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ABC transporter-driven pharmacoresistance in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a slowly progressing neurodegenerative disease that affects motor neurons of the nervous system. Despite the identification of many potential therapeutics targeting pathogenic mechanisms in *in vitro* models, there has been limited progress in translating them into a successful pharmacotherapy in the animal model of ALS. Further, efforts to translate any promising results from preclinical trials to effective pharmacotherapies for patients have been unsuccessful, with the exception of riluzole, the only FDA-approved medication, which only modestly extends survival both in the animal model and in patients. Thus, it is essential to reconsider the strategies for developing ALS pharmacotherapies. Growing evidence suggests that problems identifying highly effective ALS treatments may result from an underestimated issue of drug bioavailability and disease-driven pharmacoresistance, mediated by the ATP-binding cassette (ABC) drug efflux transporters. ABC transporters are predominately localized to the lumen of endothelial cells of the blood-brain and blood-spinal cord barriers (BBB, BSCB) where they limit the entry into the central nervous system (CNS) of a wide range of neurotoxicants and xenobiotics, but also therapeutics. In ALS, expression and function of ABC transporters is increased at the BBB/BSCB and their expression has been detected on neurons and glia in the CNS parenchyma, which may further reduce therapeutic action in target cells. Understanding and accounting for the contribution of these transporters to ALS pharmacoresistance could both improve the modest effects of riluzole and set in motion a re-evaluation of previous ALS drug disappointments. In addition, identifying pathogenic mechanisms regulating ABC transporter expression and function in ALS may lead to the development of new therapeutic strategies. It is likely that novel pharmacological approaches require counteracting pharmacoresistance to improve therapeutic efficacy.

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Keywords

Amyotrophic Lateral Sclerosis; Drug Efflux Transporters; Blood-brain barrier; Pharmacoresistance; Neurodegeneration

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) is a slowly progressing, fatal neurodegenerative disease that selectively affects both upper and lower motor neurons of the nervous system. One of the biggest challenges in the study of ALS is its complex etiology, which combines both environmental and genetic factors (Siddique and Ajroud-Driss, 2011). For ~90% of ALS cases, there is no family history and no identified cause (sporadic ALS). In the remaining 10% of cases (familial ALS), the disease is caused by genetic factors with 2/3 of disease-causative genes identified to date (Al-Chalabi et al., 2012). Currently, there is only one FDA-approved drug, riluzole, available to treat ALS, which modestly extends patient survival by 2–4 months (Miller et al., 2012). The first genetic linkage identified to contribute to ALS was $\text{Cu}^{2+}/\text{Zn}^{2+}$ superoxide dismutase 1 (SOD1) (Rosen et al., 1993). Several other genes have recently been identified as contributors to familial ALS, including the genes encoding for DNA and RNA-binding proteins TDP-43 and FUS/TLS, *TARDP* and *FUS*, as well as *UBQLN2*, *VCP*, and *C9ORF72* (DeJesus-Hernandez et al., 2011; Deng et al., 2011; Johnson et al., 2010; Kwiatkowski et al., 2009; Neumann et al., 2006; Renton et al., 2011; Vance et al., 2009).

Historically, research on the mechanisms and pathology of ALS has utilized transgenic mouse models, which overexpress the human SOD1 gene carrying one of three mutations (SOD1^{G93A}, SOD1^{G37R}, and SOD1^{G85R}). These animal models provided insight on the toxicity of the mutated gene toward neuronal and non-neuronal cell types, which has led to the development of widespread theories of the underlying disease pathogenesis. Several lines of investigations now indicate that linking ALS to a single mechanism is unlikely as multiple pathways of toxicity were found to contribute to disease (Al-Chalabi et al., 2012; Ferraiuolo et al., 2011; Garbuzova-Davis et al., 2011).

Pharmacological Interventions in ALS

The entirety of preclinical ALS research is currently focused on the transgenic mouse lines expressing human SOD1 mutations, which mimic many aspects of human ALS pathology and for which extended survival is one of the main predictors of preclinical success (Ludolph et al., 2007). Behavioral and functional assessments also measure disease onset and progression (Knippenberg et al., 2010). Several compounds have been identified that provide some degree of improvement in these functional assessment tests and in survival, but none thus far has proved to be a substantial treatment option when translated in patients.

