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

Running title: Clinical trials of "old" drugs for ALS

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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 neurons[1]. 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 system[3]. Another histopathological hallmark of ALS is the presence in degenerating neurons of ubiquitinated inclusions that often contain aggregated TDP-43 protein or its fragments[6].
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 mutations[7]. 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 sALS[10-12]. 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 references[13-17]). 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 disease[18], 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 symptoms[22]. 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 sALS[26-31].

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 protein[32].

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 patients[33]. All 13 compounds selected from a set of 3850 repurposing drugs due to their ability to prevent paralyses in two models of TDP-43-ALS (*C. elegans* expressing TARDBP^{A315T} and *D. rerio* expressing TARDBP^{G348C}) appeared to be known neuroleptics[34]. 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 transmission[34]. 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 Ca^{2+} channels[34-37]. 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 transmission[34]. 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 animals[38]. 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 excitotoxicity[39], expression of glutamatergic postsynaptic density proteins of Homer family[40,41], aberrant Notch signalling[42], apoptosis[43], 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 model[47] triggered several clinical trials in patients with ALS. In some of these trials, lithium slowed down the disease progression[47-49], but no improvement was observed in other trials[50-55]. Further studies revealed that only patients homozygous for C allele of *UNC13A* locus, which is known to be genetically linked to ALS and FTLD[56-58], response to lithium therapy[59]. 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 trial[61]. 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 disease[70-73]. 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 model[78-81]. 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-eIF2α-ATF4-CHOP pathway[82-85]. 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 patients[88]. **Sodium phenylbutyrate** (Buphenyl, Ammonaps, triButyrate), a compound used to treat urea cycle disorders, is another molecular chaperone, that also acts as an inhibitor of HDAC[89]. It has successfully passed efficiency testing in $SOD1^{G93A}$ mouse model as well as safety and tolerability tests in patients[90-94]. 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 $SOD1^{G93A}$ mice[95], and a suggestion from the first clinical trial on SOD1-ALS patients that arimoclomol treatment might have a therapeutic benefit[96], 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 coinducers[99]. 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 trials[100]. 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 channels[103]. A single dose of this drug significantly decreased excitability parameters in a study performed on a small group of ALS patients[104]. 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 neurons[105].

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 obvious[106]. Nevertheless, neuroprotective effects demonstrated in previous studies[107-109] 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 system[112]. 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 combination[110].

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 channels[116-118]. 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 activity[125]. 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 ALS[132-134], 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 expansion[136-138]. **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 groups[142].

4-aminopiridine (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 ALS[145], 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 patients[146-149]. In neurons of ALS/FTLD patients with *C9orf72* mutation ADAR2 changes its normally nuclear localisation to cytoplasmic[150]. 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 fragment[151-153]. 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 neurons[154]. 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 survival[159-162]. Chelating agents can be used to ameliorate the consequences of metal ions accumulation[163-166]. 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 agent[174-176] 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 peroxidation[177]. 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 patients[178], 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 activity[179-181], including neuroprotection against excitotoxicity[182]. 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 development[183-188]. 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 demonstrated[194-196]. 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 ALS[200].

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 activation[203-206] and protects cultured neurons against glutamate toxicity[207]. 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 disease[208]. 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 progression[215].

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 pathology[216-222].

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 year[223].

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 ALS[226]. 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 started[227]. 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 tissues[228] and increased activity of reverse transcriptase, a common retroviral marker, in blood[229-232] 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 pathogenesis[235]. 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 rate[239], 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** (Latrepirdine, 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-carbolines[242-244]. 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 systems[244-250]. Similarly, future studies in ALS models might reveal other "old drugs" or their derivates 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.

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Conflict of interests

The authors declare that there are no conflicts of interests.

