excitability due to its activity as an opener of KV7 (KCNQ) family of potassium channels¹⁰³. A single dose of this drug significantly decreased excitability parameters in a study performed on a small group of ALS patients¹⁰⁴. According to still unpublished data reported at the Motor Neurone Disease Association Symposium in December 2018, in a Phase II trial (NCT02450552) chronic administration of the drug is well tolerated by patients with ALS and decreases excitability of both upper and lower motor neurons¹⁰⁵.

Another anticonvulsant used for treatment of seizures and neuropathic pain, **lacosamide** (Vimpat), decreases neuronal excitability by interaction with two types of molecules: it enhances the slow but not affects fast inactivation of voltage-gated sodium channels, and improves neuronal connectivity via modulation of activity of collapsin response mediator protein 2 (CRMP-2), although the impact of these mechanisms is not obvious¹⁰⁶. Nevertheless, neuroprotective effects demonstrated in previous studies¹⁰⁷⁻¹⁰⁹ stimulated lacosamide testing for treatment of ALS patients and a Phase I/II open-label clinical trial (NCT03186040) is ongoing.

Results of a recently completed clinical trial (NCT01811355) demonstrated that **mexiletine** (Mexitil, NaMuscla) effectively reduces muscle cramp frequency and severity in ALS patients^{110,111}. The mechanism of this drug action is believed to be related to its ability to reduce persistent sodium currents by blocking sodium channels. It has been also demonstrated that mexiletine can cross blood brain barrier (BBB) and enter the nervous system¹¹². However, treatment with mexiletine does not affect ALS progression^{111,113}. It should be noted that riluzole also reduces persistent sodium currents but does not have any effect on muscle seizures^{114,115} and does not potentiate the effect of mexiletine when used in combination¹¹⁰.

Ranolazine (Ranexa) is used to treat chronic angina. In heart muscles, ranolazine reduces intracellular calcium levels pathologically increased due to hyperactive persistent or late inward sodium current. In addition to inhibition of sodium channels the drug affects the delayed rectifier current, i.e. potassium channels¹¹⁶⁻¹¹⁸. The ability to reduce excitability of the nervous system cells^{119,120} makes ranolazine a prospective drug for treatment of neuropathic pain^{121,122} and epilepsy^{123,124}. Ranolazine also has anti-inflammatory activity and increases survival of astrocytes in primary cultures, which led to suggestion that it might have a neuroprotective activity¹²⁵. A Phase II clinical trial on patients with ALS (NCT03472950) is currently on the way.

An antagonist of N-methyl-D-aspartate (NMDA) receptors **memantine** is an approved drug for alleviating symptoms of Alzheimer's disease (AD) and is currently considered as a treatment for ALS. Although in previous studies memantine increased the lifespan of model animals^{126,127}, preliminary data of a Phase II clinical trial (NCT02118727) did not show its efficacy in patients with ALS^{128,129}. Glutamate release by astrocytes is stimulated by prostaglandine E2, a product of a reaction catalysed by cyclooxygenase-2 (COX-2)^{130,131}. This is the rationale for testing COX-2 inhibitor **celecoxib** (Celebrex, Onsenal, etc.), a nonsteroidal anti-inflammatory drug used for treatment of pain and inflammation in various types of arthritis and certain other conditions in ALS patients. Despite promising results obtained in animal models of ALS¹³²⁻¹³⁴, celecoxib in combination with creatinine and/or minocycline failed to show efficacy in a Phase II clinical trial (NCT00355576)¹³⁵. Currently, a combination of celecoxib with an antibiotic **ciprofloxacin** is being assessed in Phase I (NCT04090684) and Phase II (NCT04165850) clinical trials.

Excitotoxicity and ERS both augment repeat associated non-AUG-dependent (RAN) translation, a process coupled with accumulation of toxic dipeptides (DPRs) from ALS-associated *C9orf72* locus carrying hexanucleotide repeat expansion¹³⁶⁻¹³⁸. **Metformin**, a drug used to treat or decrease the risk of the development of the type 2 diabetes, is considered to be an attenuator of RAN translation and thus, might be an option for treatment of certain forms of ALS as well as other neurodegenerative diseases^{139,140}. A Phase II clinical trial of metformin in *C9orf72* positive ALS patients (NCT04220021) is currently ongoing and two other drugs used to treat depression, **trazodone** and **dibenzoylmethane**, has been shown to reduce DPR levels in cellular models and suggested as potential treatments for C9-ALS^{140,141}.

Existing evidence suggests that in certain circumstances motor neurons of ALS patients can be hypoactive^{142,143}. A switch from a hyperactive to hypoactive status is associated with the late stages of the disease^{144,145}. Consequently, it is feasible

to suggest that blockers of voltage-activated potassium channels might be used for therapeutic increase of motor neuron excitability in relevant patient groups¹⁴².

4-aminopyridine (Aminopyridine, dalfampridine, etc.), a potent convulsant that in appropriate doses is used to improve walking capacity of patients with multiple sclerosis, now is testing for use in other neuromuscular disorders, including primary lateral sclerosis and ALS¹⁴⁵, including a Phase I clinical trial (NCT02868567).

It has been hypothesised that a specific mechanism behind glutamate excitotoxicity in ALS is reduced editing of a Q/R site in glutamate ionotropic receptor AMPA type subunit 2 (GRIA2), which causes increased calcium entry in neurons. Such decreased editing and reduced activity of an editing enzyme ADAR2 has been found in motor neurons of ALS patients¹⁴⁶⁻¹⁴⁹. In neurons of ALS/FTLD patients with *C9orf72* mutation ADAR2 changes its normally nuclear localisation to cytoplasmic¹⁵⁰. Moreover, ADAR2 dysfunction can cause TDP-43 pathology by increasing activity of a calcium-dependent protease calpain that cut TDP-43 molecule with production of an aggregation-prone fragment¹⁵¹⁻¹⁵³. These studies became a basis for testing **perampanel** (Fycompa), a selective non-competitive antagonist of AMPA receptors used for treatment of epilepsy, as a potential ALS drug. Studies on mice with conditional inactivation of ADAR2 in motor neurons demonstrated an ability of perampanel to prevent ALS phenotype progression, reduce the TDP-43 pathology and associated death of motor neurons¹⁵⁴. In ongoing Phase II clinical trial this drug is tested in patients with ALS (NCT03377309). Chemical structures of drugs described in this section are shown in Supplementary Figure S3.

Drugs targeting oxidative stress

Neurons are susceptible to oxidative stress and therefore it is another obvious therapeutic target in neurodegenerative diseases, including ALS. Despite failure of clinical trials for a number of antioxidants, including vitamin E, acetylcysteine

and L-methionine^{155,156}, this approach is still considered feasible and testing of other antioxidants are in progress. Additional inspiration for further search of effective antioxidant therapy is the known ability of edaravon, a drug recently approved for treatment of ALS patients, to scavenge free radicals^{17,157,158}.

Common causes of neuronal oxidative stress are disturbances in metabolism of metal ions, primarily copper, iron and zinc. For example, iron ions were found accumulating in the nervous system of ALS patients and blood concentration of ferritin reversely correlated with their survival¹⁵⁹⁻¹⁶². Chelating agents can be used to ameliorate the consequences of metal ions accumulation¹⁶³⁻¹⁶⁶. Neuroprotective effect obtained in animal models of ALS and a small Phase II clinical trial (NCT02164253) of **deferiprone**, a chelator of iron ions used for treatment of thalassemia^{167,168}, encouraged further assessment of this drug for elimination of excess iron from the brain without changes in systemic iron levels in a larger Phase II clinical trial (NCT03293069).

An alternative approach is normalisation of function of Cu-deficient SOD1, an enzyme associated with ALS pathology, by delivery of copper ion to this protein. For this purpose, the use of a copper-containing compound **CuII(atsm)** (bis(thiosemicarbazone)copper(II)compound)¹⁶⁹⁻¹⁷³, originally developed as a PET imaging agent¹⁷⁴⁻¹⁷⁶ has been suggested. An additional benefit of using CuII(atsm) is its ability to inhibit ferroptosis, a specific mechanism of cell death caused by Fe-dependent lipid peroxidation¹⁷⁷. Released results of a Phase I clinical trial (NCT02870634) suggested that CuII(atsm) treatment can slow disease progression and improve the respiratory and cognitive function of ALS patients¹⁷⁸, which justified treatment extension study (Phase II, NCT03136809) for patients participated in the Phase I as well as additional larger Phase II study (NCT04082832).

A natural antioxidant urate has a neuroprotective activity¹⁷⁹⁻¹⁸¹, including neuroprotection against excitotoxicity¹⁸². A blood level of urate is a prognostic factor for survival in ALS patients and its high level correlates with a lower risk

of ALS development¹⁸³⁻¹⁸⁸. Moreover, edaravon increases urate blood level in ALS patients^{189,190}. As a precursor of urate, **inosine** stimulates its production and thus, treatment with inosine might have a neuroprotective effect in ALS. However, it should be noted that such effect might be due to the involvement of this nucleoside in multiple other intracellular processes. Many suggested applications of inosine for treatment of various medical conditions are often not scientifically justified but following successful demonstration that inosine is well tolerated and does raise blood urate levels in patients with ALS in a Phase I clinical trial (NCT02288091)¹⁹¹ it is being tested in a Phase II trial (NCT03168711). Chemical structures of drugs described in this section are shown in Supplementary Figure S4.

Drugs targeting neuroinflammation

Neuroinflammation is a typical characteristic of various neurodegenerative diseases, including ALS^{192,193}. Activation of astrocytes and microglial cells that can be detected in the nervous system even at early stages of the disease by histological analysis of autopsies or positron emission tomography (PET) is the hallmark of neuroinflammation but infiltration of the nervous system by the peripheral immune system, including monocytes, neutrophils, T and B cells has also been demonstrated¹⁹⁴⁻¹⁹⁶. Moreover, neurodegeneration is often accompanied by systemic inflammation, i.e. changes in populations of peripheral lymphocytes and monocytes, and increased levels of blood cytokines^{193,197-199}. Although it is still not clear whether these inflammatory reactions are consequences, important elements of pathogenesis or even main causes of the disease, it is now commonly accepted that anti-inflammatory therapy should be an important part of ALS treatment. A number of specific drugs that affect immune reactions either inside the nervous system or at the systemic level are currently considered as potential options for combating ALS²⁰⁰.

A drug used in Japan for treatment of asthma and post-stroke patients, **MN-166** (Ibudilast), is a low molecular mass inhibitor of cyclic nucleotide phosphodiesterases (PDE-4 and PDE-10), macrophage migration inhibitory factor (MIF) and toll-like receptor 4^{201,202} that can cross BBB. It has been shown that MN-166 suppresses glial cell activation²⁰³⁻²⁰⁶ and protects cultured neurons against glutamate toxicity²⁰⁷. Results of recent Phase II/III clinical trials (NCT02238626, NCT04057898) demonstrated that MN-166 taken in combination with riluzole improves Amyotrophic Lateral Sclerosis Assessment Questionnaire 5 (ALSAQ-5) and ALSFRS-R scores of ALS patients with a short (less than 600 days from onset) history of the disease²⁰⁸. Further Phase III clinical trial of MN-166 administered in combination with riluzole is planned. Also, a Phase I/II of MN-166 alone (NCT02714036) for treatment of ALS patients is ongoing.