There are multiple issues that could account for this discrepancy, including the study design of preclinical trials, the lack of additional animal models available for research, and insufficient insight into pathological causes. Preclinical drug trials typically begin pharmacological intervention prior to the onset of symptoms (Benatar, 2007). This allows

the researchers to examine differences in onset and progression of the disease, but hinders the practicality of translating the treatment to patients. On average, ALS patients are diagnosed 9 months after the onset of the first symptoms, at which point, treatment can begin. The disparity between initial treatment in preclinical animal studies and realistic treatment administration in patients appears to diminish efficacy in the clinic. Furthermore, studying the mutant SOD1 transgenic mouse model has identified multiple cell types and molecular mechanisms that are affected, hence single treatments that target one pathway at a time may not be enough. Recently, a number of investigators have begun to test combination therapies, which can potentially enhance the effect of single pharmacological agents (Kong et al., 2012).

While the mutant SOD1 mice mimic ALS to some degree, multiple problems prevent accurate prediction of clinical trial success. One of the major concerns is the lack of standardization in mutant SOD1 mouse strains. Depending upon the route of administration, dose, type and gender of mutant mouse, and onset of treatment, the effects of survival can be markedly different across studies and the interpretation and extrapolation of the actual benefits of a potential ALS-treating drug becomes difficult, if not unpredictable. As evidenced by the multitude of preclinical trials, a modest extension of lifespan in the mouse model of ALS has failed in predicting success in clinical trials (Table 1 and Table 2). Recently, a workshop led by Dr. Storey Landis, Director of the National Institute for Neurological Disorders and Stroke (NINDS), defined a set of guidelines designed to mitigate such problems. They concluded that in the preclinical research community, alleviating poor reporting and improving experimental design and reporting practices should lead to greater transparency and reproducibility (Landis et al., 2012).

In addition to the many issues mentioned above, there is also a widely recognized need for more animal models that mimic ALS. Fortunately, the last couple of years have yielded a number of new genes and protein targets that are associated with ALS (see above). Developing models from these targets will likely lead to the discovery of novel pathogenic mechanisms and possibly more effective treatment options.

Combinatorial therapies

As ALS is a disease with various pathways contributing to it, combination therapies targeting more than one disease-related pathway have proved effective at the preclinical stage and may provide an effective clinical option (Turner and Talbot, 2008). Zhang et al. (2003) treated SOD1-G93A mutant mice with a combination of minocycline, an anti-inflammatory and anti-apoptotic compound, and creatine, an anti-oxidant, and found a beneficially additive effect on survival, progression and onset of disease. However, recent attempts to utilize this strategy in the clinic have proved difficult. A phase II trial in ALS patients tested riluzole in combination with Pioglitazone, a drug with anti-inflammatory properties. Pioglitazone did not provide additional benefits as compared to riluzole alone (Dupuis et al., 2012). Riluzole has also been used in combination with lithium in a clinical trial, which did not provide a benefit in slowing ALS disease progression (Aggarwal et al., 2010). This trial was based on a pilot study, which established significant effects of the addition of lithium to riluzole in ALS patients (Fornai et al., 2008). This example, in

particular, highlights the importance of selecting the proper combination therapies to test in the clinic. However, directly targeting multiple pathways and systems that are involved in ALS could establish more efficacious treatment. For example, Kong et al. (2012) treated mutant SOD1-G93A mice beginning at symptom onset with minocycline and ceftriaxone alone or in combination. Minocycline inhibits microglial activation and the apoptotic cascade, while ceftriaxone reduces excitotoxicity by activating the glutamate transporter, EAAT2. The combination treatment, significantly extended survival; no change was seen with single treatment of either compound compared to vehicle-treated mice.

Regulation of ABC transporters in health and disease

ATP-binding cassette (ABC) transporters in excretory and barrier tissues contribute to the difficulty in treating many systemic and CNS diseases using pharmacotherapy (Loscher and Potschka, 2005). These transporters are ATP-driven efflux pumps with remarkably broad substrate specificity and are responsible for the high urine-to-plasma and bile-to-plasma concentration ratios seen for some xenobiotics (excretory function) and for the inability of many xenobiotics to enter the CNS (barrier function) (Neuwelt et al., 2011). The superfamily of ABC transporters includes P-glycoprotein (P-gp), breast cancer resistance protein (Bcrp), and the family of multidrug resistant proteins (Mrp1, Mrp2, Mrp3, Mrp4, Mrp5, and Mrp6). While ABC transporters are primarily localized to the luminal plasma membrane of brain and spinal cord capillary endothelial cells, they are also expressed in other cells in the nervous system, including astrocytes, microglia and neurons (Neuwelt et al., 2011). The mRNA and protein expression patterns for many of the ABC transporters are currently being established in the cells comprising the neurovascular unit (endothelial cells, pericytes, and perivascular astrocytes) and CNS parenchyma (Hartz and Bauer, 2011). For example, the luminal membrane of capillary endothelial cells expresses P-gp, Bcrp, Mrp1, Mrp2, Mrp4, and Mrp5 (Bauer et al., 2006; Cooray et al., 2002; Nies et al., 2004). There are also reports of P-gp, Mrp1, and Mrp4 localizing to the abluminal membrane of brain capillary endothelial cells, however, efflux transporters on that membrane would not contribute to barrier function (Bendayan et al., 2006; Kilic et al., 2008; Pardridge et al., 1997; Soontornmalai et al., 2006; Zhang et al., 2004). Moreover, protein expression of P-gp, Bcrp, Mrp1, Mrp3, Mrp4, and Mrp5 has also been detected on astrocytic endfeet and microglia (Table 3).

