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Strategic approaches to developing drug treatments for ALS

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Abstract

Significant progress in understanding the cellular mechanisms of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) has not been matched with the development of therapeutic strategies to prevent disease progression. The multiple potential causes and relative rarity of the disease are two significant factors that make drug development and assessment in clinical trials extremely difficult. We review recent progress in promoting therapeutics into clinical trials and highlight the value of moderate throughput screening for the acceleration and improvement of drug design.

Keywords

amyotrophic lateral sclerosis; drug screening; motor neuron; structural analysis

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive, lethal disease with an estimated incidence of 3-5 cases per 100,000 in the U.S. [1,2]. Currently, 20,000 Americans are diagnosed with ALS, and an estimated 5,000 new cases are identified in the United States each year [3]. A patient with ALS usually dies within 3 to 5 years from the onset of symptoms, due to the lack of effective treatment. The primary consistent feature in ALS patients is the loss of upper and lower motor neurons that leads to progressive muscular paralysis [4]. The underlying etiology of sporadic ALS is unknown. Several proposed mechanisms are areas of active research in both cell culture and animal models of ALS, including oxidative stress, mitochondrial impairment, protein aggregation, glutamate cytotoxicity, transcription dysfunction, inflammation and apoptotic cell death [5]. In man, the mechanism(s) underlying the onset and progression of ALS are also influenced by genetic and environmental factors. Clearly, the apparently broad and generally unknown causes of the disease have confounded the search for a treatment [5,6].

Ten percent of patients have a familial form of ALS (FALS) [7]. Autosomal dominant FALS is an adult onset disease, clinically and pathologically indistinguishable from sporadic ALS [8]. In 1993, the protein cytosolic copper-zinc SOD (SOD1) was reported to be mutated in several FALS families [9], and, currently, more than 122 missense mutations in SOD1 have been identified in patients with FALS [10]. It is believed that mutant SOD1 may form intracellular aggregates, a prominent feature of experimental and clinical ALS. SOD1

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Drug screening is a branch of discovery science that may advance progress in treatment of ALS by fast-tracking old drugs into new clinical applications and identifying new mechanistic targets for drug development.

mutations are responsible for 20% of FALS [11]. Several other gene mutations are now known to underlie ALS. Alsin (also known as *ALS2*) is a guanine-nucleotide-exchange factor that is involved in the maintenance and normal turnover of the cellular cytoskeleton [12]. Alsin mutations lead to an early onset, but slowly progressive form of FALS [13]. There are also many as yet undiscovered genes, as linkage to chromosomes 16, 18, and 20 are reported in separate families with FALS [10].

In summary, while altered SOD and/or Alsin function may, at least partially, underlie the pathogenesis of FALS, alternative hypotheses suggest that excitotoxicity mediated *via* glutamate (Glu) receptors, oxidative stress related to mitochondrial impairment, aging or environmental factors, or disruption of the neuronal cytoskeleton also promote the development of sporadic ALS [14,15].

Understanding the mechanisms underlying ALS

While ALS is primarily characterized by upper and lower motor neuron degeneration, non-neuronal cell types are also involved in the disease. Early *in vivo* experiments examining the effect of mutant SOD1 expression exclusively in motor neurons were unsuccessful at inducing ALS-like disease in mice [16,17]. These studies led to further examination of the multifaceted interplay between motor neurons and surrounding non-neuronal cells during ALS disease onset and progression. Chimeric mice expressing a mixture of normal and mutant SOD1 demonstrate that non-neuronal cell expression of mutant SOD1 is required to produce motor neuron toxicity [18]. Microglial activation clearly plays a role in ALS pathology. Trials utilizing treatment with minocycline, which can inhibit microglial activation, demonstrated neuroprotective effects and increased survival in ALS mouse models [19-21]. In addition, removal of the mutant SOD1 in microglia does not alter disease onset, but delays death, which further supports the role of microglia in ALS disease progression [22,23].