Another drug that produced promising results in both animal studies and clinical trials (Phase II/III: NCT02588677 and ongoing Phase III: NCT03127267, both in combination with riluzole), is **masitinib** (AB1010)^{209,210}. Under the brand name Masivet this drug was used for a number of years in veterinary practice for treatment of mast cell tumours. Masitinib is an inhibitor of several tyrosine kinases, including c-Kit, PDGFR, Lck, FAK and FGFR3. It has been given a status of an orphan drug and tested for treatment of various diseases. It also suppresses proliferation and migration of microglia and expression of inflammatory mediators by inhibiting another tyrosine kinase, colony-stimulating factor 1R receptor (CSF-1R)²⁰⁹.

Reduced number and function of regulatory T lymphocytes (Tregs) that suppress microglia activation has been observed in patients with ALS^{211,212}. This triggered the development of several approaches for the correction of this deficiency. **Interleukin-2** (IL-2), which is used to treat several oncological conditions, in low doses increases the activity of Tregs and therefore a combination of autologous

Tregs infusion and IL-2 administration is currently tested in a Phase II clinical trial (NCT04055623)^{213,214}.

Dimethyl fumarate can stimulate Tregs formation and, under the brand name Tecfidera, is successfully used for treatment of relapsing forms of multiple sclerosis. First results of Phase II clinical study (ACTRN12618000534280) suggest that tecfidera treatment is able to slow ALS progression²¹⁵.

Suppression of inflammatory neurotoxic T cells responses is a well-known activity of **rapamycin** (Rapamune, Sirolimus), a drug already used as a therapeutic treatment for many conditions that benefit from immunosuppression. However, rapamycin is also able to stimulate autophagy, another mechanism that might be used for combating neurodegenerative diseases, including ALS. Inhibition of the mammalian target of rapamycin (mTOR) and consequent activation of autophagy significantly reduce accumulation of pathological inclusions and slow pathology progression in rapamycin-treated animals modelling ALS pathology²¹⁶⁻²²².

The ability of rapamycin to suppress neuroinflammation and activate autophagy makes it particularly attractive drug for ALS treatment, although potentially serious obstacles are poor penetration of BBB and potential general toxicity. Nevertheless, a Phase II (NCT03359538) is ongoing and results are expected next year²²³.

Colchicine, a drug that is used to treat gout, is another example of dual-action compound^{224,225}. In addition to the anti-inflammatory effect that is known for many hundred years, colchicine has been recently shown to upregulate the expression of a heat shock protein B8 (HSPB8) and thus, stimulate the clearance of pathological protein aggregates in various systems, including cellular and animal models of ALS²²⁶. Colchicine has serious side effects at high doses but is tolerated at low doses and its Phase II clinical trial (NCT03693781) on patients with ALS has recently started²²⁷. Chemical structures of drugs described in this section are shown in Supplementary Figure S5.

Drugs targeting endogenous retroviruses

Increased expression of human endogenous retrovirus HERV-K transcripts in the neural tissues²²⁸ and increased activity of reverse transcriptase, a common retroviral marker, in blood²²⁹⁻²³² of some but not all patients with ALS^{233,234} suggest a role of endogenous retrovirus activation or antiviral immune response triggered by viral double-stranded RNA in ALS pathogenesis²³⁵. Therefore, antiretroviral therapy using already available drugs is considered as a potential approach for treatment of ALS patients exhibiting activation of endogenous retroviruses^{233,236-238}.

In a recently completed Phase IIa clinical trial (NCT02868580), safety, tolerability and efficacy of a long-term, 24 months, antiretroviral therapy (**Triumeq**, a combination of two nucleoside analogue reverse-transcriptase inhibitors **lamivudine** and **abacavir**, and a HIV-1 integrase strand transfer inhibitor **dolutegravir**) for ALS patients have been demonstrated. A downregulation of HERV-K expression was accompanied by a decline in ALSFRS-R progression rate²³⁹, which provides a rationale for conducting further Phase III trials of Triumeq. Another combination of antiretroviral drugs (a protease inhibitor **darunavir**, its booster **ritonavir**, an integrase inhibitor **dolutegravir** and a nucleoside analogue reverse-transcriptase inhibitor **Tenofovir alafenamide**) is currently tested in a Phase I clinical trial (NCT02437110). Chemical structures of drugs described in this section are shown in Supplementary Figure S6.

Is there a perspective for other "old" drugs to be repurposed for ALS treatment?

The answer to this question is a definite “yes”, there are still a number of drugs with proven or suggested mechanisms of action that make them good candidates for testing as potential disease-modifying or at least slowing disease progression

in ALS. Example of such drug is **Dimebon** (Latrepidine, Supplementary Figure S7), an approved antihistamine drug that has demonstrated some promise in the Phase II of clinical trials for mild-to-moderate Alzheimer's disease^{240,241} and other gamma-carbolines²⁴²⁻²⁴⁴. Although not tested so far, these compounds seem to be promising candidates for ALS clinical trials because they were shown to efficiently ameliorate pathological aggregation of TDP-43 and other ALS-related aggregation-prone proteins in several in vitro and in vivo systems²⁴⁴⁻²⁵⁰. Similarly, future studies in ALS models might reveal other “old drugs” or their derivatives that deserve to be further tested in clinical trials. Hopefully, some of these drugs will become components of successful ALS treatment schemes.

Conclusions

It becomes increasingly clear that on its own neither of “old” (and probably neither of any “new”) drugs is able to cure patients with ALS by reversing the disease or halting its advancement. However, more complex therapeutic approaches based on using combinations of drugs that have different targets and thus affect different pathways and mechanisms compromised by the disease might significantly improve patients' conditions and slow down the disease progression. Together with the progress of early diagnostics of ALS, such a multitarget approach might even prevent any further loss of motor neuron function and consequently, stop the disease progression for a long time, if not completely.

Acknowledgments

This work was supported by grants from RFBR (20-34-70059) and RSF (18-15-00357).

Conflict of interests

The authors declare that there are no conflicts of interests.

References

1. Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers*. 2017;3:17071.
2. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. *The New England journal of medicine*. 2001;344(22):1688-1700.
3. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. *European journal of neurology*. 2020.
4. Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. *Brain : a journal of neurology*. 1995;118 (Pt 3):707-719.
5. Mehta P, Kaye W, Raymond J, et al. Prevalence of Amyotrophic Lateral Sclerosis - United States, 2014. *MMWR Morbidity and mortality weekly report*. 2018;67(7):216-218.
6. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130-133.
7. Brown RH, Jr., Al-Chalabi A. Amyotrophic Lateral Sclerosis. *The New England journal of medicine*. 2017;377(16):1602.
8. Mathis S, Goizet C, Soulages A, Vallat JM, Masson GL. Genetics of amyotrophic lateral sclerosis: A review. *J Neurol Sci*. 2019;399:217-226.
9. Alsultan AA, Waller R, Heath PR, Kirby J. The genetics of amyotrophic lateral sclerosis: current insights. *Degener Neurol Neuromuscul Dis*. 2016;6:49-64.
10. Nowicka N, Juranek J, Juranek JK, Wojtkiewicz J. Risk Factors and Emerging Therapies in Amyotrophic Lateral Sclerosis. *Int J Mol Sci*. 2019;20(11).
11. Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat Rev Neurol*. 2011;7(11):616-630.
12. Mejzini R, Flynn LL, Pitout IL, Fletcher S, Wilton SD, Akkari PA. ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? *Frontiers in neuroscience*. 2019;13:1310.
13. Miller RG, Bouchard JP, Duquette P, et al. Clinical trials of riluzole in patients with ALS. ALS/Riluzole Study Group-II. *Neurology*. 1996;47(4 Suppl 2):S86-90; discussion S90-82.
14. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *The Cochrane database of systematic reviews*. 2012(3):CD001447.
15. Chio A, Mazzini L, Mora G. Disease-modifying therapies in amyotrophic lateral sclerosis. *Neuropharmacology*. 2020;167:107986.

16. Jaiswal MK. Riluzole and edaravone: A tale of two amyotrophic lateral sclerosis drugs. *Medicinal research reviews*. 2019;39(2):733-748.
17. Writing G, Edaravone ALSSG. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*. 2017;16(7):505-512.
18. Taylor JP, Brown RH, Jr., Cleveland DW. Decoding ALS: from genes to mechanism. *Nature*. 2016;539(7628):197-206.
19. Phukan J, Elamin M, Bede P, et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J Neurol Neurosurg Psychiatry*. 2012;83(1):102-108.
20. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology*. 2002;59(7):1077-1079.
21. Boxer AL, Mackenzie IR, Boeve BF, et al. Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. *J Neurol Neurosurg Psychiatry*. 2011;82(2):196-203.
22. Turner MR, Goldacre R, Talbot K, Goldacre MJ. Psychiatric disorders prior to amyotrophic lateral sclerosis. *Ann Neurol*. 2016;80(6):935-938.
23. O'Brien M, Burke T, Heverin M, et al. Clustering of Neuropsychiatric Disease in First-Degree and Second-Degree Relatives of Patients With Amyotrophic Lateral Sclerosis. *JAMA Neurol*. 2017;74(12):1425-1430.
24. Byrne S, Heverin M, Elamin M, et al. Aggregation of neurologic and neuropsychiatric disease in amyotrophic lateral sclerosis kindreds: a population-based case-control cohort study of familial and sporadic amyotrophic lateral sclerosis. *Ann Neurol*. 2013;74(5):699-708.
25. McLaughlin RL, Schijven D, van Rheenen W, et al. Genetic correlation between amyotrophic lateral sclerosis and schizophrenia. *Nat Commun*. 2017;8:14774.
26. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*. 2011;72(2):245-256.
27. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*. 2011;72(2):257-268.
28. Renton AE, Chio A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci*. 2014;17(1):17-23.
29. Cammack AJ, Atassi N, Hyman T, et al. Prospective natural history study of C9orf72 ALS clinical characteristics and biomarkers. *Neurology*. 2019;93(17):e1605-e1617.
30. Jiang J, Ravits J. Pathogenic Mechanisms and Therapy Development for C9orf72 Amyotrophic Lateral Sclerosis/Frontotemporal Dementia.

- Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2019;16(4):1115-1132.
31. Silverman HE, Goldman JS, Huey ED. Links Between the C9orf72 Repeat Expansion and Psychiatric Symptoms. *Current neurology and neuroscience reports*. 2019;19(12):93.
 32. Oguro-Ando A, Zuko A, Kleijer KTE, Burbach JPH. A current view on contactin-4, -5, and -6: Implications in neurodevelopmental disorders. *Molecular and cellular neurosciences*. 2017;81:72-83.
 33. Farokhnia M, Sabzabadi M, Pourmahmoud H, et al. A double-blind, placebo controlled, randomized trial of riluzole as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia. *Psychopharmacology (Berl)*. 2014;231(3):533-542.
 34. Patten SA, Aggad D, Martinez J, et al. Neuroleptics as therapeutic compounds stabilizing neuromuscular transmission in amyotrophic lateral sclerosis. *JCI Insight*. 2017;2(22).
 35. Bancila M, Copin JC, Daali Y, Schatlo B, Gasche Y, Bijlenga P. Two structurally different T-type Ca²⁺ channel inhibitors, mibefradil and pimozide, protect CA1 neurons from delayed death after global ischemia in rats. *Fundam Clin Pharmacol*. 2011;25(4):469-478.
 36. Enyeart JJ, Biagi BA, Mlinar B. Preferential block of T-type calcium channels by neuroleptics in neural crest-derived rat and human C cell lines. *Mol Pharmacol*. 1992;42(2):364-372.
 37. Enyeart JJ, Dirksen RT, Sharma VK, Williford DJ, Sheu SS. Antipsychotic pimozide is a potent Ca²⁺ channel blocker in heart. *Mol Pharmacol*. 1990;37(5):752-757.
 38. Pozzi S, Thammisetty SS, Julien JP. Chronic Administration of Pimozide Fails to Attenuate Motor and Pathological Deficits in Two Mouse Models of Amyotrophic Lateral Sclerosis. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*. 2018.
 39. Naganska E, Matyja E, Taraszewska A, Rafalowska J. Protective effect of valproic acid on cultured motor neurons under glutamate excitotoxic conditions. Ultrastructural study. *Folia Neuropathol*. 2015;53(4):309-316.
 40. Jiang HZ, Wang SY, Yin X, et al. Downregulation of Homer1b/c in SOD1 G93A Models of ALS: A Novel Mechanism of Neuroprotective Effect of Lithium and Valproic Acid. *Int J Mol Sci*. 2016;17(12).
 41. de Bartolomeis A, Tomasetti C, Cicale M, Yuan PX, Manji HK. Chronic treatment with lithium or valproate modulates the expression of Homer1b/c and its related genes Shank and Inositol 1,4,5-trisphosphate receptor. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*. 2012;22(7):527-535.
 42. Wang SY, Ren M, Jiang HZ, et al. Notch pathway is activated in cell culture and mouse models of mutant SOD1-related familial amyotrophic lateral sclerosis, with suppression of its activation as an additional