By determining the expression and localization of ABC transporters in situ, disease-driven alterations can be identified. For example, P-gp expression in AIDS patients with HIV encephalitis demonstrated a shift from the typical dominant endothelial cell expression to expression in astrocytes and microglia, which resulted in an overall increase in P-gp levels (Langford et al., 2004). In ALS, immunofluorescent labeling of P-gp in the lumbar spinal cord of SOD1-G93A mice indicates robust P-gp expression in astrocytes of symptomatic mice compared to pre-symptomatic mice (Figure 1). This disease-driven increase in spinal cord parenchyma P-gp expression could provide a secondary barrier to drug penetration.

Because many potential therapeutic compounds are ABC transporter substrates, treatments to blunt the increases of ABC transporters have been effective towards increasing the brain:plasma ratios of these compounds. Multiple pathways are associated with alterations in

drug efflux transporter expression and transport activity. Activation of these pathways with xenobiotics or endogenous ligands serves to alter the expression and function of various ABC transporters. For instance, multiple ligand-activated transcription factors are capable of increasing P-gp expression in mouse and rat brain capillaries and in primary cultured rat and porcine brain endothelial cells (Miller, 2010). More specifically, activation of the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) by their respective ligands leads to increases in P-gp mRNA and protein expression (Chan et al., 2011). Furthermore, PXR activation in porcine brain capillary endothelial cells leads to increased expression and activity of P-gp and Bcrp (Lemmen et al., 2012). Similar types of regulation are exemplified in other ABC transporters as well. Activation of the transcription factor, peroxisome proliferator activated receptor alpha (PPAR α), leads to increased expression and activity of Bcrp (Hoque et al., 2012). With prolonged treatment of TNF-alpha and Endothelin-1, P-gp expression and activity is increased in rat brain capillaries through an NFkB-dependent mechanism (Bauer et al., 2007). Extrapolating the results to *in vivo* studies, chronic inflammatory diseases may lead to increased expression and function of P-gp, which would work in reducing the efficacy of therapeutic compounds.

Multidrug efflux transporters mediate pharmacoresistance in ALS and other CNS disorders

Alterations in ABC transporter expression have been identified in epilepsy, brain cancer, HIV, spinal cord injury, Alzheimer's disease, and Parkinson's disease. In normal aging in the human brain, there is a slight decrease in ABC transporter function based on PET imaging with [^{11}C]-verapamil, a well-characterized P-gp substrate (Bartels et al., 2009). The same finding was reported in a later study, which also identified a greater decrease in elderly men compared to elderly women (van Assema et al., 2012b). These overall reductions in normal aging could account for the susceptibility of degeneration in the elderly.

In epilepsy, excess glutamate due to seizure induction increases P-gp expression at the blood-brain barrier (Bankstahl et al., 2008). In addition, treatment with anti-epileptic drugs can result in upregulation of ABC transporters (Potschka, 2012). Combination therapy with tariquidar, a P-gp inhibitor, and phenobarbital was effective in a rat model of drug-resistant epilepsy. Therapy with phenobarbital alone did not alter seizure activity in these rats (Brandt et al., 2006). To understand how these transporters are regulated in disease, the mechanisms leading to alterations in transporters are beginning to emerge. Recent studies suggest that during seizures excess glutamate drive increased P-gp expression by signaling through COX-2, PGE2 and NFkB (Bauer et al., 2008). Consistent with this, P-gp expression levels were decreased in kainate-induced epilepsy mice when treated with a COX-2 inhibitor or a NFkB inhibitor; however it is likely that this signaling pathway is not the only one that drives increases in P-gp expression (Yu et al., 2011).