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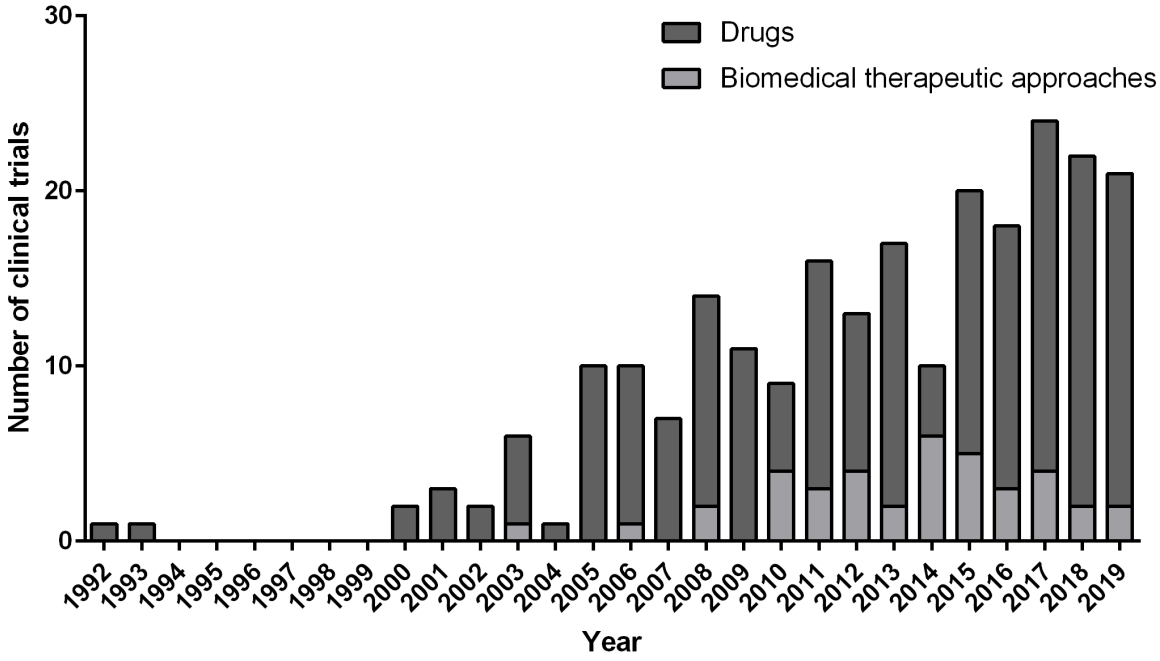


Figure 1. The number of clinical trials for potential ALS drugs and other therapeutic approaches registered in the ClinicalTrials.gov database over the last 27 years.

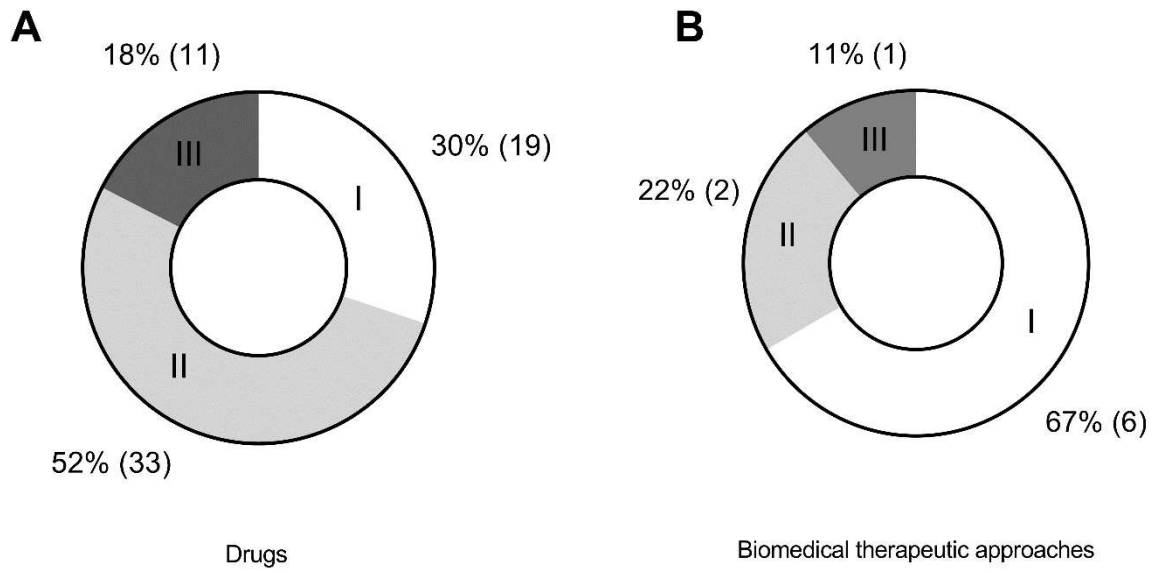
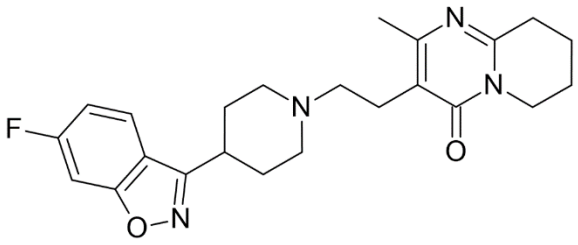
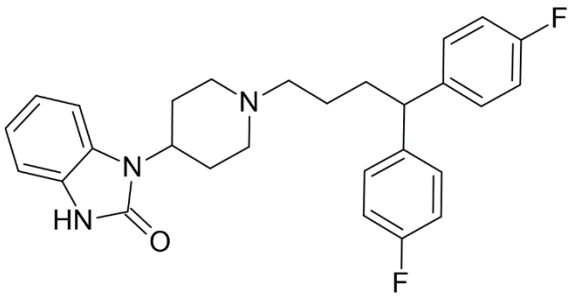