- mechanism of neuroprotection for lithium and valproate. *Neuroscience*. 2015;301:276-288.
43. Gupta A, Schulze TG, Nagarajan V, et al. Interaction networks of lithium and valproate molecular targets reveal a striking enrichment of apoptosis functional clusters and neurotrophin signaling. *Pharmacogenomics J*. 2012;12(4):328-341.
 44. Wang X, Ma M, Teng J, et al. Valproate Attenuates 25-kDa C-Terminal Fragment of TDP-43-Induced Neuronal Toxicity via Suppressing Endoplasmic Reticulum Stress and Activating Autophagy. *Int J Biol Sci*. 2015;11(7):752-761.
 45. Leng Y, Liang MH, Ren M, Marinova Z, Leeds P, Chuang DM. Synergistic neuroprotective effects of lithium and valproic acid or other histone deacetylase inhibitors in neurons: roles of glycogen synthase kinase-3 inhibition. *J Neurosci*. 2008;28(10):2576-2588.
 46. Tomasetti C, Dell'Aversano C, Iasevoli F, Marmo F, de Bartolomeis A. The acute and chronic effects of combined antipsychotic-mood stabilizing treatment on the expression of cortical and striatal postsynaptic density genes. *Progress in neuro-psychopharmacology & biological psychiatry*. 2011;35(1):184-197.
 47. Fornai F, Longone P, Cafaro L, et al. Lithium delays progression of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A*. 2008;105(6):2052-2057.
 48. Fornai F, Siciliano G, Manca ML, Murri L, Paparelli A, Ruggieri S. Lithium in ALS: from the bench to the bedside. *Amyotroph Lateral Scler*. 2008;9(2):123-124.
 49. Ferrucci M, Spalloni A, Bartalucci A, et al. A systematic study of brainstem motor nuclei in a mouse model of ALS, the effects of lithium. *Neurobiol Dis*. 2010;37(2):370-383.
 50. Verstraete E, Veldink JH, Huisman MH, et al. Lithium lacks effect on survival in amyotrophic lateral sclerosis: a phase IIb randomised sequential trial. *J Neurol Neurosurg Psychiatry*. 2012;83(5):557-564.
 51. Chio A, Borghero G, Calvo A, et al. Lithium carbonate in amyotrophic lateral sclerosis: lack of efficacy in a dose-finding trial. *Neurology*. 2010;75(7):619-625.
 52. Miller RG, Moore DH, Forshew DA, et al. Phase II screening trial of lithium carbonate in amyotrophic lateral sclerosis: examining a more efficient trial design. *Neurology*. 2011;77(10):973-979.
 53. Aggarwal SP, Zinman L, Simpson E, et al. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*. 2010;9(5):481-488.
 54. Group UK-LS, Morrison KE, Dhariwal S, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre,

- randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*. 2013;12(4):339-345.
55. Gamez J, Salvado M, Martinez de la Ossa A, Badia M. Lithium for treatment of amyotrophic lateral sclerosis: much ado about nothing. *Neurologia*. 2016;31(8):550-561.
 56. van Es MA, Veldink JH, Saris CG, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet*. 2009;41(10):1083-1087.
 57. Diekstra FP, Van Deerlin VM, van Swieten JC, et al. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. *Ann Neurol*. 2014;76(1):120-133.
 58. Placek K, Baer GM, Elman L, et al. UNC13A polymorphism contributes to frontotemporal disease in sporadic amyotrophic lateral sclerosis. *Neurobiol Aging*. 2019;73:190-199.
 59. van Eijk RPA, Jones AR, Sproviero W, et al. Meta-analysis of pharmacogenetic interactions in amyotrophic lateral sclerosis clinical trials. *Neurology*. 2017;89(18):1915-1922.
 60. Feng HL, Leng Y, Ma CH, Zhang J, Ren M, Chuang DM. Combined lithium and valproate treatment delays disease onset, reduces neurological deficits and prolongs survival in an amyotrophic lateral sclerosis mouse model. *Neuroscience*. 2008;155(3):567-572.
 61. Boll MC, Bayliss L, Vargas-Canas S, et al. Clinical and biological changes under treatment with lithium carbonate and valproic acid in sporadic amyotrophic lateral sclerosis. *J Neurol Sci*. 2014;340(1-2):103-108.
 62. Peters OM, Ghasemi M, Brown RH, Jr. Emerging mechanisms of molecular pathology in ALS. *J Clin Invest*. 2015;125(5):1767-1779.
 63. McAlary L, Plotkin SS, Yerbury JJ, Cashman NR. Prion-Like Propagation of Protein Misfolding and Aggregation in Amyotrophic Lateral Sclerosis. *Front Mol Neurosci*. 2019;12:262.
 64. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature*. 2016;529(7586):326-335.
 65. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;334(6059):1081-1086.
 66. Oakes SA, Papa FR. The role of endoplasmic reticulum stress in human pathology. *Annu Rev Pathol*. 2015;10:173-194.
 67. Urra H, Dufey E, Lisbona F, Rojas-Rivera D, Hetz C. When ER stress reaches a dead end. *Biochim Biophys Acta*. 2013;1833(12):3507-3517.
 68. Rozas P, Bargsted L, Martinez F, Hetz C, Medinas DB. The ER proteostasis network in ALS: Determining the differential motoneuron vulnerability. *Neurosci Lett*. 2017;636:9-15.

69. Saxena S, Caroni P. Selective neuronal vulnerability in neurodegenerative diseases: from stressor thresholds to degeneration. *Neuron*. 2011;71(1):35-48.
70. Atkin JD, Farg MA, Walker AK, McLean C, Tomas D, Horne MK. Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiol Dis*. 2008;30(3):400-407.
71. Hetz C, Thielen P, Matus S, et al. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. *Genes Dev*. 2009;23(19):2294-2306.
72. Ilieva EV, Ayala V, Jove M, et al. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. *Brain : a journal of neurology*. 2007;130(Pt 12):3111-3123.
73. Farg MA, Soo KY, Walker AK, et al. Mutant FUS induces endoplasmic reticulum stress in amyotrophic lateral sclerosis and interacts with protein disulfide-isomerase. *Neurobiol Aging*. 2012;33(12):2855-2868.
74. Gonzalez-Perez P, Woehlbier U, Chian RJ, et al. Identification of rare protein disulfide isomerase gene variants in amyotrophic lateral sclerosis patients. *Gene*. 2015;566(2):158-165.
75. Yang Q, Guo ZB. Polymorphisms in protein disulfide isomerase are associated with sporadic amyotrophic lateral sclerosis in the Chinese Han population. *Int J Neurosci*. 2016;126(7):607-611.
76. Parakh S, Atkin JD. Protein folding alterations in amyotrophic lateral sclerosis. *Brain Res*. 2016;1648(Pt B):633-649.
77. Ruegsegger C, Saxena S. Proteostasis impairment in ALS. *Brain Res*. 2016;1648(Pt B):571-579.
78. Watkins TA, Wang B, Huntwork-Rodriguez S, et al. DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. *Proc Natl Acad Sci U S A*. 2013;110(10):4039-4044.
79. Welsbie DS, Yang Z, Ge Y, et al. Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. *Proc Natl Acad Sci U S A*. 2013;110(10):4045-4050.
80. Poznaniak CD, Sengupta Ghosh A, Gogineni A, et al. Dual leucine zipper kinase is required for excitotoxicity-induced neuronal degeneration. *J Exp Med*. 2013;210(12):2553-2567.
81. Le Pichon CE, Meilandt WJ, Dominguez S, et al. Loss of dual leucine zipper kinase signaling is protective in animal models of neurodegenerative disease. *Sci Transl Med*. 2017;9(403).
82. Uppala JK, Gani AR, Ramaiah KVA. Chemical chaperone, TUDCA unlike PBA, mitigates protein aggregation efficiently and resists ER and non-ER stress induced HepG2 cell death. *Scientific reports*. 2017;7(1):3831.

83. Ozcan U, Yilmaz E, Ozcan L, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. 2006;313(5790):1137-1140.
84. Ackerman HD, Gerhard GS. Bile Acids in Neurodegenerative Disorders. *Frontiers in aging neuroscience*. 2016;8:263.
85. Thams S, Lowry ER, Larraufie MH, et al. A Stem Cell-Based Screening Platform Identifies Compounds that Desensitize Motor Neurons to Endoplasmic Reticulum Stress. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2019;27(1):87-101.
86. Elia AE, Lalli S, Monsurro MR, et al. Tauroursodeoxycholic acid in the treatment of patients with amyotrophic lateral sclerosis. *European journal of neurology*. 2016;23(1):45-52.
87. Min JH, Hong YH, Sung JJ, Kim SM, Lee JB, Lee KW. Oral solubilized ursodeoxycholic acid therapy in amyotrophic lateral sclerosis: a randomized cross-over trial. *Journal of Korean medical science*. 2012;27(2):200-206.
88. Ludolph AC. The TUDCA trial--innovative trial designs for amyotrophic lateral sclerosis drugs? *European journal of neurology*. 2016;23(1):11-12.
89. Kolb PS, Ayaub EA, Zhou W, Yum V, Dickhout JG, Ask K. The therapeutic effects of 4-phenylbutyric acid in maintaining proteostasis. *The international journal of biochemistry & cell biology*. 2015;61:45-52.
90. Cudkovic ME, Andres PL, Macdonald SA, et al. Phase 2 study of sodium phenylbutyrate in ALS. *Amyotroph Lateral Scler*. 2009;10(2):99-106.
91. Ryu H, Smith K, Camelo SI, et al. Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice. *Journal of neurochemistry*. 2005;93(5):1087-1098.
92. Corman A, Jung B, Haggblad M, et al. A Chemical Screen Identifies Compounds Limiting the Toxicity of C9ORF72 Dipeptide Repeats. *Cell chemical biology*. 2019;26(2):235-243 e235.
93. Del Signore SJ, Amante DJ, Kim J, et al. Combined riluzole and sodium phenylbutyrate therapy in transgenic amyotrophic lateral sclerosis mice. *Amyotroph Lateral Scler*. 2009;10(2):85-94.
94. Petri S, Kiaei M, Kipiani K, et al. Additive neuroprotective effects of a histone deacetylase inhibitor and a catalytic antioxidant in a transgenic mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis*. 2006;22(1):40-49.
95. Kieran D, Kalmar B, Dick JR, Riddoch-Contreras J, Burnstock G, Greensmith L. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nature medicine*. 2004;10(4):402-405.

