

Of Mice and Men: Reconciling Preclinical ALS Mouse Studies and Human Clinical Trials

Amyotrophic lateral sclerosis (ALS) is largely a sporadic adult-onset neurodegenerative disease. For many decades, attempts at therapies for this disease were typically small, inadequately powered clinical trials often based on anecdotal observations, with intentions of reversing the disease. In this issue of *Annals*, two separate studies provide an opportunity to reflect on how we decide to investigate drugs for neurodegenerative disease. A new clinical trial of creatine was found to be ineffective in ALS, whereas a drug trial in an ALS mouse model finds for the first time that multiple drugs, three, additively slow down the disease.

First a recap. In a well-designed and well-executed trial, Groeneveld and colleagues investigated the therapeutic efficacy of oral creatine in a randomized placebo controlled trial in ALS.¹ Using a daily dose of 10gm, and using a sequential trial design, the investigators found that creatine did not alter survival or the rate of decline in functional measurements, after over 300 days of treatment. The patient characteristics between placebo- and creatine-treated patients were well matched. Importantly, the investigators monitored for patients surreptitiously taking creatine, through urine evaluation, and found “cheaters.” Furthermore, they had few patients discontinue trial medication (<10%) but quite a small number for a trial involving a simple food supplement available to the general public. Overall, there was no effect on survival, rate of change in ALS—relevant measures such as arm strength, or vital capacity. Currently, two similar trials are under way in the United States, though using lower doses of creatine. Therefore, is this just another drug failure in ALS? Yes and no. From a practical view point—yes. This was a good trial that flatly rules out (at least at the dose used) the value of creatine in ALS. But how did we get to this point. What made them consider creatine? At this point it is important to step back and view the landscape of ALS and neurodegeneration from the vantage point of animal models and new therapeutics.

The ALS therapeutics revolution began in the early 1990s, after a series of discoveries that provided critical clinical and basic science starting points. The discovery of the first familial gene for ALS, superoxide dismutase 1 (*SOD1*), along with the contribution of glutamate toxicity and oxidative stress to cell death in the ALS nervous system provided a new and important starting

point for designing therapeutic interventions.² In parallel, the role for trophic factors in supporting injured motor neurons added to the early tools we had to use in approaching disease intervention.³

These discoveries were quickly followed by the generation of a series of transgenic mouse and rat models expressing the mutant *SOD1* protein.^{4–7} Fortunately and exciting for all in the field, these mice developed ALS in a clinical and pathological fashion quite similar (although not identical) to the human counterpart. Understanding why cells die in ALS and how to stop that devastating process had been a difficult “nut to crack” for researchers, and it was hoped that this new animal model would bring the answers—and the cure!

Why was that? Well, first, most studies examining ALS pathogenesis were indirect, looking at a patient’s blood in hopes of gaining insight into defects in the brain, a very crude, if not generally inaccurate approach to central nervous system disease, or through examination of “dead brain tissue,” postmortem samples obtained after someone died and the disease so advanced that only a few cells (motor neurons) remained. This was hardly the best starting point to unravel the complexity of a disease that slowly progresses over months to years.

Despite those limitations, important bits of insight were gained from these approaches, biochemical and immunohistochemical suggestions that oxidative stress and excitotoxicity were part of disease process, and a potential role for protein aggregation due to the presence of intracellular protein inclusions (ubiquitinated inclusions) in many motor neurons.^{2,8,9} Yet, these studies remained indirect. To understand the biology of ALS better, over the last decade, researchers have come to rely of various laboratory-based models. Disease-relevant models allow one to test hypotheses dynamically and more directly. Models of disease initially consisted of pure motor neurons cultured in vitro, and slices of the spinal cord, a more complex, yet still in vitro paradigm to study motor neurons.^{10,11} These systems provided better control of cellular/molecular events and could be used to quickly (days to weeks) screen many drugs that could alter motor neuron degeneration. These early systems in fact identified and/or validated the early approaches to ALS therapies, including most of the trophic factors considered in

ALS as well as antiexcitotoxic therapies. By crude estimates, more than 50 drugs and trophic factors were evaluated in these systems by 1995. They were responsible, in part, for the first drug to be approved for ALS, riluzole, in 1995. They also provided the preclinical data used to start at least a half a dozen ALS clinical trials.