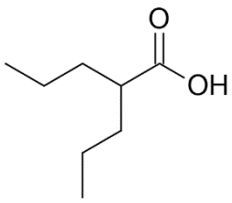
Figure 2. Distribution of currently active clinical trials for drugs (A) and biomedical therapeutic approaches (B) between phases of trails.



Risperidone

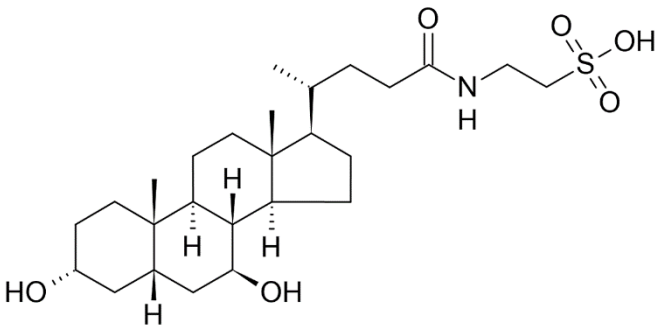


Pimozide

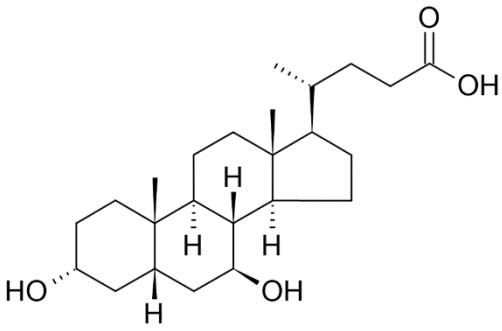


Valproic acid

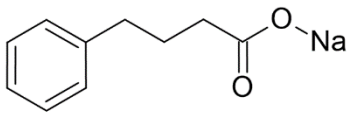
Figure S1. Available structures for drugs used for treatment of psychiatric disorders.



