REVIEW

Amyotrophic lateral sclerosis and the clinical potential of dexpramipexole

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that leads to progressive weakness from loss of motor neurons and death on average in less than 3 years after symptom onset. No clear causes have been found and just one medication, riluzole, extends survival. Researchers have identified some of the cellular processes that occur after disease onset, including mitochondrial dysfunction, protein aggregation, oxidative stress, excitotoxicity, inflammation, and apoptosis. Mitochondrial disease may be a primary event in neurodegeneration or occur secondary to other cellular processes, and may itself contribute to oxidative stress, excitotoxicity, and apoptosis. Clinical trials currently aim to slow disease progression by testing drugs that impact one or more of these pathways. While every agent tested in the 18 years after the approval of riluzole has been ineffective, basic and clinical research methods in ALS have become dramatically more sophisticated. Dexpramipexole (RPPX), the R(+) enantiomer of pramiprexole, which is approved for symptomatic treatment of Parkinson disease, carries perhaps the currently largest body of pre- and early clinical data that support testing in ALS. The neuroprotective properties of RPPX in various models of neurodegeneration, including the ALS murine model, may be produced through protective actions on mitochondria. Early phase trials in human ALS suggest that the drug can be taken safely by patients in doses that provide neuroprotection in preclinical models. A Phase III trial to test the efficacy of RPPX in ALS is underway.

Keywords: dexpramipexole, amyotrophic lateral sclerosis, survival, clinical trials, neurodegeneration

Introduction

Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative disorder in developed countries,[1] with an estimated worldwide incidence of 1.2–4.0/100,000 people.[2–6] The illness is characterized by progressive degeneration of upper and lower motor neurons in bulbar as well as spinal myotomes. The result for patients is progressive weakness of voluntary muscles that can lead to complete paralysis. Cognition is impaired in 20%–50% of patients who undergo neuropsychological testing, and 5%–15% of ALS patients develop overt frontotemporal dementia.[7,8] Oculomotor, sphincter, and extrapyramidal function are spared in the majority of patients. Two major clinical forms, bulbar and spinal ALS, are described based on the site-of-onset of motor weakness. The two phenotypes are associated with a particular sex predominance, age-of-onset, and disease duration. Limb-onset disease, which makes up about 75% of ALS, predominates in men, while bulbar-onset cases are more frequent in women and the elderly, and have shorter survival.[9] Rarely, ALS begins in

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respiratory myotomes, which carries the poorest prognosis.[10] The mechanisms underlying the different sites-of-onset are just beginning to be explored.[11]

The disease is almost always fatal, with a median survival time of 30 months from onset and 19 months from diagnosis,[12] but some patients live just a few months while others survive for several decades. Death is usually, but not invariably, caused by respiratory failure.[13,14]

A principal difficulty in the diagnosis and management of ALS is the absence of biomarkers, which contributes to a diagnostic delay of 9–12 months and to initial false-negative diagnosis in up to 10% of cases.[15] Electromyography aids the diagnosis, and laboratory assessments as well as neuroimaging help exclude alternative diagnoses, including compressive cervical myelopathy or multifocal motor neuropathy, among others.[15,16] Ultimately, the diagnosis of ALS is based on the identification of progressive upper and lower motor neuron signs by history and examination. To aid in standardizing enrollment in clinical research studies, diagnostic criteria were developed in 1994, revised in 1998, and revised again in 2006 on Awaji-Shima Island.[17,18] Four stages of diagnostic certainty are defined as clinically definite or probable, laboratory-supported probable, and clinically possible based on the number of affected regions (bulbar, cervical, thoracic, and lumbosacral).[17] The consensus meeting in Awaji-Shima Island modified the electromyographic criteria, equating electromyographic abnormalities with clinical findings and restoring the diagnostic importance of fasciculation potentials to that of fibrillation potentials.[18] The modified criteria may improve diagnostic sensitivity in patients with bulbar-onset or upper motor neuron predominant disease.[19]

Pathogenesis

ALS is thought to be a complex genetic disorder in which genetic and environmental risk factors combine in the pathogenesis, but cigarette smoking is the only universally accepted environmental risk factor,[20] and no genes have been consistently identified that contribute to sporadic ALS. While 85%–90% of ALS is sporadic, the remaining familial cases are usually inherited in an autosomal dominant pattern.[21] During the past 20 years, numerous genes and loci have been linked to familial ALS (Table 1). The most frequently identified gene to date is the *c9orf72* gene, which is involved in approximately 40% of familial cases.[21] Mutations in the superoxide dismutase (*SOD1*) gene, the first identified in ALS, cause between 5% and 10% of familial ALS and were used in the advent of an important rodent model of the disease.[22] Increasing numbers of mutated genes are being