However, the generation of transgenic mice and rats based on the familial ALS SOD1 gene defects provided the real opportunity to unravel the biochemical/molecular steps responsible for why motor neurons degenerate.^{5,7} Transgenic rat and mouse models have now proved to be one of the most useful tools to understand the complexity of neurodegenerative disease, and not for just ALS, but comparable models exist for Huntington's disease, Parkinson's disease, Alzheimer's disease, spinal muscular atrophy, and spinocerebellar atrophy. These tools have proved useful in dissecting the steps that underlie the neuronal dysfunction responsible for disease. They are also extremely useful in evaluating new therapeutics. Drug therapies, gene therapies, and even stem cell therapies are now being actively pursued by both academic and pharmaceutical industry laboratories in hopes of finding agents or approaches capable of altering the disease. These efforts already have resulted in the study of more than 60 different therapies in ALS mice.² Most, at least from the published literature, have extremely marginal effects. And that brings us back to why creatine was studied by clinicians.

Several years ago, Beal and colleagues¹² administered creatine, orally at various doses (1 and 2% of diet) to the G93A SOD1 mouse. Interestingly, they found that creatine chronically administered before clinical disease onset could substantially delay onset of disease and prolong survival of the mice. It also delayed degeneration of motor neurons. That was great news at the time. As it turns out, many drugs can slow disease in these mice, at last by statistical evaluation. But, in retrospect, most of these effects were quite small. Furthermore, there are different versions of the mutant ALS mice, that is, some with many copies of the mutant gene, resulting in a more virulent, rapid disease (survival approximately 125 days) as compared with mice with a lower number of the mutant genes per cell, and a mild form of the disease (survival more than 250 days).^{4,6,13} Simply knowing that a drug increases survival by 10 or 30 days may not be not adequate in evaluating efficacy. A more important index is how much longer, in terms of percentage of life span, did they live? Thus, a 30-day increase in survival in the "high-expressing" mutant SOD1 mice is roughly equivalent to a 25% increase in survival, whereas the same 30-day increase in a mouse living 250 days is merely a 12% increase in survival, a big difference! Creatine increased survival by 18%. In comparison, the

only FDA-approved drug, riluzole, increased survival in the same mice by approximately 10%.¹⁴ For this reason, groups around the world soon began trials of creatine in ALS, and many patients now take the compound outside of trials. So, the mouse trial suggested the agent might be useful, but the human trial found otherwise. Why was that?

The most obvious answer is that all preclinical models inherently have certain limitations. The culture models typically reflected immature, embryonic, or young postnatal motor neurons that are essentially on a short path to death (cultured motor neurons only survive a few weeks in vitro), and, at least in some paradigms, they are devoid of their neighbors—no surrounding astrocytes, oligodendroglia, or microglia. This turns out to be very important in ALS; multiple studies are suggesting that motor neurons do not die "alone."^{15–17} It appears that surrounding neuropil (microglia, astrocytes, and possible other cells) all contribute to and are essential for the death of motor neurons. Similarly, before the mutant SOD1 mouse, other motor neuron injury models were used to evaluate how motor neurons die, at least from the vantage point of axonal injury. These models, including facial nerve axotomy,¹⁸ and to a lesser extent, inherited axonopathies (eg, *pnn* mouse),¹⁹ were quite useful in evaluating trophic factors but in the end not so good at predicted success in the more complex disease such as ALS (or transgenic rodent models of ALS). More recently, *Drosophila* and *Caenorhabditis elegans* have been utilized as screening methods for other neurodegenerative disease, but for now it appears that at least the fly may not be a suitable model for ALS.²⁰

Even the ALS transgenic rodents have limitations. First and the most important consideration when planning human trials based on preclinical rodent research was the dose equivalent? The authors of the ALS creatine trial chose a daily dose of 10gm to fall within the effective range used in the mouse trial. But many other factors also provide for differences. The pharmacokinetics of the drug, daily chronic oral administration in a mouse, could lead to a very different "steady" state of the drug/agent from the 5gm twice a day dosing in humans, a possible difference in pharmacology. Studies in the ALS mouse might not always predict human response, and the reasons for this are extensive and include (1) pharmacokinetics, difference in metabolism of the drug; (2) modes of delivery, different routes of administration can alter central nervous system distribution and outcome; (3) timing of drug intervention, most drugs are given well before or at the time of disease onset in the mice, whereas ALS patients receive drug when they are well into their disease; (4) potential inappropriateness of a familial model, the ALS mutant SOD1 mouse is based on a single defect that only accounts for approximately 1 to 2% of all ALS case. Cer-

tainly, the downstream toxicity associated with the mutant SOD1 may be common to multiple pathways for neuronal and glial injury in the disease, but a therapy that only acts on the mutant protein or the immediate toxic effects of the mutant protein is not very likely to be beneficial to the large sporadic ALS population.