96. Benatar M, Wu J, Andersen PM, et al. Randomized, double-blind, placebo-controlled trial of arimoclomol in rapidly progressive SOD1 ALS. *Neurology*. 2018;90(7):e565-e574.
97. Batulan Z, Shinder GA, Minotti S, et al. High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1. *J Neurosci*. 2003;23(13):5789-5798.
98. Manzerra P, Brown IR. Expression of heat shock genes (hsp70) in the rabbit spinal cord: localization of constitutive and hyperthermia-inducible mRNA species. *Journal of neuroscience research*. 1992;31(4):606-615.
99. Batulan Z, Nalbantoglu J, Durham HD. Nonsteroidal anti-inflammatory drugs differentially affect the heat shock response in cultured spinal cord cells. *Cell stress & chaperones*. 2005;10(3):185-196.
100. Kuta R, Larochelle N, Fernandez M, et al. Depending on the stress, histone deacetylase inhibitors act as heat shock protein co-inducers in motor neurons and potentiate arimoclomol, exerting neuroprotection through multiple mechanisms in ALS models. *Cell stress & chaperones*. 2020;25(1):173-191.
101. van den Bos MAJ, Geevasinga N, Higashihara M, Menon P, Vucic S. Pathophysiology and Diagnosis of ALS: Insights from Advances in Neurophysiological Techniques. *Int J Mol Sci*. 2019;20(11).
102. King AE, Woodhouse A, Kirkcaldie MT, Vickers JC. Excitotoxicity in ALS: Overstimulation, or overreaction? *Experimental neurology*. 2016;275 Pt 1:162-171.
103. Deeks ED. Retigabine (ezogabine): in partial-onset seizures in adults with epilepsy. *CNS Drugs*. 2011;25(10):887-900.
104. Kovalchuk MO, Heuberger J, Sleutjes B, et al. Acute Effects of Riluzole and Retigabine on Axonal Excitability in Patients With Amyotrophic Lateral Sclerosis: A Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. *Clin Pharmacol Ther*. 2018;104(6):1136-1145.
105. Wainger B. Ezogabine treatment shown to reduce motor neuron excitability in ALS patients. 2018; <https://www.massgeneral.org/neurology/als/news/ezogabine-reduces-motor-neuron-excitability>. Accessed 30.01.2020, 2020.
106. Wilson SM, Khanna R. Specific binding of lacosamide to collapsin response mediator protein 2 (CRMP2) and direct impairment of its canonical function: implications for the therapeutic potential of lacosamide. *Mol Neurobiol*. 2015;51(2):599-609.
107. Ahn JY, Yan BC, Park JH, et al. Novel antiepileptic drug lacosamide exerts neuroprotective effects by decreasing glial activation in the hippocampus of a gerbil model of ischemic stroke. *Experimental and therapeutic medicine*. 2015;10(6):2007-2014.

108. Kim GH, Byeon JH, Eun BL. Neuroprotective Effect of Lacosamide on Hypoxic-Ischemic Brain Injury in Neonatal Rats. *Journal of clinical neurology*. 2017;13(2):138-143.
109. Gencpinar P, Basaranlar G, Sati L, Duman O, Derin N. Effects of Chronic Topiramate, Lacosamide, and Levetiracetam Pre-treatment on a Status Epilepticus Model in Rat Pups. *Neurophysiology*. 2019;51(1):35-42.
110. Oskarsson B, Moore D, Mozaffar T, et al. Mexiletine for muscle cramps in amyotrophic lateral sclerosis: A randomized, double-blind crossover trial. *Muscle Nerve*. 2018.
111. Weiss MD, Macklin EA, Simmons Z, et al. A randomized trial of mexiletine in ALS: Safety and effects on muscle cramps and progression. *Neurology*. 2016;86(16):1474-1481.
112. Stys PK, Lesiuk H. Correlation between electrophysiological effects of mexiletine and ischemic protection in central nervous system white matter. *Neuroscience*. 1996;71(1):27-36.
113. Shibuya K, Misawa S, Kimura H, et al. A single blind randomized controlled clinical trial of mexiletine in amyotrophic lateral sclerosis: Efficacy and safety of sodium channel blocker phase II trial. *Amyotroph Lateral Scler Frontotemporal Degener*. 2015;16(5-6):353-358.
114. Bensimon G, Lacomblez L, Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *The New England journal of medicine*. 1994;330(9):585-591.
115. Bensimon G, Lacomblez L, Delumeau JC, et al. A study of riluzole in the treatment of advanced stage or elderly patients with amyotrophic lateral sclerosis. *J Neurol*. 2002;249(5):609-615.
116. Reddy BM, Weintraub HS, Schwartzbard AZ. Ranolazine: a new approach to treating an old problem. *Tex Heart Inst J*. 2010;37(6):641-647.
117. Schram G, Zhang L, Derakhchan K, Ehrlich JR, Belardinelli L, Nattel S. Ranolazine: ion-channel-blocking actions and in vivo electrophysiological effects. *Br J Pharmacol*. 2004;142(8):1300-1308.
118. Chaitman BR. Ranolazine for the treatment of chronic angina and potential use in other cardiovascular conditions. *Circulation*. 2006;113(20):2462-2472.
119. Peters CH, Sokolov S, Rajamani S, Ruben PC. Effects of the antianginal drug, ranolazine, on the brain sodium channel Na(V)1.2 and its modulation by extracellular protons. *Br J Pharmacol*. 2013;169(3):704-716.
120. Park YY, Johnston D, Gray R. Slowly inactivating component of Na⁺ current in peri-somatic region of hippocampal CA1 pyramidal neurons. *J Neurophysiol*. 2013;109(5):1378-1390.
121. Gould HJ, 3rd, Garrett C, Donahue RR, Paul D, Diamond I, Taylor BK. Ranolazine attenuates behavioral signs of neuropathic pain. *Behav Pharmacol*. 2009;20(8):755-758.

122. Gould HJ, 3rd, Diamond I. Ranolazine: A potential treatment for refractory neuropathic pain. *J Neurol Sci.* 2016;369:310-311.
123. Kahlig KM, Hirakawa R, Liu L, George AL, Jr., Belardinelli L, Rajamani S. Ranolazine reduces neuronal excitability by interacting with inactivated states of brain sodium channels. *Mol Pharmacol.* 2014;85(1):162-174.
124. Kahlig KM, Lepist I, Leung K, Rajamani S, George AL. Ranolazine selectively blocks persistent current evoked by epilepsy-associated Nanu1.1 mutations. *Br J Pharmacol.* 2010;161(6):1414-1426.
125. Aldasoro M, Guerra-Ojeda S, Aguirre-Rueda D, et al. Effects of Ranolazine on Astrocytes and Neurons in Primary Culture. *PLoS One.* 2016;11(3):e0150619.
126. Joo IS, Hwang DH, Seok JI, Shin SK, Kim SU. Oral administration of memantine prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis. *Journal of clinical neurology.* 2007;3(4):181-186.
127. Wang R, Zhang D. Memantine prolongs survival in an amyotrophic lateral sclerosis mouse model. *Eur J Neurosci.* 2005;22(9):2376-2380.
128. Levine TD, Bowser R, Hank N, Saperstein D. A pilot trial of memantine and riluzole in ALS: correlation to CSF biomarkers. *Amyotroph Lateral Scler.* 2010;11(6):514-519.
129. de Carvalho M, Pinto S, Costa J, Evangelista T, Ohana B, Pinto A. A randomized, placebo-controlled trial of memantine for functional disability in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler.* 2010;11(5):456-460.
130. Bezzi P, Carmignoto G, Pasti L, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature.* 1998;391(6664):281-285.
131. Sanzgiri RP, Araque A, Haydon PG. Prostaglandin E(2) stimulates glutamate receptor-dependent astrocyte neuromodulation in cultured hippocampal cells. *J Neurobiol.* 1999;41(2):221-229.
132. Drachman DB, Frank K, Dykes-Hoberg M, et al. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann Neurol.* 2002;52(6):771-778.
133. Klivenyi P, Kiaei M, Gardian G, Calingasan NY, Beal MF. Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *Journal of neurochemistry.* 2004;88(3):576-582.
134. Kiaei M, Kipiani K, Petri S, et al. Integrative role of cPLA with COX-2 and the effect of non-steroidal anti-inflammatory drugs in a transgenic mouse model of amyotrophic lateral sclerosis. *Journal of neurochemistry.* 2005;93(2):403-411.
135. Cudkowicz ME, Shefner JM, Schoenfeld DA, et al. Trial of celecoxib in amyotrophic lateral sclerosis. *Ann Neurol.* 2006;60(1):22-31.

136. Zu T, Liu Y, Banez-Coronel M, et al. RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. *Proc Natl Acad Sci U S A*. 2013;110(51):E4968-4977.
137. Mori K, Arzberger T, Grasser FA, et al. Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. *Acta Neuropathol*. 2013;126(6):881-893.
138. Tabet R, Schaeffer L, Freyermuth F, et al. CUG initiation and frameshifting enable production of dipeptide repeat proteins from ALS/FTD C9ORF72 transcripts. *Nat Commun*. 2018;9(1):152.
139. Rotermund C, Machetanz G, Fitzgerald JC. The Therapeutic Potential of Metformin in Neurodegenerative Diseases. *Front Endocrinol (Lausanne)*. 2018;9:400.
140. Westergard T, McAvoy K, Russell K, et al. Repeat-associated non-AUG translation in C9orf72-ALS/FTD is driven by neuronal excitation and stress. *EMBO Mol Med*. 2019;11(2).
141. Kaneb HM, Sharp PS, Rahmani-Kondori N, Wells DJ. Metformin treatment has no beneficial effect in a dose-response survival study in the SOD1(G93A) mouse model of ALS and is harmful in female mice. *PLoS One*. 2011;6(9):e24189.
142. Naujock M, Stanslowsky N, Bufler S, et al. 4-Aminopyridine Induced Activity Rescues Hypoexcitable Motor Neurons from Amyotrophic Lateral Sclerosis Patient-Derived Induced Pluripotent Stem Cells. *Stem Cells*. 2016;34(6):1563-1575.
143. Martinez-Silva ML, Imhoff-Manuel RD, Sharma A, et al. Hypoexcitability precedes denervation in the large fast-contracting motor units in two unrelated mouse models of ALS. *Elife*. 2018;7.
144. Devlin AC, Burr K, Borooh S, et al. Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. *Nat Commun*. 2015;6:5999.
145. Peikert K, Naumann M, Gunther R, Wegner F, Hermann A. Off-Label Treatment of 4 Amyotrophic Lateral Sclerosis Patients With 4-Aminopyridine. *J Clin Pharmacol*. 2019;59(10):1400-1404.
146. Aizawa H, Hideyama T, Yamashita T, et al. Deficient RNA-editing enzyme ADAR2 in an amyotrophic lateral sclerosis patient with a FUS(P525L) mutation. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia*. 2016;32:128-129.
147. Hideyama T, Yamashita T, Aizawa H, et al. Profound downregulation of the RNA editing enzyme ADAR2 in ALS spinal motor neurons. *Neurobiol Dis*. 2012;45(3):1121-1128.
148. Kawahara Y, Ito K, Sun H, Aizawa H, Kanazawa I, Kwak S. Glutamate receptors: RNA editing and death of motor neurons. *Nature*. 2004;427(6977):801.