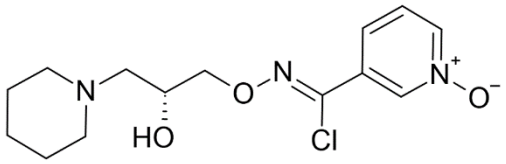
Tauroursodeoxycholic acid



Ursodeoxycholic acid



Sodium phenylbutyrate



Arimoclomol

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

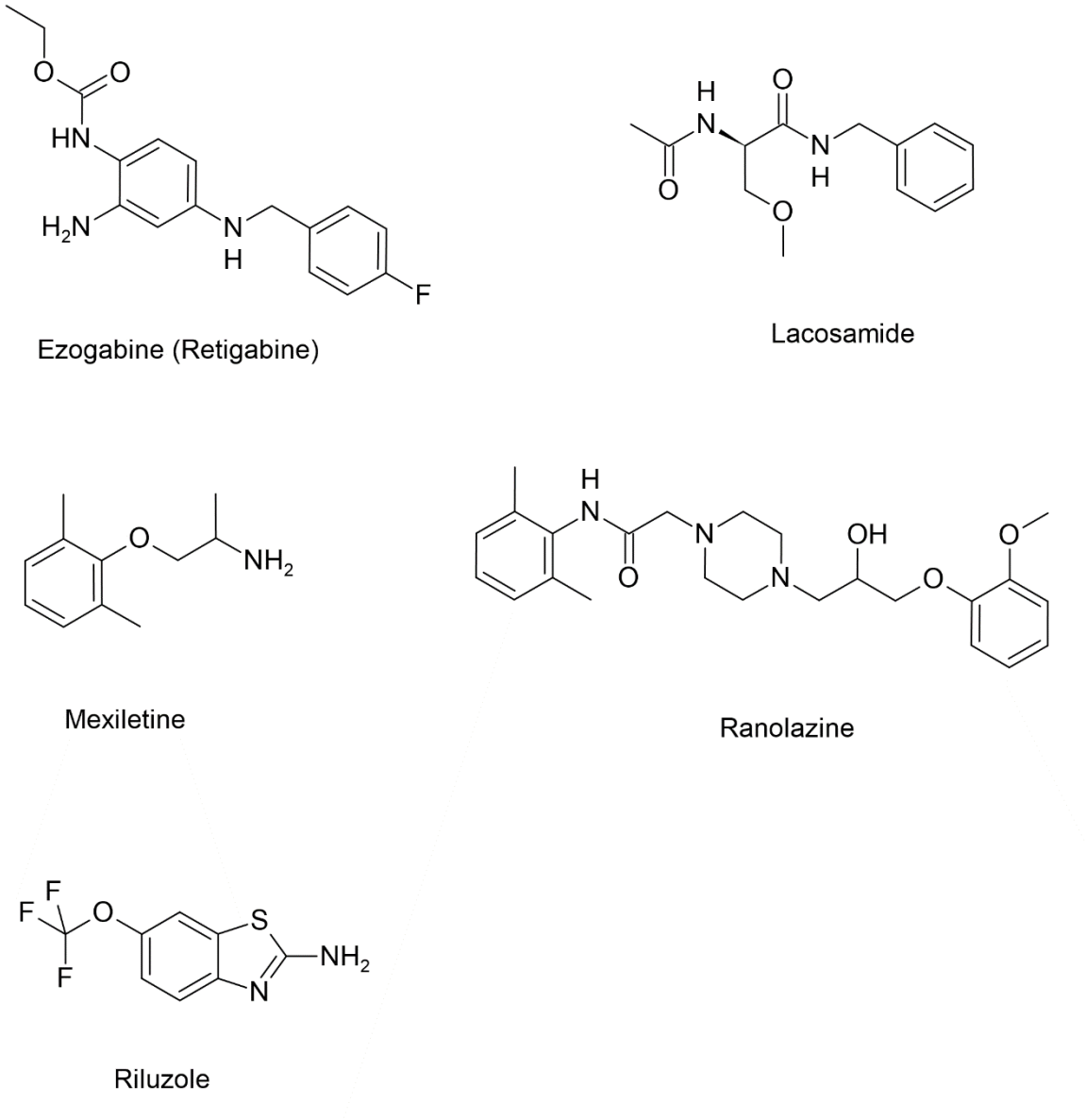


Figure S3. Available structures for drugs targeting excitotoxicity.