Yet, despite these limitations, the transgenic rodent models are the best tools to reveal potential new human therapies. This brings one to the two recent studies published in *Annals*. In a recent *Annals*, Friedlander and colleagues²⁶ utilized a combination of creatine and minocycline in the ALS mice, showing, an enhanced survival beyond the effects of creatine alone, described above. Importantly, in the current issue, Kriz and colleagues provide the first, well described, combination therapy involving three agents, in the ALS mice. Using a combination of minocycline, nifedipine, and riluzole, the authors demonstrate an additive effect on survival, when compared with previous studies, using minocycline alone. Similar to creatine and celecoxib, minocycline has received attention recently because of its ability to prolong survival in the G93A SOD1 mice. Perhaps, more than any previous drug, minocycline has been shown to increase survival in at least three independent laboratories, using two different G93A SOD1 mouse lines, and using various modes of drug delivery.^{22–24} Like creatine, the minocycline studies alone were sufficient to plan and initiate clinical trials in ALS. So how do we now interpret and extrapolate data—at least involving creatine—in deciding human use of the compound in ALS patients? Simply—the drug failed in humans. This makes the extrapolation of the mouse combination of creatine and minocycline to patients very hard to fathom in decisions on human use. Obviously knowledge of the clinical efficacy of minocycline alone in ALS will be useful. Furthermore, how should we consider combination trials in ALS patients based on these two preclinical studies? Should we now consider combination trials in ALS patients based on this preclinical data? Combinatorial therapeutic trials can be complex in their design and execution and likely require large numbers of patients. A poorly executed trial will not help us find effective treatments for this terrible disease. Before we venture into these complex issues, we should tackle a more fundamental question: can we adequately predict human outcomes from ALS mouse trials? No—at least not yet.

As described above there are several variables that one has to consider when making these decisions, should a drug be studied in multiple independent laboratory experiments, with different mutant SOD1 mice (others exist such as the G37R SOD1 and the G85R SOD1 mice), at different drug concentrations and at different times of drug delivery (eg, before clinical disease onset, at disease onset, and after disease begins in the mice)? Ideally, all of these variables should be eval-

uated before starting a human trial. But they are not all practical and economically feasible. A single mouse “trial” takes at least 5 to 7 months to complete and costs approximately \$50,000 per trial (mouse housing and care costs are not insubstantial at universities today). Another reason some object to the ALS mouse trials is because they reflect only the familial form of ALS. Although as described above, that argument might be reasonable for some therapies aimed at the mutant SOD1 protein, many studies have documented that there is a wide range of cytotoxic events common to the ALS mouse and the sporadic ALS patient, including evidence for excitotoxicity (eg, loss of glutamate transport protein), oxidative injury, markers and genes reflecting programmed cell death, and neuroinflammation. Furthermore, at least one drug, riluzole, is effective in both the SOD1 mouse and large populations of sporadic ALS.

Should every drug considered for human trial first be evaluated in the mouse? The single failure of creatine does not invalidate the need for this correlative approach. At minimum, it would be useful to prospectively study all ALS trial candidates in mice and patients and then develop a database of these drug studies in hopes of eventually being able use these models effectively in decision trees. The need for this will be soon apparent. The National Institute of Neurological Disease and Stroke recently completed a screen, involving more than 2 dozen academic laboratories evaluating over 1,000 FDA-approved drugs and nutritional compounds in high throughput in vitro neurodegeneration model systems.²⁵ From this screen, many candidate compounds emerged in ALS-relevant assays (eg, motor neuron death, excitotoxicity, oxidative motor neuron damage). How we move dozens of FDA-approved drugs from these simple assays to human trials will likely require preclinical evaluation in the ALS mice. ALS clinical trials are expensive, ranging from a million to many millions of dollars per trial. With so many new drug possibilities on the horizon, we may have to reconsider trial design in ALS to develop methods to effectively and rapidly study these agents. Identification of reliable surrogate markers of disease progression (eg, motor neurons) or drug actions (eg, prostaglandin levels for COX2 inhibitors) will be required. Our mouse models will facilitate these studies. An effective strategy to make decisions about drug choices based on these preclinical tools will be necessary in times of limited resources.