Table 1 Genes mutated in familial ALS

Locus	Gene	Inheritance	Phenotype
ALS1	SOD1	AD, AR	ALS, PMA
ALS2	ALS2	AR	Juvenile ALS, Juvenile PLS
ALS3	Not defined	AD	ALS
ALS4	SETX	AD	ALS, dHMN
ALS5	SPG11	AR	Juvenile ALS
ALS6	FUS	AD, AR, De Novo	ALS, ALS-FTD
ALS7	Not defined	AD	ALS
ALS8	VAPB	AD	ALS, PMA
ALS9	ANG	AD	ALS, ALS-FTD, PBP
ALS10	TARDBP	AD	ALS, ALS-FTD
ALS11	FIG4	AD	ALS, PLS
ALS12	OPTN	AD, AR	ALS
ALS13	ATXN2	AD	ALS
	C9orf72	AD	ALS, FTD, ALS-FTD

Abbreviations: ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; FTD, frontotemporal dementia; dHMN, distal hereditary motor neuropathy; PBP, progressive bulbar palsy; AD, autosomal dominant; AR, autosomal recessive.

described in ALS, but the mechanisms underlying their contribution to the disorder await elucidation.[21]

Pathophysiology

While the causes for most ALS are still mysterious, more is known from human and animal studies about the cellular events that transpire after disease onset.[23,24] Excitotoxicity, protein aggregation, oxidative stress due to free radical production, abnormal axonal transport, poor RNA function, mishandling of glutamate by glial cells, mitochondrial dysfunction, inflammation and apoptosis all appear to play a role in cell death in ALS.[24] The timing and sequence of the different mechanisms have proved more difficult to explore.[25] A central theme in many of the pathways is the role of mitochondrial impairment.[26]

Mitochondria are the major source of cellular energy. They are enveloped by two membranes, separated by an intermembrane space. The mitochondrial respiratory chain is located in the inner membrane and consists of four complexes that manage the reduction-oxidation reactions that transfer electrons from one complex to another. Complex IV, cytochrome c oxidase, transfers electrons to molecular oxygen, creating water, and in the process helps to generate the transmembrane electrochemical gradient that drives adenosine triphosphate (ATP) production. Other roles have recently been attributed to mitochondria such as buffering intracellular calcium and triggering apoptotosis.[27]

Because mitochondria play a critical role in all energetic processes in motor neurons, which are the largest nerve cells and whose metabolism requires the greatest energetic supply,

mitochondrial integrity appears crucial for motor neuron viability. Mitochondrial dysfunction could be a primary or secondary event in motor neuron cell death. Evidence for mitochondrial impairment in ALS first came over 40 years ago from microscopic studies that revealed abnormal mitochondrial structural morphology.[28] Later, alterations of the mitochondrial machinery were shown through research that described an increase in mitochondrial activity in the frontal cortex of ALS patients carrying the *SOD1* mutation.[29] Numerous disturbances of mitochondrial function have subsequently been implicated in the pathophysiology of ALS, from oxidative stress to glutamate-mediated excitotoxicity, promoting an increase of intracellular calcium and reduced calcium-buffering capacity.[30] All of these events, including excitotoxicity, increased oxidative stress and activation of proapoptotic enzymes, could, in theory, stem from a single mitochondrial event, the formation of the mitochondrial permeability transition pore, which depletes the mitochondrial membrane potential, reducing generation of ATP. Mitochondrial impairment, excitotoxicity, and oxidative stress are linked because excess glutamate, increased intracellular calcium and failure to reduce free radical production are interrelated.[31] Mitochondrial dysfunction and poor energy production are thought to eventually lead to motor neuron death through apoptosis.[27,32]

Management

There is no cure yet for ALS, in no small part because the causes are elusive. Riluzole, the only drug approved so far for the treatment of ALS, extends survival without effect on motor function.[33,34] The gain in survival, statistically significant in repeated studies,[35] is approximately 11% or 3 months.[36] The precise action of riluzole, a benzothiazole derivative, is unknown.[25] The drug is a low-potency and non-specific modulator of many pharmaceutical targets, including the inhibition of presynaptic glutamate release, originally thought to be the mechanism of action in ALS. However, other functions have been identified, including inactivation of voltage-dependent ion channels and prevention of protein aggregation,[25,37] and other drugs with antiglutamate properties are ineffective in ALS. Currently, the final common pathway is thought to rely on modulation of motor neuron excitotoxicity.[37,38] Blockade of voltage-dependent ion channels could represent a major mode of this action since riluzole improves survival without an effect on muscular function and increases feelings of asthenia (a frequent side effect).[38]

Until there is a curative treatment, most large centers manage symptoms to the extent possible using multidisciplinary

clinics, which contribute to longer survival and better quality of life for patients.[15,39] Evaluations of nutritional and respiratory status are key components of the multidisciplinary approach to care. Non-invasive ventilation and supplemental feedings may improve quality of life and survival if introduced early enough in the disease course.[40–42]

Continuing research for new and stronger agents is also a major thrust of ALS centers. More effective symptomatic and life-extending treatments of all types are badly needed and participation in research offers hope to patients.[43] When really effective treatments are found, the successes will stand on the shoulders of all the research, including the participants, that preceded it.