Assessment of the different cell types implicated in the development of ALS and their interactions with motor neurons has identified several additional pathogenic mechanisms. In co-cultures of motor neurons with glial cells, the glial cells have a direct effect on motor neuron survival [24]. An astrocyte-motor neuron co-culture system demonstrated that astrocytes expressing mutant SOD1 secrete toxic factors that selectively kill motor neurons through a Bax-dependent mechanism [25]. Overexpression of mutant SOD1 in organotypic spinal cord slice cultures causes downregulation of the astrocyte glutamate transporter GLT1, leading to accumulation of extracellular glutamate and motor neuron excitotoxicity [26]. These data together suggest that astrocytes and microglia might play complementary roles in ALS by affecting disease onset and progression. In addition to spinal cord cells, other organ systems are implicated. One such system is skeletal muscle, which is a primary site of action of mutant SOD1 [27]. Muscle expression of growth factors can lead to preservation of spinal motor neurons [27,28]. Recently, the vascular system was also implicated in ALS through the discovery that mice lacking the hypoxia-response element in the VEGF promoter (*Vegf*^{δ/δ} mice) develop motor neuron disease with a phenotype identical to that of ALS. Low VEGF levels may impair spinal cord perfusion and cause chronic ischemia of motor neurons, while also depriving motor neurons of VEGF-mediated survival signals. Both mechanisms result in progressive motor neuron dysfunction that leads to ALS [29-32].

From mechanisms to drug targets

For the purposes of both confirming pathogenic mechanisms and also for therapeutic strategies, compounds with known activities against the injury mechanisms in experimental systems have been tested in mice overexpressing human SOD1 with the G93A mutation. These compounds include antiepileptic agents, antibiotics, antioxidants, protease inhibitors, platelet inhibitors and several growth factors [33,34]. In mice, silencing of SOD1 using a lentivirus-delivered

shSOD1 delays disease onset and progression when given prior to the symptomatic phase [35]. The neuroprotective agent rasagiline improves motor function and increases survival, which is further enhanced by combination with riluzole [36]. The β -lactam antibiotic, ceftriaxone, delays motor neuron loss, improves muscle strength and increases survival in the SOD1G93A mouse, either through increased glutamate transporter expression [26] or by acting as a metal ion chelator [37]. Pioglitazone, a thiazolidinedione antidiabetic agent, with reported anti-inflammatory properties, also improves muscle strength and survival [38]. Finally, a new study indicates that manganese porphyrin extends survival of the SOD1G93A mouse up to 3-fold; this study also has the advantage that this marked improvement is seen when the drug is given at symptom onset (as would occur in patients), rather than during the presymptomatic phase as in the previous studies discussed [39]. A recent review summarizes the 78 individual treatments and the 12 combination treatments that have been evaluated in ALS animal models [33]. Many compounds that showed some efficacy in the ALS mice were then moved to patient trials. A thorough review of current ALS clinical trials also is available [40].

Although we have advanced our understanding of the pathology of ALS, effective drug treatments for patients have remained elusive [41]. Research over the last decade produced a single FDA-approved drug for ALS, riluzole [42], despite the many treatments that were effective in mouse ALS as discussed above [19,43-46]. In man in the past 12 years, 11 double blind placebo-controlled trials have failed for the treatment of ALS (www.alsa.org). Possibly a major reason for the discrepancy between preclinical and clinical studies is the limitation of the animal models. While a mouse will develop an ALS-like syndrome when mutant SOD1 is highly expressed [47-49], these animals are a poor representation of the human patient population. To date, almost all rodent trials have focused upon overexpression of mutant SOD1. There is a clear “disconnect” between effective therapies in the ALS mouse and effective treatment in man, likely due to the more complex mechanism(s) underlying disease development in sporadic ALS [10]. Transfer of information gained using this mouse model is further weakened by the modest results generally obtained in these studies. An average extension of life of 2 weeks is considered significant in a mouse; it is difficult to estimate how this would translate to patient survival and the number of patients in a trial that would be required to demonstrate a statistically significant effect. In this regard, both mouse and human patient clinical trials would benefit from the development of disease biomarkers that would provide more useful trial end-points. Attempts to define biomarkers for ALS are ongoing [50-52].