149. Takuma H, Kwak S, Yoshizawa T, Kanazawa I. Reduction of GluR2 RNA editing, a molecular change that increases calcium influx through AMPA receptors, selective in the spinal ventral gray of patients with amyotrophic lateral sclerosis. *Ann Neurol.* 1999;46(6):806-815.
150. Moore S, Alsop E, Lorenzini I, et al. ADAR2 mislocalization and widespread RNA editing aberrations in C9orf72-mediated ALS/FTD. *Acta Neuropathol.* 2019;138(1):49-65.
151. Yamashita T, Kwak S. Cell death cascade and molecular therapy in ADAR2-deficient motor neurons of ALS. *Neuroscience research.* 2019;144:4-13.
152. Yamashita T, Kwak S. The molecular link between inefficient GluA2 Q/R site-RNA editing and TDP-43 pathology in motor neurons of sporadic amyotrophic lateral sclerosis patients. *Brain Res.* 2014;1584:28-38.
153. Aizawa H, Sawada J, Hideyama T, et al. TDP-43 pathology in sporadic ALS occurs in motor neurons lacking the RNA editing enzyme ADAR2. *Acta Neuropathol.* 2010;120(1):75-84.
154. Akamatsu M, Yamashita T, Hirose N, Teramoto S, Kwak S. The AMPA receptor antagonist perampanel robustly rescues amyotrophic lateral sclerosis (ALS) pathology in sporadic ALS model mice. *Scientific reports.* 2016;6:28649.
155. Orrell RW, Lane RJ, Ross M. Antioxidant treatment for amyotrophic lateral sclerosis / motor neuron disease. *The Cochrane database of systematic reviews.* 2007(1):CD002829.
156. Orrell RW, Lane RJ, Ross M. A systematic review of antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. *Amyotroph Lateral Scler.* 2008;9(4):195-211.
157. Brooks BR, Jorgenson JA, Newhouse BJ, Shefner JM, Agnese W. Edaravone in the treatment of amyotrophic lateral sclerosis: efficacy and access to therapy - a roundtable discussion. *The American journal of managed care.* 2018;24(9 Suppl):S175-S186.
158. Takei K, Watanabe K, Yuki S, Akimoto M, Sakata T, Palumbo J. Edaravone and its clinical development for amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener.* 2017;18(sup1):5-10.
159. Veyrat-Durebex C, Corcia P, Mucha A, et al. Iron metabolism disturbance in a French cohort of ALS patients. *BioMed research international.* 2014;2014:485723.
160. Adachi Y, Sato N, Saito Y, et al. Usefulness of SWI for the Detection of Iron in the Motor Cortex in Amyotrophic Lateral Sclerosis. *Journal of neuroimaging : official journal of the American Society of Neuroimaging.* 2015;25(3):443-451.
161. Kwan JY, Jeong SY, Van Gelderen P, et al. Iron accumulation in deep cortical layers accounts for MRI signal abnormalities in ALS: correlating 7 tesla MRI and pathology. *PLoS One.* 2012;7(4):e35241.

162. Ignjatovic A, Stevic Z, Lavrnjic D, et al. Inappropriately chelated iron in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler.* 2012;13(4):357-362.
163. Sheykhansari S, Kozielski K, Bill J, et al. Redox metals homeostasis in multiple sclerosis and amyotrophic lateral sclerosis: a review. *Cell death & disease.* 2018;9(3):348.
164. Galey JB. Potential use of iron chelators against oxidative damage. *Advances in pharmacology.* 1997;38:167-203.
165. Jomova K, Valko M. Importance of iron chelation in free radical-induced oxidative stress and human disease. *Current pharmaceutical design.* 2011;17(31):3460-3473.
166. Petillon C, Hergesheimer R, Puy H, et al. The Relevancy of Data Regarding the Metabolism of Iron to Our Understanding of Deregulated Mechanisms in ALS; Hypotheses and Pitfalls. *Frontiers in neuroscience.* 2018;12:1031.
167. Zarbin M. Treatment of oxidative stress with chelation therapy. *Archives of ophthalmology.* 2012;130(12):1597-1598.
168. Moreau C, Danel V, Devedjian JC, et al. Could Conservative Iron Chelation Lead to Neuroprotection in Amyotrophic Lateral Sclerosis? *Antioxidants & redox signaling.* 2018;29(8):742-748.
169. Hilton JB, Mercer SW, Lim NK, et al. Cu(II)(atsm) improves the neurological phenotype and survival of SOD1(G93A) mice and selectively increases enzymatically active SOD1 in the spinal cord. *Scientific reports.* 2017;7:42292.
170. McAllum EJ, Lim NK, Hickey JL, et al. Therapeutic effects of CuII(atsm) in the SOD1-G37R mouse model of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener.* 2013;14(7-8):586-590.
171. Soon CP, Donnelly PS, Turner BJ, et al. Diacetyl-bis(N(4)-methylthiosemicarbazone) copper(II) (CuII(atsm)) protects against peroxynitrite-induced nitrosative damage and prolongs survival in amyotrophic lateral sclerosis mouse model. *J Biol Chem.* 2011;286(51):44035-44044.
172. Roberts BR, Lim NK, McAllum EJ, et al. Oral treatment with Cu(II)(atsm) increases mutant SOD1 in vivo but protects motor neurons and improves the phenotype of a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci.* 2014;34(23):8021-8031.
173. Vieira FG, Hatzipetros T, Thompson K, et al. CuATSM efficacy is independently replicated in a SOD1 mouse model of ALS while unmetallated ATSM therapy fails to reveal benefits. *IBRO Rep.* 2017;2:47-53.
174. Ikawa M, Okazawa H, Arakawa K, et al. PET imaging of redox and energy states in stroke-like episodes of MELAS. *Mitochondrion.* 2009;9(2):144-148.

175. Ikawa M, Okazawa H, Kudo T, Kuriyama M, Fujibayashi Y, Yoneda M. Evaluation of striatal oxidative stress in patients with Parkinson's disease using [62Cu]ATSM PET. *Nucl Med Biol.* 2011;38(7):945-951.
176. Ikawa M, Okazawa H, Tsujikawa T, et al. Increased oxidative stress is related to disease severity in the ALS motor cortex: A PET study. *Neurology.* 2015;84(20):2033-2039.
177. Southon A, Szostak K, Acevedo KM, et al. Cu(II) (atSM) inhibits ferroptosis: Implications for treatment of neurodegenerative disease. *Br J Pharmacol.* 2020;177(3):656-667.
178. Rowe D, Mathers S, Smith G, et al. Modification of ALS disease progression in a phase 1 trial of CuATSM. *Amyotroph Lateral Scler Frontotemporal Degener.* 2018;19(sup1):280-281.
179. Chen X, Burdett TC, Desjardins CA, et al. Disrupted and transgenic urate oxidase alter urate and dopaminergic neurodegeneration. *Proc Natl Acad Sci U S A.* 2013;110(1):300-305.
180. Chamorro A, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *The Lancet Neurology.* 2016;15(8):869-881.
181. Paganoni S, Schwarzschild MA. Urate as a Marker of Risk and Progression of Neurodegenerative Disease. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics.* 2017;14(1):148-153.
182. Yu ZF, Bruce-Keller AJ, Goodman Y, Mattson MP. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *Journal of neuroscience research.* 1998;53(5):613-625.
183. O'Reilly EJ, Liu D, Johns DR, et al. Serum urate at trial entry and ALS progression in EMPOWER. *Amyotroph Lateral Scler Frontotemporal Degener.* 2017;18(1-2):120-125.
184. Paganoni S, Nicholson K, Chan J, et al. Urate levels predict survival in amyotrophic lateral sclerosis: Analysis of the expanded Pooled Resource Open-Access ALS clinical trials database. *Muscle Nerve.* 2018;57(3):430-434.
185. Paganoni S, Zhang M, Quiroz Zarate A, et al. Uric acid levels predict survival in men with amyotrophic lateral sclerosis. *J Neurol.* 2012;259(9):1923-1928.
186. Kuffner R, Zach N, Norel R, et al. Crowdsourced analysis of clinical trial data to predict amyotrophic lateral sclerosis progression. *Nature biotechnology.* 2015;33(1):51-57.
187. Abraham A, Drory VE. Influence of serum uric acid levels on prognosis and survival in amyotrophic lateral sclerosis: a meta-analysis. *J Neurol.* 2014;261(6):1133-1138.

188. Zhang F, Zhang Q, Ke Y, et al. Serum uric acid levels in patients with amyotrophic lateral sclerosis: a meta-analysis. *Scientific reports*. 2018;8(1):1100.
189. Nagase M, Yamamoto Y, Miyazaki Y, Yoshino H. Increased oxidative stress in patients with amyotrophic lateral sclerosis and the effect of edaravone administration. *Redox report : communications in free radical research*. 2016;21(3):104-112.
190. Yamamoto Y. Plasma marker of tissue oxidative damage and edaravone as a scavenger drug against peroxy radicals and peroxy nitrite. *Journal of clinical biochemistry and nutrition*. 2017;60(1):49-54.
191. Nicholson K, Chan J, Macklin EA, et al. Pilot trial of inosine to elevate urate levels in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. 2018;5(12):1522-1533.
192. 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.
193. McCauley ME, Baloh RH. Inflammation in ALS/FTD pathogenesis. *Acta Neuropathol*. 2019;137(5):715-730.
194. Graves MC, Fiala M, Dinglasan LA, et al. Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. *Amyotrophic lateral sclerosis and other motor neuron disorders : official publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases*. 2004;5(4):213-219.
195. Henkel JS, Engelhardt JI, Siklos L, et al. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol*. 2004;55(2):221-235.
196. Engelhardt JI, Tajti J, Appel SH. Lymphocytic infiltrates in the spinal cord in amyotrophic lateral sclerosis. *Archives of neurology*. 1993;50(1):30-36.
197. Murdock BJ, Zhou T, Kashlan SR, Little RJ, Goutman SA, Feldman EL. Correlation of Peripheral Immunity With Rapid Amyotrophic Lateral Sclerosis Progression. *JAMA Neurol*. 2017;74(12):1446-1454.
198. Zhang R, Gascon R, Miller RG, et al. Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (sALS). *Journal of neuroimmunology*. 2005;159(1-2):215-224.
199. Prinz M, Priller J. The role of peripheral immune cells in the CNS in steady state and disease. *Nat Neurosci*. 2017;20(2):136-144.
200. Khalid SI, Ampie L, Kelly R, Ladha SS, Dardis C. Immune Modulation in the Treatment of Amyotrophic Lateral Sclerosis: A Review of Clinical Trials. *Front Neurol*. 2017;8:486.
201. Cho Y, Crichton GV, Vermeire JJ, et al. Allosteric inhibition of macrophage migration inhibitory factor revealed by ibudilast. *Proc Natl Acad Sci U S A*. 2010;107(25):11313-11318.