Drug efflux transporter-mediated chemoresistance has been identified and studied extensively in certain cancers such as glioblastoma multiforme (GBM) and other metastatic and primary brain cancers (Agarwal et al., 2011). The importance of P-gp contribution to chemoresistance remains a perplexing issue and the challenges associated with chemoresistance have proven difficult to address in the clinic (Shaffer et al., 2012).

Inflammation, which accompanies all CNS diseases, also signals changes in ABC transporter expression. As P-gp is known to increase under conditions of inflammation, treatment with an anti-inflammatory drug, licofelone, improved the bioavailability of riluzole, as well as reduced levels of P-gp expression *in vivo* (Dulin et al., 2013). However, all conditions of inflammation do not consistently produce similar change. For example, P-gp expression was decreased in a rat brain capillary endothelial cell line after exposure to TNF-alpha (Theron et al., 2003). From available evidence, it is evident that whether P-gp increases or decreases in response to inflammation depends on the nature of the stimulus and the time of exposure. For example, in rat brain capillaries, Hartz et al. (2006, 2004) found a rapid and reversible decrease in P-gp transport function with activation of innate immune signaling through TNF-alpha and endothelin-1 (ET-1). However, at later time points, P-gp expression and activity was upregulated with TNF-alpha and ET-1 exposure of isolated rat brain capillaries (Bauer et al., 2007).

In contrast, two neurodegenerative diseases lead to decreases in expression and function of ABC transporters. One proposed cause of excess A β protein accumulation in Alzheimer's disease (AD) is reduced efflux of the protein from the brain (Cirrito et al., 2005). Brains from patients with AD show decreased P-gp expression (van Assema et al., 2012a). Studies with animal models indicate that A β protein can be transported by both P-gp and Bcrp, interesting observations given the reduced A β protein clearance from the brain in AD models. In AD model mice, there is a significant accumulation of A β if P-gp is inhibited, indicating that clearance, rather than the increased production of A β is contributing to its removal and plaque formation (Cirrito et al., 2005). Consistent with that observation, increasing P-gp expression in those mice reduces brain A β accumulation (Hartz et al., 2010). However, it appears that not all transporters are modulated in the same direction as increases in Bcrp in AD patients and animal models have been found (Xiong et al., 2009). Bcrp-null mice accumulate more A β in brain parenchyma compared to wild-type mice, so it is not clear at present how changes in Bcrp expression contribute to AD pathology (Xiong et al., 2009).

One proposed contributing factor to the etiology of Parkinson's disease is exposure to environmental toxins that may target dopaminergic neurons in the striatum. Some of these toxins are substrates for P-gp and a subset of human Parkinson's disease patients exhibit reductions in ABCB1 (P-gp) gene expression in brain (Westerlund et al., 2008).

Understanding ABC transporter alterations in neurodegeneration, therefore, may lead to improvements in current and future treatments.

Although ABC transporter expression levels at the blood-brain barrier have been shown to change in several neurodegenerative diseases, parallel studies for ALS are just beginning. Multiple reports have identified increases in P-gp expression in ALS mutant mice (Boston-howes et al., 2008; Jablonski et al., 2012; Milane et al., 2010). Furthermore, Bcrp has been implicated as another transporter with increased expression of mRNA and protein in ALS mutant mice (Jablonski et al., 2012), although another report did not detect mRNA and protein increases of Bcrp (Milane et al., 2010). The reason for this discrepancy could be that Milane et al. (2010) isolated mRNA and capillaries from whole brain, which is not the primary region of degeneration in the mouse, while Jablonski et al. (2012) isolated mRNA

and capillaries from the spinal cord of ALS mutant mice. Examination of the BBB and BSCB at areas of degeneration in the mutant ALS mice reveals specific, disease-driven expression and functional increases of two ABC transporters, P-gp and Bcrp, in the spinal cord and cortex of SOD1-G93A mice (Jablonski et al., 2012). In isolated cortex and spinal cord capillaries from mutant SOD1-G93A mice, P-gp and Bcrp expression levels are increased as the disease progresses. These increases are specific to the CNS as protein expression of P-gp and Bcrp in the liver of these mice is unchanged. Furthermore, the functional activity of P-gp and Bcrp in the isolated capillaries is increased in symptomatic compared to presymptomatic mice, indicating the functional relevance in P-gp and Bcrp in extruding pharmacological agents from the CNS. Increased protein levels of P-gp and Bcrp have been detected in post-mortem human lumbar spinal cords of ALS patients, confirming the relevance of the mouse findings for the human disease (Jablonski et al., 2012).