Further considerations for drug targeting-ALS is a progressive disease

One underlying theme emerging from these and other studies provides evidence for distinct phases of ALS - onset and early stages, symptomatic and end stages - and dynamic contributions of motor neurons and glia throughout these stages [53,54]. These complex interactions between motor neurons and glia make cell type selection a critical consideration when designing mechanistic and intervention studies. Another consideration is the animal model and the outcomes that are being measured, as the contribution of different cell types to disease progression changes over the course of the disease. Based on these results, drugs acting on microglia might have an impact on ALS progression and survival, whereas drugs that exert their effects on astrocytes might be more effective at onset and early disease stages. Drugs that promote motor neuron survival, the central focus of most approaches to date, likely will slow the progression of the disease and delay the end-stage. In each case, the choice of the animal model would also affect the experimental outcome. The most effective compounds in mouse trials to date have operated at the onset stage of the disease [19]. Since patients present during disease progression when significant motor neuron loss has already occurred, this markedly narrows the window of opportunity for therapeutic intervention and drugs that prevent early disease progression in mice may be past the point of utility before a patient is diagnosed. Much

more investigation into cell-cell interactions in different animal models of ALS is warranted in order to exploit these interactions for targeted drug development. As well as drug development, understanding non-neuronal derived motor neuron toxic and protective factors will also contribute to future gene therapy and stem cell based therapeutic strategies [55].

IGF-I as an example of a promising candidate ALS treatment

Our own approach to experimental and clinical ALS has focused, in part, on using growth factors to promote neuroprotective and regenerative processes in affected neurons. Growth factors have the distinct advantage of offering both neuroprotection, as seen with the factors described in the above paragraph, and regenerative capacity through increased neurite regrowth and extension [56,57]. The potent neuroprotective effects of IGF-I led us to examine IGF-I in *in vitro* ALS models where IGF-I was potentially neuroprotective [56]. Viral gene therapy for targeted delivery of IGF-I to motor neurons in animal models of ALS by our group [56, 58-63] as well as the collaborative group from the Salk Institute and Johns Hopkins [64], found that retrograde transport of viruses to motor neurons occurs and that viral delivery of IGF-I delivery to motor neurons prolongs survival in the SOD1G93A mouse model of ALS. Yet, subcutaneous administration of IGF-I to ALS patients has been of uncertain clinical benefit [1,65,66]. Because of some positive outcomes for ALS patients, a redesigned multicenter phase III clinical trial is currently ongoing (www.alsa.org). The overall unsatisfying clinical results with this experimentally-effective compound further support the need for initiatives to discover new therapies for ALS.

Discovery Science and High Throughput Screening

New initiatives aimed at aggressively seeking therapeutic strategies for ALS have turned to drug screening. These screens can be low throughput, with a goal to also gain mechanistic information, or high throughput where tens of thousands of compounds are tested against a single disease target. Recently, drug screening has begun to produce data that has led to trials in mice and man [67,68]. So far, this review has highlighted the difficulty in designing therapeutics because of the multiple cell types and mechanisms that may lead to ALS. Yet, the ability to identify drugs that target a specific pathological pathway is a powerful tool that may lead to the rational design of combination therapies that effectively slow disease progression [69].

In order to increase the power of the screening strategy, multiple assays may be used. In 2001, the National Institute of Neurological Disorders and Stroke (NINDS) and three voluntary organizations, the ALS Association, the Huntington's Disease Society of America and the Hereditary Disease Foundation formed a consortium of 26 laboratories to screen a collection of 1040 compounds in 29 different assays [68,70,71]. Initially, it was thought that a compound that "hit" in multiple assays would be an excellent candidate for a new therapy. Later, mining through the compiled data proved that this was not the most useful information to be gained from the study. A compound that was effective in several assays was more likely to be producing a non-specific effect without clinical utility. The compound with the most hits in different assays was mercurochrome that was probably inhibiting mitochondria and killing cells without apoptosis. However, the different assays identified classes of compounds that operated on specific cellular mechanisms that could be important therapeutic targets. Many candidate compounds were identified and because the majority of the compounds were pre-selected FDA-approved drugs, researchers are in a strong position to move rapidly into clinical trials.