202. Fox RJ, Coffey CS, Conwit R, et al. Phase 2 Trial of Ibudilast in Progressive Multiple Sclerosis. *The New England journal of medicine*. 2018;379(9):846-855.
203. Schwenkgrub J, Zaremba M, Joniec-Maciejak I, Cudna A, Mirowska-Guzel D, Kurkowska-Jastrzebska I. The phosphodiesterase inhibitor, ibudilast, attenuates neuroinflammation in the MPTP model of Parkinson's disease. *PLoS One*. 2017;12(7):e0182019.
204. Suzumura A, Ito A, Yoshikawa M, Sawada M. Ibudilast suppresses TNFalpha production by glial cells functioning mainly as type III phosphodiesterase inhibitor in the CNS. *Brain Res*. 1999;837(1-2):203-212.
205. Kawanokuchi J, Mizuno T, Kato H, Mitsuma N, Suzumura A. Effects of interferon-beta on microglial functions as inflammatory and antigen presenting cells in the central nervous system. *Neuropharmacology*. 2004;46(5):734-742.
206. Mizuno T, Kurotani T, Komatsu Y, et al. Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. *Neuropharmacology*. 2004;46(3):404-411.
207. Tominaga Y, Nakamura Y, Tsuji K, Shibata T, Kataoka K. Ibudilast protects against neuronal damage induced by glutamate in cultured hippocampal neurons. *Clinical and experimental pharmacology & physiology*. 1996;23(6-7):519-523.
208. Matsuda K, Iwaki Y, Makhay M, Dojillo J, Yasui S. *Interaction (nonuniformity) of ALS progression and the efficacy of MN-166 (ibudilast)*. 30th International Symposium on ALS/MND2019.
209. Trias E, Ibarburu S, Barreto-Nunez R, et al. Post-paralysis tyrosine kinase inhibition with masitinib abrogates neuroinflammation and slows disease progression in inherited amyotrophic lateral sclerosis. *J Neuroinflammation*. 2016;13(1):177.
210. Mora JS, Genge A, Chio A, et al. Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. *Amyotroph Lateral Scler Frontotemporal Degener*. 2019:1-10.
211. Henkel JS, Beers DR, Wen S, et al. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. *EMBO Mol Med*. 2013;5(1):64-79.
212. Beers DR, Zhao W, Wang J, et al. ALS patients' regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. *JCI Insight*. 2017;2(5):e89530.
213. Kosmaczewska A. Low-dose interleukin-2 therapy: a driver of an imbalance between immune tolerance and autoimmunity. *Int J Mol Sci*. 2014;15(10):18574-18592.

214. Thonhoff JR, Beers DR, Zhao W, et al. Expanded autologous regulatory T-lymphocyte infusions in ALS: A phase I, first-in-human study. *Neurol Neuroimmunol Neuroinflamm*. 2018;5(4):e465.
215. Vucic S, Ryder J, Mekhael L, et al. Phase 2 randomized placebo controlled double blind study to assess the efficacy and safety of tecfidera in patients with amyotrophic lateral sclerosis (TEALS Study): Study protocol clinical trial (SPIRIT Compliant). *Medicine (Baltimore)*. 2020;99(6):e18904.
216. Cheng CW, Lin MJ, Shen CK. Rapamycin alleviates pathogenesis of a new Drosophila model of ALS-TDP. *J Neurogenet*. 2015;29(2-3):59-68.
217. Ryu HH, Jun MH, Min KJ, et al. Autophagy regulates amyotrophic lateral sclerosis-linked fused in sarcoma-positive stress granules in neurons. *Neurobiol Aging*. 2014;35(12):2822-2831.
218. Nalbandian A, Llewellyn KJ, Nguyen C, Yazdi PG, Kimonis VE. Rapamycin and chloroquine: the in vitro and in vivo effects of autophagy-modifying drugs show promising results in valosin containing protein multisystem proteinopathy. *PLoS One*. 2015;10(4):e0122888.
219. Wang IF, Guo BS, Liu YC, et al. Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. *Proc Natl Acad Sci U S A*. 2012;109(37):15024-15029.
220. Barmada SJ, Serio A, Arjun A, et al. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol*. 2014;10(8):677-685.
221. Madill M, McDonagh K, Ma J, et al. Amyotrophic lateral sclerosis patient iPSC-derived astrocytes impair autophagy via non-cell autonomous mechanisms. *Mol Brain*. 2017;10(1):22.
222. Lattante S, de Calbiac H, Le Ber I, Brice A, Ciura S, Kabashi E. Sqstm1 knock-down causes a locomotor phenotype ameliorated by rapamycin in a zebrafish model of ALS/FTLD. *Hum Mol Genet*. 2015;24(6):1682-1690.
223. Mandrioli J, D'Amico R, Zucchi E, et al. Rapamycin treatment for amyotrophic lateral sclerosis: Protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). *Medicine (Baltimore)*. 2018;97(24):e11119.
224. Ben-Chetrit E, Bergmann S, Sood R. Mechanism of the anti-inflammatory effect of colchicine in rheumatic diseases: a possible new outlook through microarray analysis. *Rheumatology (Oxford)*. 2006;45(3):274-282.
225. Gasparyan AY, Ayvazyan L, Yessirkepov M, Kitas GD. Colchicine as an anti-inflammatory and cardioprotective agent. *Expert Opin Drug Metab Toxicol*. 2015;11(11):1781-1794.
226. Crippa V, D'Agostino VG, Cristofani R, et al. Transcriptional induction of the heat shock protein B8 mediates the clearance of misfolded proteins responsible for motor neuron diseases. *Scientific reports*. 2016;6:22827.
227. Mandrioli J, Crippa V, Cereda C, et al. Proteostasis and ALS: protocol for a phase II, randomised, double-blind, placebo-controlled, multicentre

- clinical trial for colchicine in ALS (Co-ALS). *BMJ Open*. 2019;9(5):e028486.
228. Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. *Ann Neurol*. 2011;69(1):141-151.
 229. MacGowan DJ, Scelsa SN, Imperato TE, Liu KN, Baron P, Polsky B. A controlled study of reverse transcriptase in serum and CSF of HIV-negative patients with ALS. *Neurology*. 2007;68(22):1944-1946.
 230. McCormick AL, Brown RH, Jr., Cudkowicz ME, Al-Chalabi A, Garson JA. Quantification of reverse transcriptase in ALS and elimination of a novel retroviral candidate. *Neurology*. 2008;70(4):278-283.
 231. Steele AJ, Al-Chalabi A, Ferrante K, Cudkowicz ME, Brown RH, Jr., Garson JA. Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives. *Neurology*. 2005;64(3):454-458.
 232. Andrews WD, Tuke PW, Al-Chalabi A, et al. Detection of reverse transcriptase activity in the serum of patients with motor neurone disease. *Journal of medical virology*. 2000;61(4):527-532.
 233. Mayer J, Harz C, Sanchez L, et al. Transcriptional profiling of HERV-K(HML-2) in amyotrophic lateral sclerosis and potential implications for expression of HML-2 proteins. *Mol Neurodegener*. 2018;13(1):39.
 234. Garson JA, Usher L, Al-Chalabi A, Huggett J, Day EF, McCormick AL. Quantitative analysis of human endogenous retrovirus-K transcripts in postmortem premotor cortex fails to confirm elevated expression of HERV-K RNA in amyotrophic lateral sclerosis. *Acta neuropathologica communications*. 2019;7(1):45.
 235. Shelkownikova TA, An H, Skelt L, Tregoning JS, Humphreys IR, Buchman VL. Antiviral Immune Response as a Trigger of FUS Proteinopathy in Amyotrophic Lateral Sclerosis. *Cell reports*. 2019;29(13):4496-4508 e4494.
 236. Alfahad T, Nath A. Retroviruses and amyotrophic lateral sclerosis. *Antiviral Res*. 2013;99(2):180-187.
 237. Li W, Lee MH, Henderson L, et al. Human endogenous retrovirus-K contributes to motor neuron disease. *Sci Transl Med*. 2015;7(307):307ra153.
 238. Bowen LN, Tyagi R, Li W, et al. HIV-associated motor neuron disease: HERV-K activation and response to antiretroviral therapy. *Neurology*. 2016;87(17):1756-1762.
 239. Gold J, Rowe DB, Kiernan MC, et al. Safety and tolerability of Triumeq in amyotrophic lateral sclerosis: the Lighthouse trial. *Amyotroph Lateral Scler Frontotemporal Degener*. 2019;20(7-8):595-604.
 240. Doody RS, Gavrilova SI, Sano M, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with

- mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. *Lancet*. 2008;372(9634):207-215.
241. Cano-Cuenca N, Solis-Garcia del Pozo JE, Jordan J. Evidence for the efficacy of latrepirdine (Dimebon) treatment for improvement of cognitive function: a meta-analysis. *Journal of Alzheimer's disease : JAD*. 2014;38(1):155-164.
 242. Rosini M, Simoni E, Bartolini M, et al. The bivalent ligand approach as a tool for improving the in vitro anti-Alzheimer multitarget profile of dimebon. *ChemMedChem*. 2013;8(8):1276-1281.
 243. Skvortsova VI, Bachurin SO, Ustyugov AA, et al. Gamma-Carbolines Derivatives As Promising Agents for the Development of Pathogenic Therapy for Proteinopathy. *Acta naturae*. 2018;10(4):59-62.
 244. Strekalova T, Bahzenova N, Trofimov A, et al. Pro-neurogenic, Memory-Enhancing and Anti-stress Effects of DF302, a Novel Fluorine Gamma-Carboline Derivative with Multi-target Mechanism of Action. *Mol Neurobiol*. 2018;55(1):335-349.
 245. Bachurin SO, Shelkovernikova TA, Ustyugov AA, et al. Dimebon slows progression of proteinopathy in gamma-synuclein transgenic mice. *Neurotoxicity research*. 2012;22(1):33-42.
 246. Yamashita M, Nonaka T, Arai T, et al. Methylene blue and dimebon inhibit aggregation of TDP-43 in cellular models. *FEBS letters*. 2009;583(14):2419-2424.
 247. Coughlan KS, Mitchem MR, Hogg MC, Prehn JH. "Preconditioning" with latrepirdine, an adenosine 5'-monophosphate-activated protein kinase activator, delays amyotrophic lateral sclerosis progression in SOD1(G93A) mice. *Neurobiol Aging*. 2015;36(2):1140-1150.
 248. Ninkina N, Peters O, Millership S, Salem H, van der Putten H, Buchman VL. Gamma-synucleinopathy: neurodegeneration associated with overexpression of the mouse protein. *Hum Mol Genet*. 2009;18(10):1779-1794.
 249. Peters OM, Millership S, Shelkovernikova TA, et al. Selective pattern of motor system damage in gamma-synuclein transgenic mice mirrors the respective pathology in amyotrophic lateral sclerosis. *Neurobiol Dis*. 2012;48(1):124-131.
 250. Bronovitsky EV, Deikin AV, Ermolkevich TG, et al. Gamma-carboline inhibits neurodegenerative processes in a transgenic model of amyotrophic lateral sclerosis. *Doklady Biochemistry and biophysics*. 2015;462:189-192.

Michail Kukharsky is a Research Associate in IPAC RAS. Graduated from Tomsk State University (TSU), Faculty of Cytology and Genetics in 2010. In 2013 received the Ph.D. degree in Biochemistry. His scientific career started in the Institute of Medical Genetics while he was still an undergraduate student. At that time, he has joined a research group that worked on genetics of neurodegenerative diseases. For further two years Michail worked in the Laboratory of Drug Toxicology of the Institute of Pharmacology where he conducted research dedicated to testing of new drugs, including assessment of their genetic toxicity and impact on the central nervous system of model organisms. From 2012 till the present time Michail works in the Laboratory of Genetic Modeling of Neurodegenerative Processes of the Institute of Physiologically Active Compounds of Russian Academy of Sciences (IPAC RAS). From 2018 M. Kukharsky is a lecturer in the Faculty of Medical Biology, Pirogov Russian National Research Medical University. Current research activities include studies of molecular mechanisms of pathogenesis and searching for new therapeutic approaches for treatment of neurodegenerative and psychiatric diseases using various in vitro and vivo models of neurodegenerative processes.