Strategies to improve treatment for ALS

Improving the bioavailability of therapeutics: Overcoming Pharmacoresistance

One of the limiting factors associated with preclinical drug trials in ALS, as well as other CNS disorders, is the ability of therapeutic drugs to enter the CNS and remain there. For ALS, the issue of pharmacoresistance and the consequences of altered bioavailability have been largely ignored. One can combat limited drug movement across the blood-brain and blood-spinal cord barriers by modifying the route of drug delivery, e.g., systemic vs direct CNS delivery. Although not discussed in detail in this review, modifying or altering the delivery route of a compound requires the systematic analysis of the altered pharmacokinetics, dynamics, and penetration of that compound. Another way to increase the bioavailability and improve efficacy of compounds is to target mechanisms that limit the accumulation of therapeutics in the CNS. Many proposed ALS therapeutics are substrates for or interact with P-gp and/or Bcrp. These include, Riluzole, Minocycline, and Ceftriaxone (Boston-howes et al., 2008; Gosland et al., 1989; Milane et al., 2009, 2007). Thus, for drugs like Riluzole, disease-driven increases in transporter expression at the blood-brain and blood-spinal cord barriers limit penetration into the CNS (Milane et al., 2007). In addition, consider NDGA, a compound that increases glutamate uptake that was tested as a potential therapeutics for ALS (Boston-howes et al., 2008). In a preclinical trial with wild-type mice, NDGA increased glutamate uptake over 30 days of treatment. In the SOD1-G93A mice, the increase in glutamate uptake was only sustained for 10 days, before a return to lower levels. Concomitant with the decrease of glutamate uptake, was a steady increase in expression levels of the ABC transporter, P-gp. Further experiments identify NDGA as a substrate of P-gp (Figure 2). As an initial proof of principle to inhibiting P-gp in ALS mice, transgenic mutant SOD1-G93A/P-gp knockout mice were treated with a known P-gp substrate and protectant against mitochondrial-mediated apoptosis, cyclosporine A. Survival was extended by 12% in the SOD1-G93A/P-gp knockout mice compared to mutant SOD1-G93A mice given cyclosporine A (Kirkinezos et al., 2004). The combined effect would seem marginal, however, cyclosporine A was shown to be toxic to neuronal cells expressing mutant SOD1 (Maxwell et al., 2004) and perhaps was not an appropriate therapeutic agent.

While it is possible to inhibit efflux transporters with various pharmacological agents, these approaches tend to produce severe side-effects. This can be evidenced by the lack of efficacy in various types of clinical cancer trials (Agarwal et al., 2011; Shaffer et al., 2012). Inhibition does not necessarily target specific tissues, leading to issues of selectivity that could potentially exacerbate disease conditions with chronic treatment. However, subsequent generations of inhibitors are becoming more specific for individual transporters and much more effective at reducing side-effects. Various third generation inhibitors (i.e. tariquidar and elacridar) have more potency and specificity than earlier inhibitors. They are also tolerated well in human trials (Bauer et al., 2012). An alternative approach would be to target the signaling pathways that regulate basal transporter activity. In a recent study using rats, Cannon et al. (2012) decreased P-gp activity at the blood-brain barrier by targeting the sphingosine-1-phosphate (S1P) receptor 1. Using fingolimod, a S1P receptor agonist approved for use in the clinic, they demonstrated increased brain accumulation *in vivo* of several drugs that are P-gp substrates. A second recent publications shows that spinal cord capillaries possess the same S1P-based signaling pathway (Cartwright et al., 2012).

For ALS, the clinical relevance of pharmacoresistance is still somewhat uncertain. When we pay attention to lessons learned from other diseases, the arguments for induced pharmacoresistance in ALS are strengthened and potential mechanisms of induction are disclosed. For example, epilepsy and ALS are characterized by glutamate excitotoxicity, which can lead to up-regulation of P-gp. In epilepsy, excess glutamate released from neurons in the synaptic cleft activates the NMDA receptor on endothelial cells to release arachidonic acid, which is converted to PGE2 (via COX-2), activating the EP1 receptor and NFkB and increasing ABCB1 gene transcription and translation (Bauer et al., 2008). Identification of the pathways controlling P-gp and Bcrp up-regulation in ALS could lead to more targeted manipulation of the transporters.