In the 2001 drug screen, one screen for the increased expression of astrocyte glutamate transporters demonstrated that β -lactam antibiotics increased glial glutamate transporter GLT1, also known as EAAT2. Subsequently, the β -lactam antibiotic Ceftriaxone proved

effective in a mouse model of ALS [72] and this group proposed trials in ALS patients. Although previous clinical studies of β -lactam antibiotics proved equivocal [73,74], the new data are promising and a large multicenter clinical trial is currently enrolling patients (www.alsa.org). Another group suggested that β -lactam antibiotics also operate as metal chelators in neurodegenerative diseases [37]. We would contend that therapies need to be designed for individual patients, an approach known as personalized medicine [75,76]. In trials, Riluzole was effective in some patients who began with high serum or CSF glutamate that was lower following a Riluzole regimen [77,78]. In a similar manner, we would predict that the 25 % of patients with reduced expression of EAAT [79], would benefit from a therapy that increases EAAT2 expression. Patient populations for clinical trials ideally should be enriched for target mechanisms prior to the trial, in order to be able to demonstrate efficacy. Dilution of positive effects by patients with a different underlying etiology will cause trial failure for potentially important therapeutics. This increases the challenge of recruiting patients as many more would need to be screened for entry into the trial. However, an enriched population would statistically require fewer patients to demonstrate clinical efficacy so this strategy should decrease overall trial costs.

Other assays in the NINDS-led consortium screen of 1040 FDA-approved compounds examined mutant SOD1 cytotoxicity, glutamate excitotoxicity, protein aggregation and other cytotoxic agents, including polyglutamine proteins, alpha-synuclein and kainate [68,70,71]. The degree of hits overlap was not insignificant, but largely unfruitful. One exception was the finding of celastrol in several of the screens [67]. Celastrol has many potentially important cellular effects that could modulate the progression of ALS, including direct antioxidant and anti-inflammatory actions [80,81], activation of the heat shock response [82] and inhibition of proteasomes [83]. Celastrol prevents neuronal injury *in vitro* [84] and delays disease progression and death in a mouse model of ALS [43]. All of this evidence has placed celastrol on the list of potential trials, although no trial is currently proposed. Another prominent hit from the consortium screen was thalidomide[34]. A small clinical trial aimed at recruiting 24 patients at a single center is enrolling for treatment with this compound (see www.alsa.org).

What Do We Do With Screening Data?

Drug screening has proved an important tool to identify classes of compounds that may be important for the treatment of ALS [67-69,71]. In 6 years since the consortium screen a few compounds have entered clinical trials but the outcomes are not yet known. More significantly, hits in classes of compounds such as anti-inflammatories, non-steroidal anti-inflammatory drugs, antioxidants and antibiotics have led us and others to examine further the cellular effects of such compounds, in order to design new compounds with altered specificities [85]. Our own laboratory is focusing on understanding how anti-inflammatory compounds promote survival in a cell culture of enriched motor neurons (unpublished data). Figure 1 illustrates how screening, animal trials and patient trials can feed forward to continue to improve our understanding of disease pathogenesis and develop therapeutic strategies. Clearly, rapid and effective data sharing are important to eliminate redundancy between researchers working within this type of drug development system.

We distinguished the following potential outcomes for the compound hits from a single trial:

1. Test the compound in several screens. If the compound has broad effect on multiple pathogenic mechanisms, could this already be a strong candidate therapy? Rationally eliminate non-specific compound effects[86].
2. Test the compound in an animal model of the disease. If known mechanisms of action suggest that this compound targets a significant pathologic mechanism, it should be confirmed in an appropriate animal model. By appropriate, remember the

consideration that animal models generally represent a single disease-causing gene mutation or toxic agent [33]. The experimental system should model the potential target mechanism.

3. Instigate a clinical trial: If the screen incorporated FDA-approved compounds, fast-tracking the hits to clinical trials is well underway[70]. Clinical trials, notwithstanding, require substantial pre-clinical testing. One important consideration here will also be the pharmacokinetics of the compound. A compound with FDA approval for one application may be unsuitable for in vivo ALS treatment because of blood-brain barrier impermeability. A promising compound could be modified at this stage to increase CNS penetration.
4. Refine the compound. Structural comparisons of compound hits may reveal particular domain motifs that are active in the screening assay. Libraries of structurally-similar compounds may be obtained and also screened to obtain more hits or to predict a novel therapeutic small molecule. Such structural analyses also can be used to predict the mechanistic actions of unexpected compound hits.
5. Finally, screening data and the known mechanisms of action should be compared and contrasted with a goal to design combination therapies aimed at the full spectrum of known cellular dysfunctions in ALS. A safe combination cocktail that can prevent oxidative stress, provide neuronal trophic support, normalize glutamate balance, promote neuronal energy levels, improve blood flow, and prevent protein aggregate formation will clearly be a good therapy for ALS patients. Individual compounds that can somewhat impact on these mechanisms are known, but they are rarely tested in combinations. The combinations should be tested in several animal models, and then promoted to patient clinical trials.