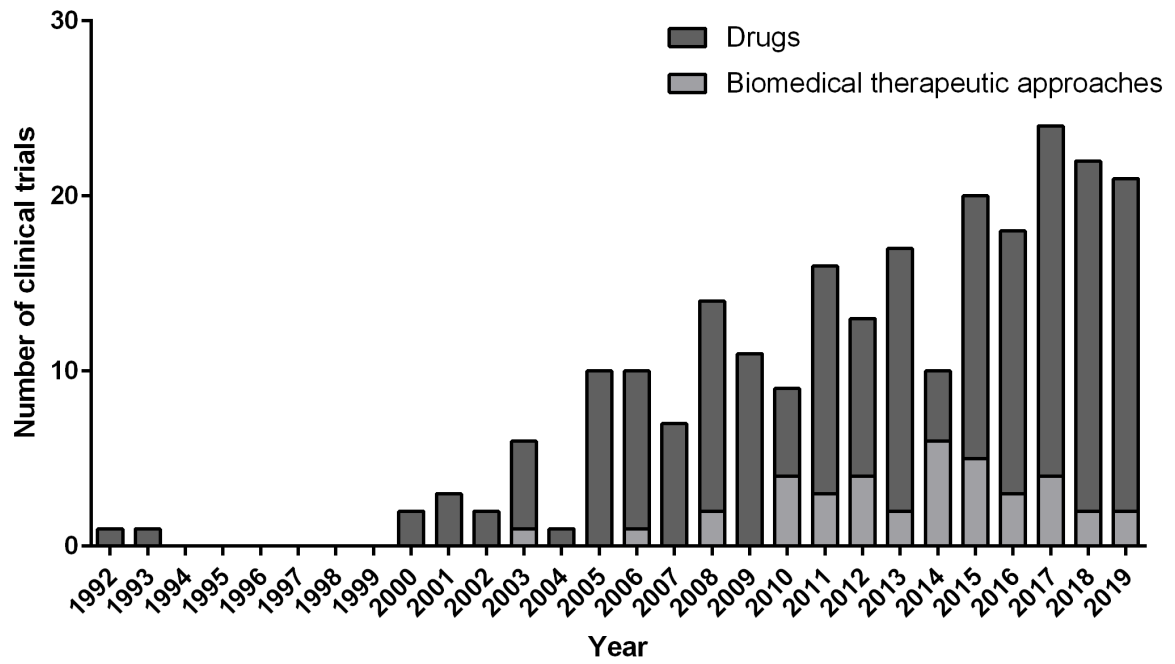


Figure 1. The number of clinical trials for potential ALS drugs and other therapeutic approaches registered in the ClinicalTrials.gov database over the last 27 years.

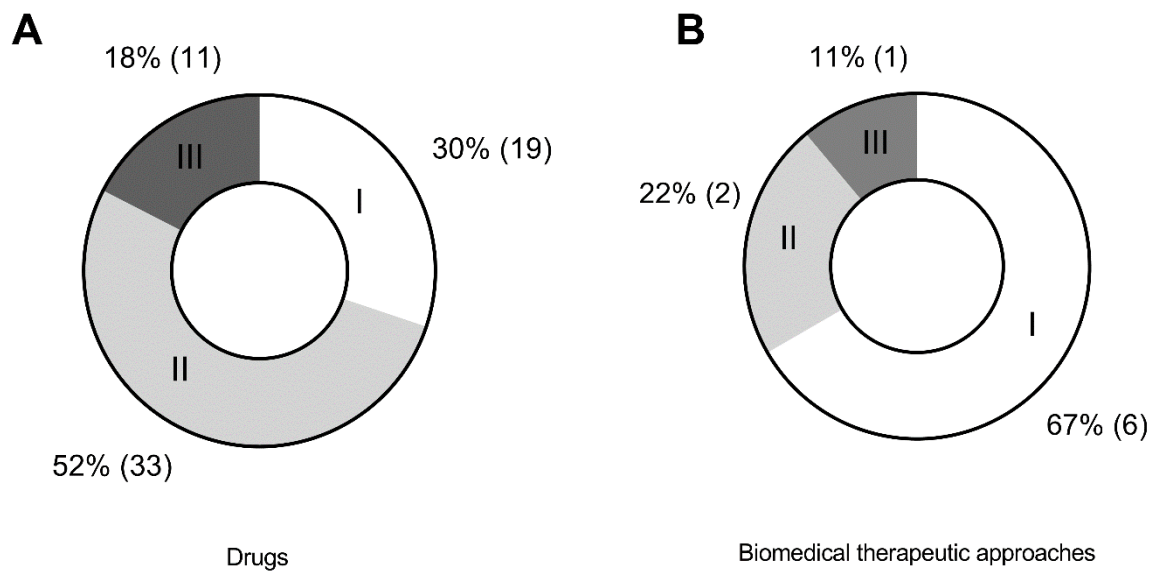
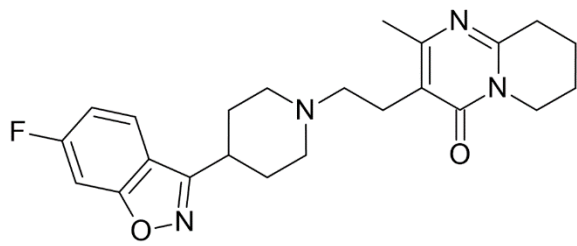
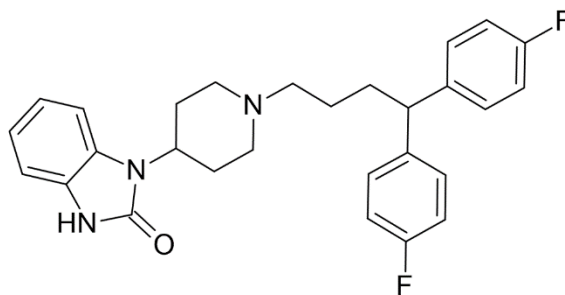


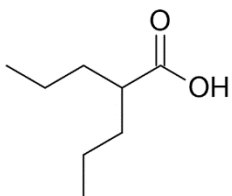
Figure 2. Distribution of currently active clinical trials for drugs (A) and biomedical therapeutic approaches (B) between phases of trails.



Risperidone

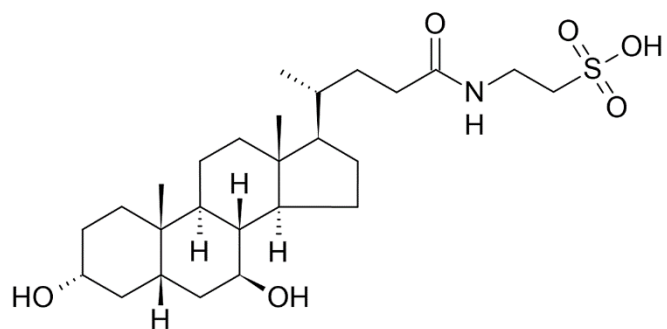


Pimozide

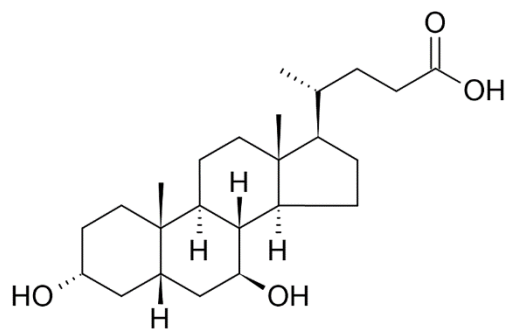


Valproic
acid

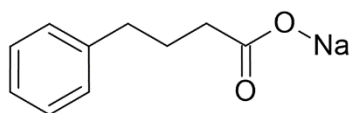
Figure S1. Available structures for drugs used for treatment of psychiatric disorders.



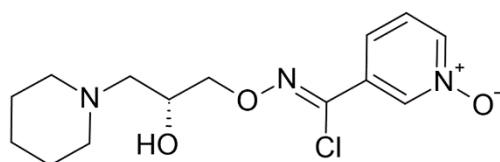
Tauroursodeoxycholic acid



Ursodeoxycholic acid

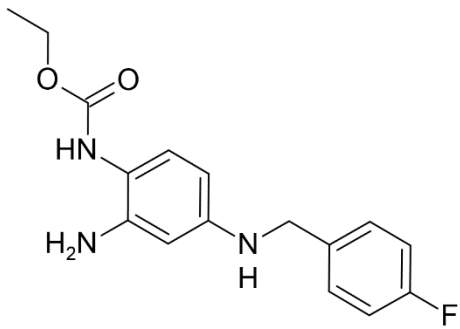


Sodium phenylbutyrate

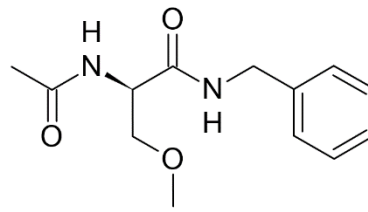


Arimoclomol

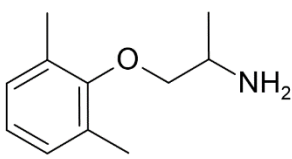
Figure S2. Available structures for drugs targeting endoplasmic reticulum stress.



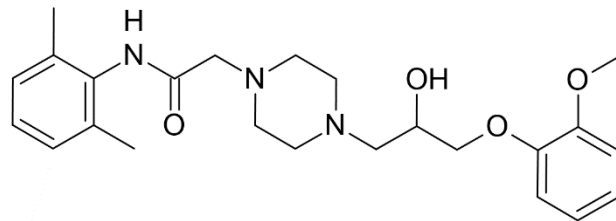
Ezogabine (Retigabine)



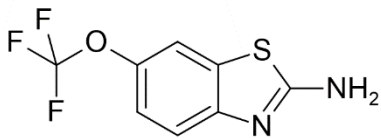
Lacosamide



Mexiletine

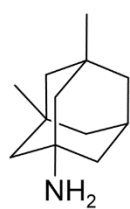


Ranolazine

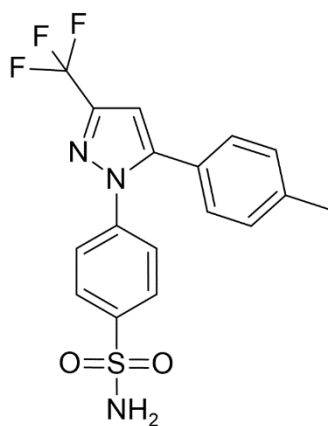


Riluzole

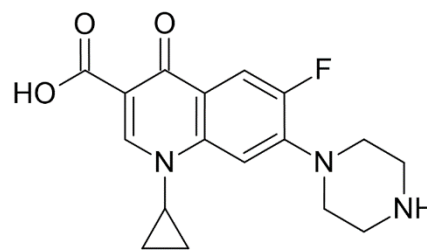
Figure S3. Available structures for drugs targeting excitotoxicity.



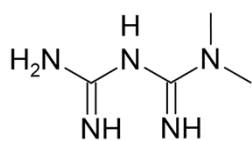
Memantine



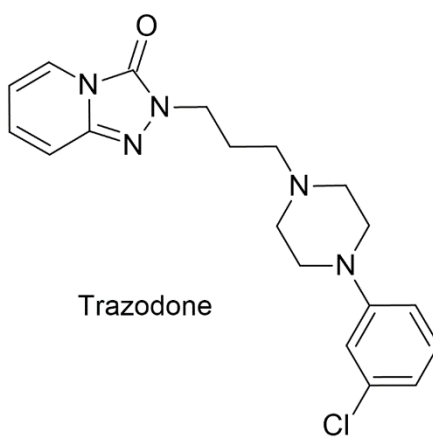
Celecoxib



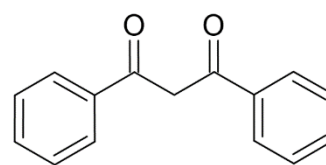
Ciprofloxacin



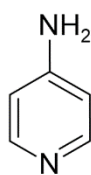
Metformin



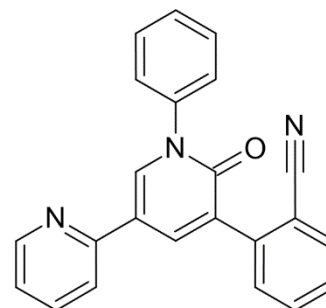
Trazodone



Dibenzoylmethane

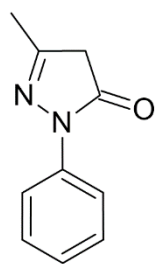


4-aminopyridine

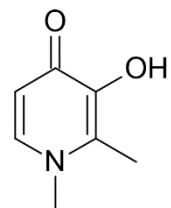


Perampanel

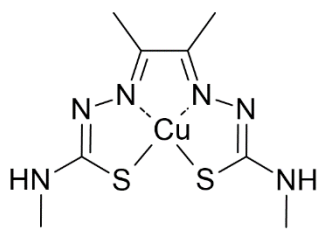
Figure S3 (continues). Available structures for drugs targeting excitotoxicity.