Conclusion

ALS is a disease of the upper and lower motor neurons, however, these are not the only cell types affected. The non-cell autonomous nature of ALS results in a variety of pathogenic mechanisms contributing to disease. With only one moderately effective drug that can be used in the clinic, riluzole, there is a desperate need for the development of more efficacious treatments. The standard method of identifying a potential therapeutic agent, demonstrating an extension of lifespan in a mouse model, and moving to a clinical trial has not produced effective therapies. The complexity of disease and the disparity between effective preclinical trials and ineffective clinical trials requires further research on underlying ALS disease mechanisms and a re-thinking of clinical treatment strategies in light of new information. In recent years, some combination therapies have proven successful at the preclinical stage. Indeed, combination treatment can begin at the onset of symptoms in the ALS mice and produce a significant extension of lifespan, making this strategy particularly appealing. Translating this strategy to the clinic may prove more beneficial than the use of single therapeutic treatments initially tested in presymptomatic ALS mice.

Finally, given the recent discovery of a selective and disease-driven pharmacoresistance seen in ALS mice and the upregulation of ABC transporters in human patients with the

disease, targeting pharmacoresistance appears to be one way to improve the efficacy of ALS therapeutics. The ability to control P-gp and BCRP transport activity in ALS patients could prove invaluable. Activities of P-gp and Bcrp could be controlled by use of pharmacologic inhibitors of the transporters or by targeting pathways that regulate activity or expression. However, first we must identify how ABC transporters are upregulated in the brain and spinal cord endothelial barriers and in brain parenchymal cells of the CNS. These studies will initially have to be done using ALS mouse models rather than healthy, wild-type controls. Moreover, the determination of bioavailability and proper dosage should be studied throughout the course of the disease so that changes in dosage of therapeutics can be tied to increases of P-gp and Bcrp expression. In this instance, the issues of pharmacokinetics and pharmacodynamics require persistent analysis throughout the ALS disease course and should be studied appropriately.

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Highlights

- ABC drug efflux transporters are a potential cause for pharmacoresistance in ALS
- Pre-clinical drug trials have failed to cure the ALS mouse model
- Novel pharmacological approaches require combating pharmacoresistance in ALS

Figure 1.

Confocal imaging of *in vivo* P-gp expression in spinal cord astrocytes. Mutant SOD1-G93A mice at the presymptomatic stage (A–E) do not express detectable levels of P-gp (green) in astrocytes (red). Conversely, P-gp expression is increased in astrocytes at the symptomatic stage of SOD1-G93A mice (F–J), suggesting the disease-driven increase is partly due to increased astrocytic expression of P-gp.

Figure 2.

P-gp activity is decreased in isolated mouse spinal cord capillaries exposed to nordihydroguaiaretic acid (NDGA) as well as the specific P-gp inhibitor, PSC-833 (***) ($P < 0.01$). Specific P-gp activity was determined by quantification of luminal fluorescence in presence of inhibitor subtracted from the luminal fluorescence in the absence of inhibitor. Data analysis was performed using one-way analysis of variance (ANOVA). Data are shown as mean $\pm$ SEM.

Table 1**Current and Recently Completed Clinical Trials**

Compound	Study ID	Study Design	Dosage	Route	Primary Outcome Measure	Prospective End Date	Location
VEGF165	NCT01384162	Phase 1/2 single arm	Not specified	ICV	Safety and tolerability	December 2013	Belgium
Rasagiline	NCT01232738	Phase 2 RCT	1mg/day	P.O.	Difference in rate of decline in function, ALSFRS-R	May 2013	USA & Canada
NP001	NCT01281631	Phase 2 RCT	Low/High	I.V.	Measures of clinical function	October 2012	USA
Creatine and Tamoxifen	NCT01257581	Phase 2 RCT	30g/40mg/day	P.O.	Decline in ALSFRS-R	December 2012	USA
Mecobalamin	NCT00444613	Phase 2/3 RCT	25mg or 50mg twice weekly	I.M.	Survival rate, functional rating scale	March 2014	Japan
Mecobalamin	NCT00445172	Phase 2/3 NRSA	Not specified	I.M.	Safety and tolerability	January 2014	Japan
Tirasemtiv (CK-2017357)	NCT01709149	Phase 2b RCT	250–500mg twice daily	P.O.	Change from baseline to average of ALSFRS-R	October 2013	USA
Anakinra and Riluzole	NCT01277315	Phase 2	100mg	SubQ	Safety and tolerability	June 2012	Germany
Arimoclomol	NCT00706147	Phase 2/3 RCT	200mg three times daily	P.O.	Decline of ALSFRS-R	December 2012	USA
MCI-186	NCT01492686	Phase 3 RCT	60mg/day	I.V.	Decline of ALSFRS-R	March 2015	Japan