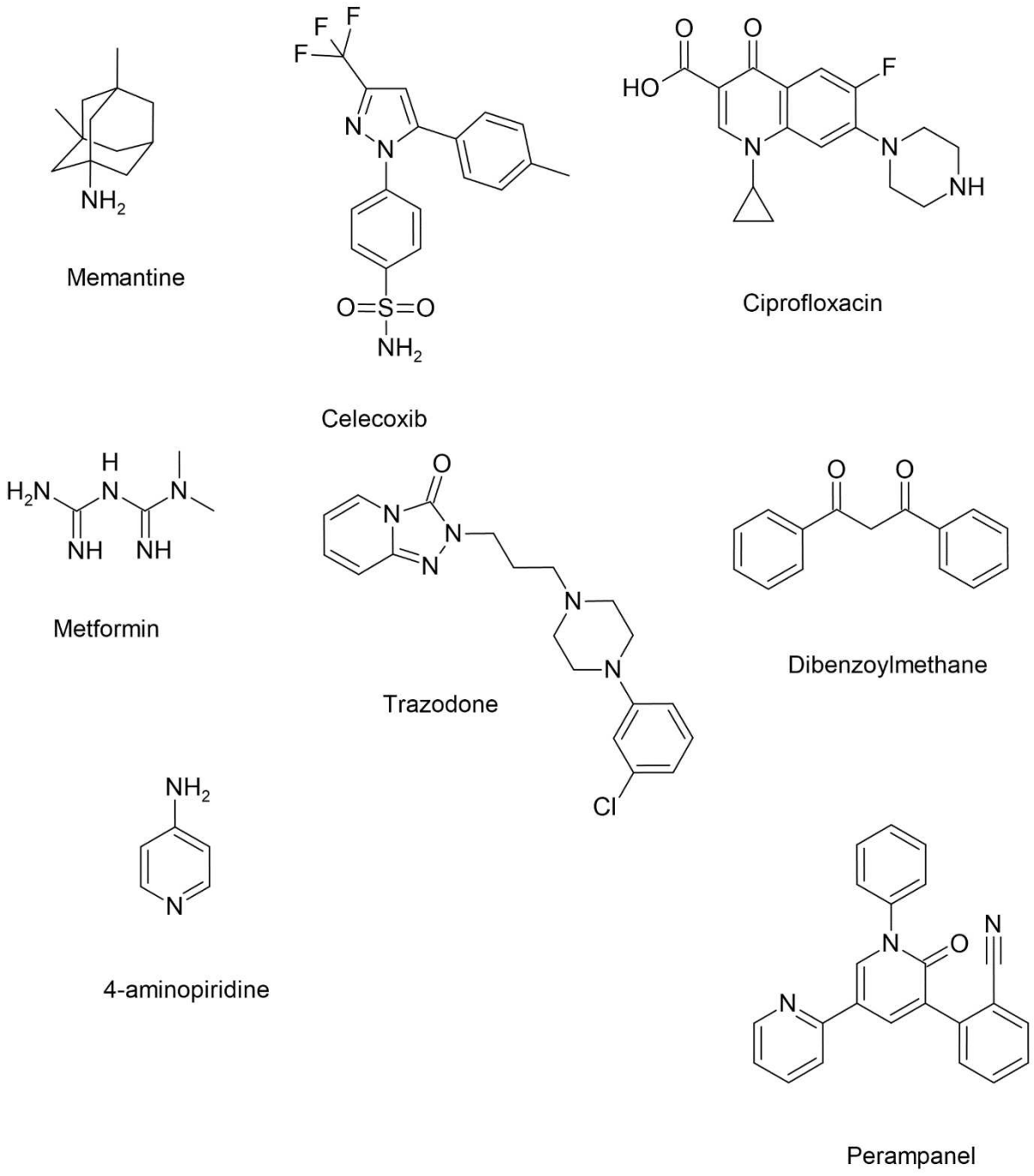


Figure S3 (continues). Available structures for drugs targeting excitotoxicity.

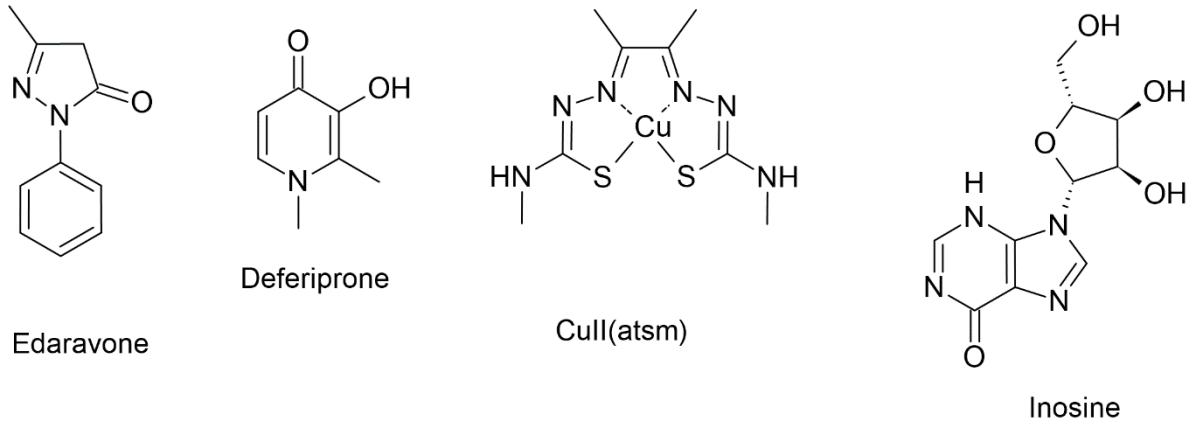


Figure S4. Available structures for drugs targeting oxidative stress.