Computational Modeling as a Novel Approach to Drug Design

Our laboratory is embarking upon a new initiative to create computational models and methods for determining how SOD1 mutations ultimately lead to the development of ALS [87]. We are comparing the known tertiary and quaternary structures of known SOD1 mutants and modeling how these alter the functional motion of the proteins. We predict that we will find a change in atomic motion that is consistent between different SOD1 mutations and that this consistent change will help understand the toxic gain of function of the mutants. Ultimately, these studies could lead to the design of a small molecule therapeutic that blocks the toxic gain-of-function. While this is a targeted approach that may initially benefit only patients with FALS resulting from SOD1 mutations, the results may have much broader mechanistic implications than we initially can predict.

Conclusion

We present a strategy whereby drug screening is an integral part of the drug development process. This process also incorporates clinical and animal trials. We acknowledge that animal trials have previously proved poor predictors of efficacy in patients [33] and that returning to *in vitro* systems is apparently removing the research further from the bedside. However, screening strategies that are carefully designed with disease mechanisms in mind and followed-up with systematic testing, assessment, and improvement may be the way forward for the ALS patient population with a critical need to see some progress.

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References

1. Feldman, EL., et al. Cecil Textbook of Medicine. W.B. Saunders; 2004. Amyotrophic lateral sclerosis and other motor neuron diseases; p. 2316-2319.
2. Cronin S, et al. Ethnic variation in the incidence of ALS: a systematic review. *Neurology* 2007;68(13): 1002–1007. [PubMed: 17389304]
3. Hirtz D, et al. How common are the “common” neurologic disorders? *Neurology* 2007;68(5):326–337. [PubMed: 17261678]
4. Gonzalez de Aguilar JL, et al. Amyotrophic lateral sclerosis: all roads lead to Rome. *J Neurochem* 2007;101(5):1153–1160. [PubMed: 17250677]
5. Bruijn LI, et al. Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu.Rev.Neurosci* 2004;27:723–749. [PubMed: 15217349]
6. Noonan, CW., et al. Health Investigations Branch, Agency for Toxic Substances and Disease Registry. Division of Health Studies; Atlanta: Mar 5. 2002 Motor neuron disease/amyotrophic lateral sclerosis: Preliminary review of environmental risk factors and mortality in Bexar County, Texas. <http://www.atsdr.cdc.gov/NEWS/alsreport.html>
7. de Belleruche J, et al. Familial amyotrophic lateral sclerosis motor neurone disease (FALS): A review of current developments. *Journal of Medical Genetics* 1995;32:841–847. [PubMed: 8592323]
8. Brown JRH. Amyotrophic lateral sclerosis: recent insights from genetics and transgenic mice. *Cell* 1995;80:687–692. [PubMed: 7889564]
9. Rosen DR, et al. Mutations in Cu/Zn superoxide dismutase are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59–62. [PubMed: 8446170]
10. Gros-Louis F, et al. Genetics of familial and sporadic amyotrophic lateral sclerosis. *Biochim Biophys Acta* 2006;1762(1112):956–972. [PubMed: 16503123]
11. Hart PJ. Pathogenic superoxide dismutase structure, folding, aggregation and turnover. *Curr Opin Chem Biol* 2006;10(2):131–138. [PubMed: 16516535]
12. Hadano S, et al. Molecular and cellular function of ALS2/alsin: Implication of membrane dynamics in neuronal development and degeneration. *Neurochem Int* 2007;51(24):74–84. [PubMed: 17566607]
13. Yang Y, et al. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet* 2001;29(2):160–165. [PubMed: 11586297]
14. Rothstein JD. Excitotoxicity hypothesis. *Neurology* 1996;47:S19–S26. [PubMed: 8858047]
15. Rothstein JD. Of mice and men: reconciling preclinical ALS mouse studies and human clinical trials. *Ann Neurol* 2003;53:423–426. [PubMed: 12666108]
16. Pramatarova A, et al. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *Journal of Neuroscience* 2001;21:3369–3374. [PubMed: 11331366]
17. Lino MM, et al. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci* 2002;22(12):4825–4832. [PubMed: 12077179]
18. Clement AM, et al. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 2003;302(5642):113–117. [PubMed: 14526083]
19. Kriz J, et al. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2002;10(3):268–278. [PubMed: 12270689]
20. Van Den Bosch L, et al. Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport* 2002;13(8):1067–1070. [PubMed: 12060810]
21. Zhu S, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 2002;417:74–78. [PubMed: 11986668]
22. Beers DR, et al. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 2006;103(43):16021–16026. [PubMed: 17043238]