Table 2

Preclinical Trials and Current Progress

Compound	Mechanism of Action	Preclinical Impact	Preclinical Survival Effect	Clinical Trials	Reference
Arimoclomol	Protein Aggregation Inhibition	Prolonged lifespan Increased motor neuron survival	~9–22%	Ongoing	(Kalmar et al., 2008) (Kieran et al., 2004)
AEOL-10150	Antioxidant and inhibits lipid peroxidation	Preserved SC motor neurons, decreased astrogliosis	~26%	No data	(Crow et al., 2005)
AM-1241	Anti-inflammatory (CB2 receptor agonist)	Delayed progression	~10%	No data	(Shoemaker et al., 2007)
Ammonium Tetrathiomolybdate	Copper chelation	Prolonged survival and delayed onset	~25%	No data	(Tokuda et al., 2008)
BDNF	TrkB receptor activation	Improved motor performance, decreased atrophy and loss of ventral root myelinated fibers	No effect	Failed	(Ikeda et al., 1995) (Mitsumoto, Ikeda, Klinkosz, et al., 1994)
Ceftriaxone	Increased EAAT2 transcription	Prolonged survival Increased EAAT2 levels, delayed loss of motor neurons	8%	Failed	(Rothstein et al., 2005)
Celastrol	Anti-inflammatory, anti-oxidant, prevents protein aggregation	Delayed onset, Improved weight loss and motor performance	9–13%	No data	(Kiaei et al., 2005)
Celecoxib	COX-2 inhibitor	Prolonged survival and delayed onset preserved motor neurons and decreased gliosis	25%	Failed	(Drachman et al., 2002)
CGP 3466B	Anti-apoptotic	Conflicting results: No effect seen in G93A mice. Slowing of motor neuron loss in pnm/pnm mice	No effect in SOD1-G93A 57% in pnm/pnm	Failed	(Sagot et al., 2000) (Groeneveld et al., 2004) (R. Miller et al., 2007)
Ciliary Neurotrophic Factor	Promotes motor neuron survival	Improved motor performance, decreased muscle atrophy, reduced loss of ventral root myelinated fibers	No effect	Failed	(Mitsumoto, Ikeda, Holmlund, et al., 1994)
Cobalamin	Anti-glutamatergic, anti-oxidant, anti-apoptotic	Prolonged survival and delayed onset improved motor performance	~3%	Ongoing	(X. Zhang et al., 2008)
Coenzyme Q	Anti-oxidant	Prolonged survival	~4%	Failed	(Matthews et al., 1998)
Creatine	Anti-oxidant, mitochondrial cofactor	Prolonged survival and delayed onset	14.6%	Failed	(Andreassen et al., 2001) (Shefner et al., 2004)

Compound	Mechanism of Action	Preclinical Impact	Preclinical Survival Effect	Clinical Trials	Reference
Dexramipexole	Anti-oxidant Anti-apoptotic	No Data	No Data	Failed	(Pattee et al., 2003) (H. Wang et al., 2008)
Edaravone	Anti-oxidant	Improved motor performance and decreased SOD1 deposits	No effect	Ongoing	(Hidelfumi Ito et al., 2008)
Erythropoietin	Anti-inflammatory, anti-apoptotic	Conflicting results: Some studies prolonged survival and delayed onset; while others see no effect	~10%	Failed	(Koh et al., 2007) (Lauria et al., 2009)
G-CSF	Neuroprotective Antiapoptotic	Increased MN survival	~7%	None	(Pitzer et al., 2008)
Gabapentin	Anti-glutamatergic	Prolonged survival	~5%	Failed	(Gurney et al., 1996)
GDNF	Promotes motor neuron survival	Prolonged survival and delayed onset improved motor function, reduced motor neuron loss	~12%	Failed	(Acsadi et al., 2002) (M. Suzuki et al., 2008)
Glatiramer acetate	Anti-inflammatory Anti-glutamatergic	Conflicting results: some studies prolonged survival, while others did not	No effect	Failed	(Gordon et al., 2006) (Haenggeli et al., 2007) (Meininger et al., 2009)
HGF	Neuroprotective Anti-apoptotic Anti-glutamatergic	Prevented MN cell death prolonged survival	18.8%	No data	(Sun et al., 2002) (Kadoyama et al., 2007)
IGF-I	Neuroprotective Anti-oxidant	Suppression of reactive species, protection against glutamate induced toxicity	No effect	Failed/Inconclusive	(Lai et al., 1997) (Sakowski et al., 2009) (Saenger et al., 2012)
L-Arginine	Anti-glutamatergic	Prolonged survival, delayed onset and slowed neuropathology	9–20%	No data	(J. Lee et al., 2009)
Lithium	Promotes autophagy	Delayed disease onset and progression	~36%	Failed	(Aggarwal et al., 2010) (Fornai et al., 2008)
Mechano-growth factor	Neuroprotective	Increased survival of motor neurons	No effect	No data	(Riddoch-Contreras et al., 2009)
Memantine	Anti-glutamatergic	Prolonged survival and delayed progression	~5–7%	No data	(R. Wang and D. Zhang, 2005) (Joo et al., 2007)
Minocycline	Anti-apoptotic Anti-inflammatory	Delayed disease progression and onset	~6–15%	Failed	(Kriz et al., 2002) (Zhu et al., 2002) (Van Den Bosch et al., 2002) (Gordon et al., 2004)
N-acetylated alpha linked acidic dipeptidase	Anti-glutamatergic	Prevented MN cell death	15%	No data	(Ghadge et al., 2003)