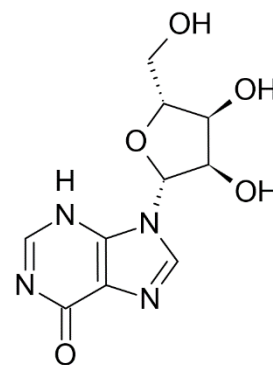
Edaravone



Deferiprone

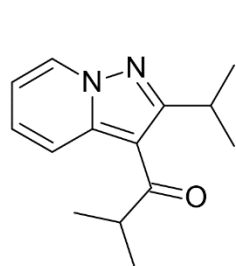


Cull(atsm)

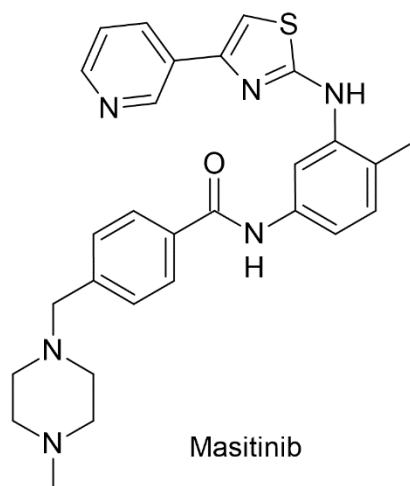


Inosine

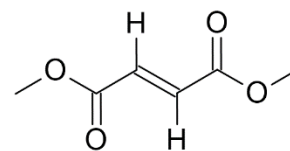
Figure S4. Available structures for drugs targeting oxidative stress.



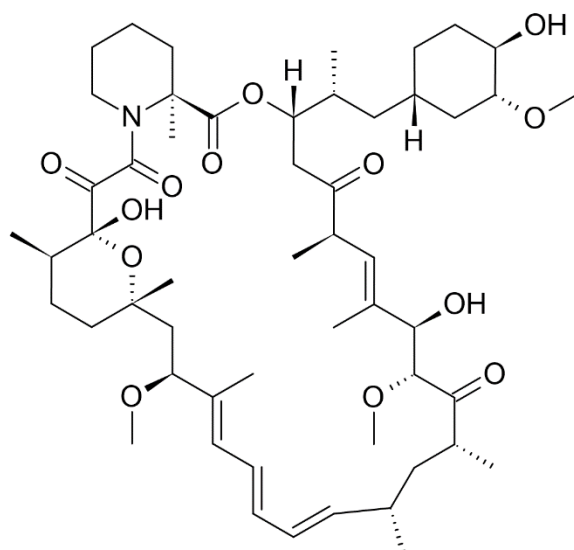
MN-166



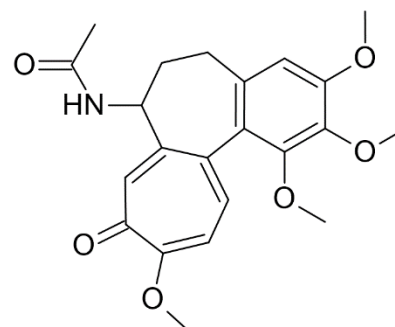
Masitinib



Dimethyl fumarate

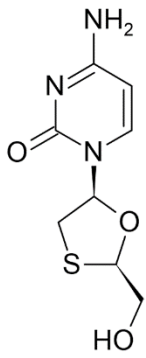


Rapamycin

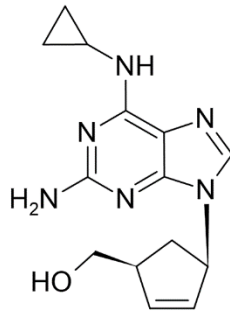


Colchicine

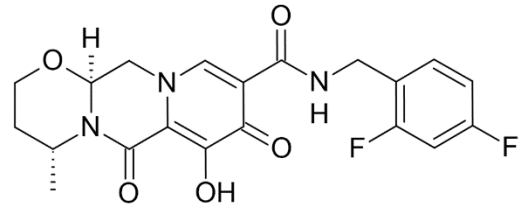
Figure S5. Available structures for drugs targeting neuroinflammation.



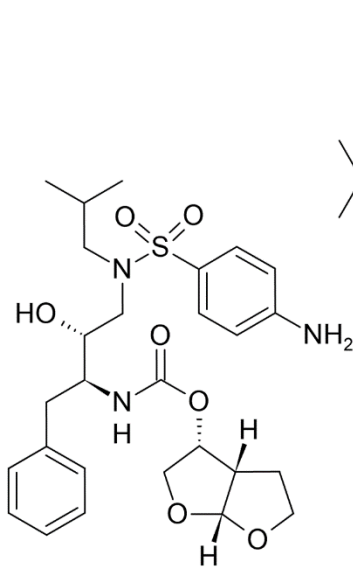
Lamivudine



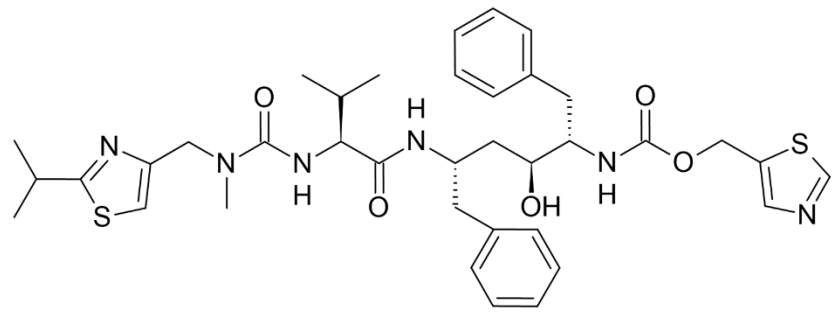
Abacavir



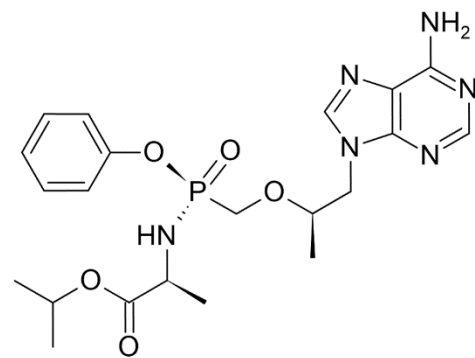
Dolutegravir



Darunavir

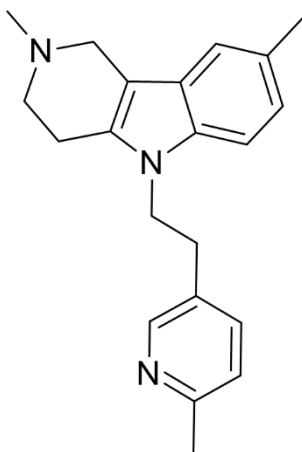


Ritonavir



Tenofovir alafenamide

Figure S6. Available structures for drugs targeting endogenous retroviruses.



Dimebon

Figure S7. Structure of Dimebon.

Supplementary table 1. The list of drugs that are currently being tested in clinical trials and their anticipated mechanism of action for ALS treatment

Drugs	Anticipated mechanism of action for ALS treatment	References
Pimozide	Improves synaptic transmission in neuromuscular junctions by blocking of T-type of Ca ²⁺ channels.	34-37
Lithium and Valproic acid	Inhibitor of glycogen synthase kinase 3 and histone deacetylases respectively. Interfere with many pathways involved in neuronal death.	39-46
GDC-0134	Inhibitor of dual leucine zipper kinase. Modulates cell response to endoplasmic reticulum stress.	
Tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA)	Chemical chaperones. Alleviate endoplasmic reticulum stress.	86-88
Sodium phenylbutyrate	Molecular chaperone and inhibitor of histone deacetylases.	89-94
Arimoclomol	Coinducer of heat shock proteins	95, 96
Ezogabine	Opener of KV7 (KCNQ) family of potassium channels. Decreases neuronal excitability.	103-105
Lacosamide	Enhances the slow inactivation of voltage-gated sodium channels and improves neuronal connectivity via modulation of activity of collapsin response mediator protein 2. Decreases neuronal excitability.	106-109
Mexiletine	Blocks sodium channels. Reduces muscle cramp.	110-113
Riluzole	Inhibits voltage-gated sodium channels and glutamatergic neurotransmission.	13, 14, 16, 114, 115
Ranolazine	Inhibitor of sodium and potassium channels. Reduces excitability of nervous system cells and also has anti-inflammatory activity.	116-125

Memantine	Antagonist of N-methyl-D-aspartate (NMDA) receptors.	126-129
Celecoxib	Decreases prostaglandine E2-dependent release of glutamate by astrocytes via inhibiting of cyclooxygenase-2. A nonsteroidal anti-inflammatory drug.	130-135
Metformin	Attenuator of repeat associated non-AUG-dependent (RAN) translation. Reduces accumulation of toxic dipeptides from ALS-associated <i>C9orf72</i> .	139-141
4-aminopiridine	Blocks voltage-activated potassium channels and therefore increases motor neuron excitability.	142, 145
Perampanel	Selective non-competitive antagonist of AMPA receptors.	154
Edaravon	Free radicals scavenger.	17, 157, 158
Deferiprone	Chelator of iron ions.	167, 168
CuII(at5m)	A copper-containing compound. Suggested for normalisation of function of Cu-deficient SOD1 and inhibition of ferroptosis.	169-173, 178
Inosine	A precursor of a natural antioxidant urate.	191
MN-166	Inhibitor of cyclic nucleotide phosphodiesterases (PDE-4 and PDE-10), macrophage migration inhibitory factor (MIF) and toll-like receptor 4. Suppresses glial cell activation and protects against glutamate toxicity.	201, 204, 206-208
Masitinib	Inhibitor of tyrosine kinases. Suppresses proliferation and migration of microglia and expression of inflammatory mediators.	209, 210
Interleukin-2 (IL-2)	Increases the activity of regulatory T lymphocytes (Tregs).	212, 213
Dimethyl fumarate	Stimulates Tregs formation.	215
Rapamycin	Suppressor of inflammatory neurotoxic T cells response and stimulator of autophagy.	216-219, 222, 223

Colchicine	Upregulates the expression of a heat shock protein B8 (HSPB8) and has an anti-inflammatory effect.	226, 227
Triumeq (lamivudine, abacavir, dolutegravir)	A combination of two nucleoside analogue reverse-transcriptase inhibitors and a HIV-1 integrase strand transfer inhibitor, respectively. Antiretroviral drugs.	239
Darunavir, ritonavir, dolutegravir, Tenofovir alafenamide	A protease inhibitor, its booster, an integrase inhibitor and a nucleoside analogue reverse-transcriptase inhibitor respectively. Antiretroviral drugs.	238