23. Boillee S, et al. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 2006;312(5778):1389–1392. [PubMed: 16741123]
24. Di Giorgio FP, et al. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci* 2007;10(5):608–614. [PubMed: 17435754]
25. Nagai M, et al. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 2007;10(5):615–622. [PubMed: 17435755]
26. Rothstein JD, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* 2005;433:73–77. [PubMed: 15635412]
27. Dobrowolny G, et al. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *J Cell Biol* 2005;168(2):193–199. [PubMed: 15657392]
28. Mohajeri MH, et al. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. *Human Gene Therapy* 1999;10:1853–1866. [PubMed: 10446925]
29. Storkebaum E, Carmeliet P. VEGF: a critical player in neurodegeneration. *J Clin Invest* 2004;113:14–18. [PubMed: 14702101]
30. Cleveland JL. A new piece of the ALS puzzle. *Nat. Genet* 2003;34:357–358. [PubMed: 12923539]
31. Greenberg DA, Jin K. VEGF and ALS: the luckiest growth factor? *Trends Mol Med* 2004;10:1–3. [PubMed: 14720577]
32. Carmeliet P. Blood vessels and nerves: common signals, pathways and diseases. *Nat. Rev. Genet* 2003;4:710–720. [PubMed: 12951572]
33. Benatar M. Lost in translation: treatment trials in the SOD1 mouse and in human ALS. *Neurobiol Dis* 2007;26(1):1–13. [PubMed: 17300945]
34. Traynor BJ, et al. Neuroprotective agents for clinical trials in ALS: a systematic assessment. *Neurology* 2006;67(1):20–27. [PubMed: 16832072]
35. Raoul C, et al. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nature Medicine* 2005;11:423–428.
36. Waibel S, et al. Rasagiline alone and in combination with riluzole prolongs survival in an ALS mouse model. *J Neurol* 2004;251:1080–1084. [PubMed: 15372249]
37. Ji HF, et al. Beta-lactam antibiotics are multipotent agents to combat neurological diseases. *Biochem. Biophys Res. Commun* 2005;333:661–663. [PubMed: 15907788]
38. Schutz B, et al. The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice. *J Neurosci* 2005;25:7805–7812. [PubMed: 16120782]
39. Crow JP, et al. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. *Annals of Neurology* 2005;58:258–265. [PubMed: 16049935]
40. Bhatt JM, Gordon PH. Current clinical trials in amyotrophic lateral sclerosis. *Expert Opin Investig Drugs* 2007;16(8):1197–1207.
41. Weiss MD, et al. Current pharmacological management of amyotrophic [corrected] lateral sclerosis and a role for rational polypharmacy. *Expert Opin. Pharmacother* 2004;5:735–746. [PubMed: 15102560]
42. Festoff BW, et al. Prospects for the pharmacotherapy of amyotrophic lateral sclerosis: old strategies and new paradigms for the third millennium. *CNS. Drugs* 2003;17:699–717. [PubMed: 12873154]
43. Kiaei M, et al. Celastrol blocks neuronal cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. *Neurodegener Dis* 2005;2(5):246–254. [PubMed: 16909005]
44. Chiba T, et al. Colivelin prolongs survival of an ALS model mouse. *Biochem Biophys Res Commun* 2006;343(3):793–798. [PubMed: 16564029]
45. Petri S, et al. Cell-permeable peptide antioxidants as a novel therapeutic approach in a mouse model of amyotrophic lateral sclerosis. *J Neurochem* 2006;98(4):1141–1148. [PubMed: 16895581]
46. Koh SH, et al. Recombinant human erythropoietin suppresses symptom onset and progression of G93A-SOD1 mouse model of ALS by preventing motor neuron death and inflammation. *Eur J Neurosci* 2007;25(7):1923–1930. [PubMed: 17439481]