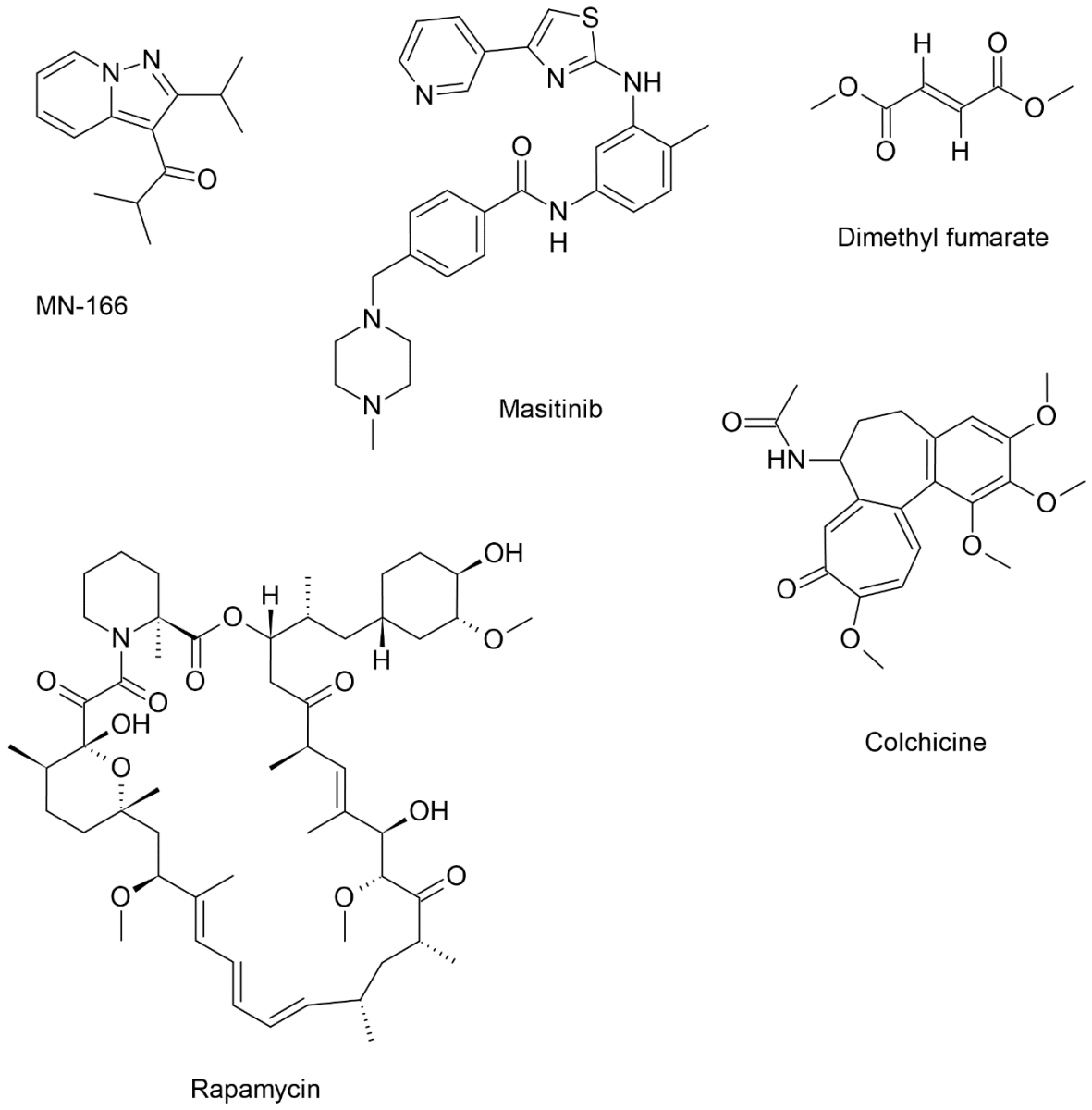


Figure S5. Available structures for drugs targeting neuroinflamation.