Jeffrey D. Rothstein, MD, PhD

*Department of Neurology
The Robert Packard Center for ALS Research
Johns Hopkins University
Baltimore, MD*

References

1. Groeneveld GJ, Veldink JH, van der Tweel I. A randomized sequential trial of creatine monohydrate in amyotrophic lateral sclerosis. *Ann Neurol* 2003;53:437–445.
2. Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2001;2:806–819.
3. Henderson CE. Neurotrophic factors as therapeutic agents in amyotrophic lateral sclerosis. Potential and pitfalls. *Adv Neurol* 1995;68:235–240.
4. Wong PC, Pardo CA, Borchelt DR, et al. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 1995;14:1105–1116.
5. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 1994;264:1772–1775.
6. Bruijn LI, Becher MW, Lee MK, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 1997;18:327–338.
7. Howland DS, Liu J, She Y, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci USA* 2002;99:1604–1609.
8. Julien JP. Amyotrophic lateral sclerosis. Unfolding the toxicity of the misfolded. *Cell* 2001;104:581–591.
9. Shaw PJ. Excitatory amino acid neurotransmission, excitotoxicity and excitotoxins. *Curr Opin Neurol Neurosurg* 1992;5:383–390.
10. Camu W, Henderson CE. Rapid purification of embryonic rat motoneurons: an in vitro model for studying MND/ALS pathogenesis. *J Neurol Sci* 1994;124(suppl):73–74.
11. Rothstein JD, Jin L, Dykes-Hoberg M, et al. Chronic inhibition of glutamate uptake produces a model of slow neurotoxicity. *Proc Natl Acad Sci USA* 1993;90:6591–6595.
12. Ferrante RJ, Andreassen OA, Jenkins BG, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 2000;20:4389–4397.
13. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 1994;264:1772–1775.
14. Gurney ME, Cutting FB, Zhai P, et al. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann Neurol* 1996;39:147–157.
15. Gong YH, Parsadanian AS, Andreeva A, et al. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J Neurosci* 2000;20:660–665.
16. Lino MM, Schneider C, Caroni P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci* 2002;22:4825–4832.
17. Raoul C, Estevez AG, Nishimune H, et al. Motoneuron death triggered by a specific pathway downstream of Fas. Potentiation by ALS-linked SOD1 mutations. *Neuron* 2002;35:1067–1083.
18. Haenggeli C, Kato AC. Differential vulnerability of cranial motoneurons in mouse models with motor neuron degeneration. *Neurosci Lett* 2002;335:39–43.
19. Sagot Y, Vejsada R, Kato AC. Clinical and molecular aspects of motoneuron diseases: animal models, neurotrophic factors and Bcl-2 oncoprotein. *Trends Pharmacol Sci* 1997;18:330–337.
20. Mockett RJ, Radyuk SN, Benes JJ, et al. Phenotypic effects of familial amyotrophic lateral sclerosis mutant Sod alleles in transgenic *Drosophila*. *Proc Natl Acad Sci USA* 2003;100:301–306.
21. Kriz J, Gowing G, Julien J. An efficient three-drug cocktail for motor neuron disease induced by mutant superoxide dismutase. *Ann Neurol* 2003;53:429–436.
22. Chen M, Ona VO, Li M, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 2000;6:797–801.
23. Kriz J, Nguyen MD, Julien JP. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2002;10:268–278.
24. Van den BL, Tilkin P, Lemmens G, et al. Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport* 2002;13:1067–1070.
25. Heemskerk J, Tobin AJ, Ravina B. From chemical to drug: neurodegeneration drug screening and the ethics of clinical trials. *Nat Neurosci* 2002;5(suppl):1027–1029.
26. Zhang W, Narayanan M, Friedlander RM. Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. *Ann Neurol* 2003;53:267–270.

DOI: 10.1002/ana.10561