47. Tu PH, et al. Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis lesions. *Proc Natl Acad Sci U S A* 1996;93(7):3155–3160. [PubMed: 8610185]
48. Nagai M, et al. Rats expressing human cytosolic copper-zinc superoxide dismutase transgenes with amyotrophic lateral sclerosis: associated mutations develop motor neuron disease. *J Neurosci* 2001;21(23):9246–9254. [PubMed: 11717358]
49. Lemmens R, et al. Overexpression of mutant superoxide dismutase 1 causes a motor axonopathy in the zebrafish. *Hum Mol Genet.* 2007
50. Mitsumoto H, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. *Neurology* 2007;68(17):1402–1410. [PubMed: 17452585]
51. Wang LY, et al. Molecular Diagnosis and Biomarker Identification on SELDI proteomics data by ADTBoost method. *Conf Proc IEEE Eng Med Biol Soc* 2005;5:4771–4774. [PubMed: 17281308]
52. Kolarcik C, Bowser R. Plasma and cerebrospinal fluid-based protein biomarkers for motor neuron disease. *Mol Diagn Ther* 2006;10(5):281–292. [PubMed: 17022691]
53. Boillee S, et al. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 2006;52(1):39–59. [PubMed: 17015226]
54. Sargsyan SA, et al. Microglia as potential contributors to motor neuron injury in amyotrophic lateral sclerosis. *Glia* 2005;51(4):241–253. [PubMed: 15846792]
55. Miller TM, Cleveland DW. Has gene therapy for ALS arrived? *Nature Medicine* 2003;9:1256–1257.
56. Vincent AM, et al. IGF-I prevents glutamate-induced motor neuron programmed cell death. *Neurobiology of Disease* 2004;16:407–416. [PubMed: 15193297]
57. Vincent AM, Feldman EL. Control of cell survival by IGF signaling pathways. *Growth Hormone and IGF Research* 2002;12:193–197. [PubMed: 12175651]
58. Boulis NM, et al. Characterization of adenoviral gene expression in spinal cord after remote vector delivery. *Neurosurgery* 1999;45:131–138. [PubMed: 10414575]
59. Turner DE, et al. Remote adenoviral gene delivery to the spinal cord: Contralateral delivery and reinjection. *Neurosurgery* 2001;48:1309–1317. [PubMed: 11383735]
60. Rubin AD, et al. Remote delivery of rAAV-GFP to the rat brainstem via the recurrent laryngeal nerve. *Laryngoscope* 2001;111:2041–2045. [PubMed: 11801993]
61. Boulis NM, et al. Neuronal survival following remote adenovirus gene delivery. *Journal of Neurosurgery* 2002;96(2 Suppl):212–219. [PubMed: 12450285]
62. Boulis NM, et al. Intraneural colchicine inhibition of adenoviral and adeno-associated viral vector remote spinal cord gene delivery. *Neurosurgery* 2003;52:381–387. [PubMed: 12535368]
63. Rubin A, et al. Delivery of an adenoviral vector to the crushed recurrent laryngeal nerve. *Laryngoscope* 2003;113:985–989. [PubMed: 12782809]
64. Kaspar BK, et al. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 2003;301:839–842. [PubMed: 12907804]
65. Lai EC, et al. The North America ALS/IGF-I Study Group. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. *Neurology* 1997;49:1621–1630. [PubMed: 9409357]
66. Ackerman SJ, et al. Cost effectiveness of recombinant human insulin-like growth factor I therapy in patients with ALS. *Pharmacoeconomics* 1999;15:179–195. [PubMed: 10351191]
67. Aiken CT, et al. A cell-based screen for drugs to treat Huntington's disease. *Neurobiol Dis* 2004;16(3):546–555. [PubMed: 15262266]
68. Heemskerk J. High throughput drug screening. *Amyotroph.Lateral.Scler.Other Motor Neuron Disord* 2004;5(Suppl 1):19–21. [PubMed: 15512863]
69. Carri MT, et al. Targets in ALS: designing multidrug therapies. *Trends Pharmacol Sci* 2006;27(5):267–273. [PubMed: 16690390]
70. Heemskerk J, et al. Teaching old drugs new tricks. Meeting of the Neurodegeneration Drug Screening Consortium, 7-8 April 2002, Washington, DC, USA. *Trends in Neurosciences* 2002;25:494–496. [PubMed: 12220871]
71. Heemskerk J, et al. From chemical to drug: neurodegeneration drug screening and the ethics of clinical trials. *Nat.Neurosci* 2002;5(Suppl):1027–1029. [PubMed: 12403977]