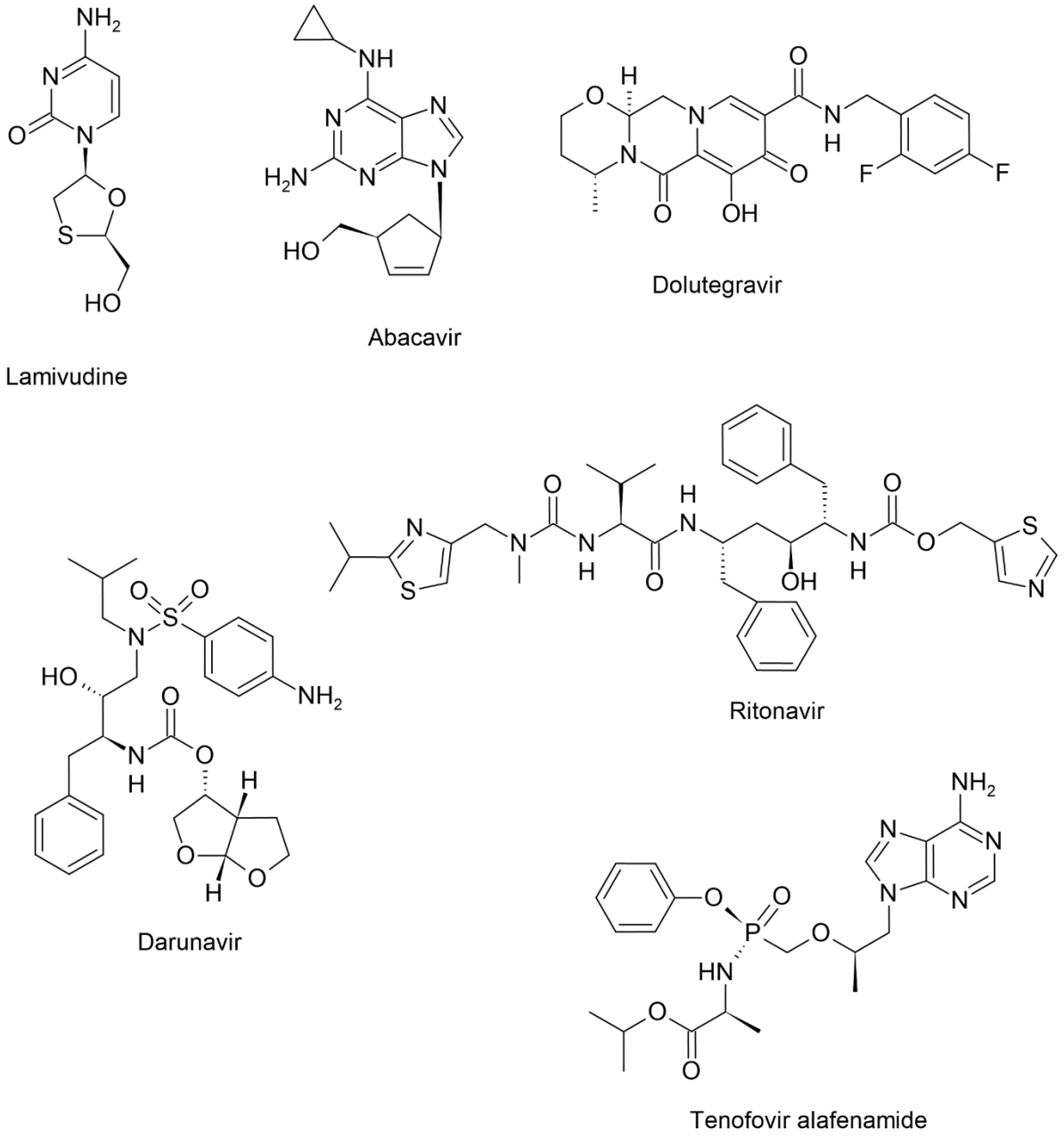
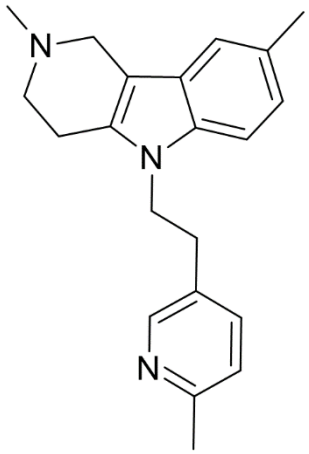


Figure S6. Available structures for drugs targeting endogenous retroviruses.



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 Ca2+ 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 *C9orf72*.	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(atsm)	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