Compound	Mechanism of Action	Preclinical Impact	Preclinical Survival Effect	Clinical Trials	Reference
N-acetylcysteine	Anti-oxidant	Prolonged survival and delayed onset	~7%	Failed	(Louwerse et al., 1995) (Andreassen et al., 2000)
Nordihydroguaiaretic acid	Anti-inflammatory Anti-glutamatergic	No effect on survival Increased glutamate uptake	No effect	No data	(Boston-howes et al., 2008)
ONO-2506	Prevents astrogliosis Anti-inflammatory Anti-glutamatergic	See Valproate (parent compound)	No data	Failed	(dePaulis, 2003)
Pentoxifylline	Anti-apoptotic Anti-inflammatory	No preclinical data	No data	Failed	(Meininger et al., 2006)
Pioglitazone	Neuroprotective Anti-inflammatory Anti-apoptotic	Prolonged survival and delayed onset	~8%	Failed	(Schütz et al., 2005)
Rasagiline	Anti-apoptotic	Prolonged survival	~14%	Ongoing	(Waibel et al., 2004)
Riluzole	Anti-glutamatergic	Prolonged survival and delayed onset	~10%	Only effective treatment known	(Gurney et al., 1996) (Gurney et al., 1998)
RO-26-2853	Anti-inflammatory (inhibition of MMPs)	Prolonged survival and improved motor performance	~11%	No data	(Lorenzl et al., 2006)
Sodium phenylbutyrate	Protein aggregation inhibitor	Prolonged survival with conflicting results	21.9%	Phase I Completed	(Ryu et al., 2005) (Cudkowicz et al., 2009)
Talampanel	Anti-glutamatergic	Reduces MN calcium	No effect	Phase II Completed	(Faizs et al., 2011) (Pascuzzi et al., 2010)
Thalidomide	Anti-inflammatory	Prolonged survival and decreased motor neuron cell death	~11%	Failed	(Kiaei et al., 2006) (Stommel et al., 2009)
Topiramate	Anti-glutamatergic	Prevents MN degeneration in culture	No effect	Failed	(Maragakis et al., 2003) (Cudkowicz et al., 2003)
TRO19622	Anti-oxidant	Prolonged survival and delayed onset	10%	No data	(Bordet et al., 2007)
Valproate	Histone deacetylase inhibitor	Conflicting results; two studies found prolonged survival, while two others showed no effect	No effect - 10%	Failed	(Sugai et al., 2004) (Feng et al., 2008) (Rouaux et al., 2007) (Crochemore et al., 2009) (Piepers et al., 2009)
VEGF	Neuroprotective	Decreased astrogliosis and increased neuromuscular junctions; Decreased motor neuron death	No effect	Ongoing	(Zheng et al., 2007) (Tovar-Y-Romo et al., 2007)

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Compound	Mechanism of Action	Preclinical Impact	Preclinical Survival Effect	Clinical Trials	Reference
Vitamin E	Anti-oxidant	Delayed onset and slowed progression; does not prolong survival	No effect	Failed	(Gurney et al., 1996) (Terro et al., 1996) (Desnuelle et al., 2001) (Graf et al., 2005)
zVAD-fmk	Anti-apoptotic	Prolonged survival and delayed onset	22%	No data	(M. Li et al., 2000)

Table 3
Transporters detected on cell types of the CNS. Plus (+) indicates detection of transporters at the protein level.

	Endothelial Cells	Astrocytes	Pericytes	Neurons	Microglia	Oligodendrocytes	Choroid Plexus
P gp	+	+	+	+	+		+
BCRP	+	+	+	+	+		
Mtp1	+	+	+	+	+		+
Mtp2	+						+
Mtp3	+						+
Mtp4	+	+			+		+
Mtp5	+	+		+	+		