72. Rothstein JD, Kuncel RW. Neuroprotective strategies in a model of chronic glutamate-mediated motor neuron toxicity. *Journal of Neurochemistry* 1995;65:643–651. [PubMed: 7616220]
73. Couratier P, et al. Report of six sporadic cases of ALS patients receiving ceftriaxone. *Therapie* 1994;49(2):146. [PubMed: 7817342]
74. Carelli V, et al. Ceftriaxone is ineffective in ALS. *Ital J Neurol Sci* 1994;15(1):66. [PubMed: 8206750]
75. Collins CD, et al. The application of genomic and proteomic technologies in predictive, preventive and personalized medicine. *Vascul.Pharmacol* 2006;45:258–267. [PubMed: 17030152]
76. Susztak K, Bottinger EP. Diabetic nephropathy: a frontier for personalized medicine. *J Am.Soc.Nephrol* 2006;17:361–367. [PubMed: 16407421]
77. Bensimon G, et al. A controlled trial of riluzole in amyotrophic lateral sclerosis. *New England Journal of Medicine* 1994;330:585–591. [PubMed: 8302340]
78. Niebroj-Dobosz I, et al. Effect of Riluzole on serum amino acids in patients with amyotrophic lateral sclerosis. *Acta Neurologica Scandinavica* 2002;106:39–43. [PubMed: 12067327]
79. Rothstein JD, et al. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Annals of Neurology* 1995;38:73–84. [PubMed: 7611729]
80. Allison AC, et al. Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 2001;25(7):1341–1357. [PubMed: 11513350]
81. Lee JH, et al. Inhibition of NF-kappa B activation through targeting I kappa B kinase by celastrol, a quinone methide triterpenoid. *Biochem Pharmacol* 2006;72(10):1311–1321. [PubMed: 16984800]
82. Hieronymus H, et al. Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators. *Cancer Cell* 2006;10(4):321–330. [PubMed: 17010675]
83. Yang H, et al. Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. *Cancer Res* 2006;66(9):4758–4765. [PubMed: 16651429]
84. Cleren C, et al. Celastrol protects against MPTP- and 3-nitropropionic acid-induced neurotoxicity. *J Neurochem* 2005;94(4):995–1004. [PubMed: 16092942]
85. Vincent AM, et al. Identification of candidate drugs for the treatment of ALS. *Amyotrophic Lateral Sclerosis* 2005;6:29–36. [PubMed: 16036423]
86. Rothstein JD. Preclinical studies: how much can we rely on? *Amyotroph Lateral Scler Other Motor Neuron Disord* 2004;5(Suppl 1):22–25. [PubMed: 15512864]
87. Schuyler AD, Feldman EL. Iterative Cluster Normal Mode Analysis (icNMA): A Tool for Traversing the Conformation Energy Landscape with an Application to ALS. *Biophysical Society*. 2007abstract number 3831 (Protein Conformation and Dynamics)

Fig. 1. The Cycle of Screening and Testing Therapeutics. Drug screening can lead to further understanding of pathogenesis and drug development. Thorough examination of screening data with follow-up studies and testing of new drug candidates is predicted to speed the process of drug development.