Numerous clinical trials testing agents that target known mechanisms in the pathophysiology of motor neurons have been conducted during the last 18 years (Table 2). The benefit of riluzole led investigators to examine other agents with antiglutamatergic properties, but several trials showed no benefit,[44,45] and trials of protein-clearing agents,[46–49] anti-inflammatory agents,[50,51] antiapoptotic agents[52] and immune modulators[53] were also negative. Use of different types and different approaches to administration of stem cells are being tried, but no efficacy has been demonstrated to date.[35]

Several problems currently face ALS researchers. First, the etiologies of ALS are still unknown, so it is not yet possible to target primary disease mechanisms. The rodent model, developed using an overexpression of the human mutated *SOD-1* gene, has become a major source of drug screening, but many of the negative trials in humans have followed positive studies in the ALS model. These discordant findings have raised questions about the utility of the rodent models and support the need for the development of other models based on different genes. Consensus criteria are now published on the use of the *SOD-1* model to standardize drug testing in rodents,[54] and additional models using *TARDBP* or *FUS* mutated genes are under development. In addition, the absence of biomarkers means that therapeutic trials rely on clinical endpoints, which have high inter-individual variability, rendering trials long, large and expensive in order to detect meaningful changes in outcome measures. Finally, the disease is rare and rapidly progressive so that entering and maintaining patients in large trials has been problematic. Trial methodology is being refined,[47,55–57] but truly meaningful treatment in ALS remains a matter of continued research for more effective molecules.

Dexpramipexole

Considering the potential central role of mitochondrial impairment in motor neuron death, neuroprotection through

Table 2 Recent clinical trials in ALS

Drug	Trial design	Mechanism	Sample	Endpoint	Outcome
Glatiramer	Phase III	Immune modulator	360	ALSFRS-R	Negative
Lithium	Phase II	Antiglutamatergic, Protein clearance	Up to 171	Survival/ALSFRS-R	Negative
Ceftriaxone	Phase II–III	Antiglutamatergic	600	Survival/ALSFRS-R	Ongoing
Memantine	Phase II	Antiglutamatergic	63	ALSFRS-R	Negative
Arimoclomol	Phase II	Heat shock protein inducer	84	Safety	Adequate safety
Talampanel	Phase II	Antiglutamatergic	59	Arm strength	Nonsignificant improvement
CoQ10	Phase II dose selection/futility	Antioxidant/mitochondrial cofactor	185	ALSFRS-R	Negative
Minocycline	Phase III	Anti-inflammatory/antiapoptotic	412	ALSFRS-R	Negative
Xaliproden	Phase III	Antiapoptotic	Up to 1210	Survival Breathing capacity	Negative
Gabapentin	Phase III	Antiglutamatergic	204	Arm strength	Negative
Celecoxib	Phase III	Anti-inflammatory	300	Arm strength	Negative
Riluzole	Phase III	Antiglutamatergic, unknown	Up to 959	Survival	Positive

Abbreviation: ALSFRS-R, revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale.

maintenance of normally functioning mitochondria is one promising therapeutic avenue in ALS.

Perhaps the best researched agent currently under study is the mitochondrial protector pramipexole (PPX).[58] The S(−) enantiomer of PPX, a nonergot dopamine analog that possesses D2, D3, and D4 autoreceptor agonist activity, is approved for the treatment of Parkinson disease and restless legs syndrome.[59,60] PPX, like riluzole, belongs to the benzothiazole family that has neuroprotective properties in models of acute and chronic neurodegeneration.[61] The neuroprotective properties of PPX have been at the core of numerous recent studies, primarily in models of Parkinson disease, where PPX appears to exert its neuroprotective effects through actions on mitochondria[62,63] and reducing activation of proapoptotic pathways.[62–64] In rats treated with 3-AP, a nicotinamide antagonist, PPX exhibits stronger effects than riluzole against neurodegeneration.[62] Glutamate-induced dopaminergic neuronal death is blocked by adding PPX to the culture medium, and continuous subcutaneous injection of PPX in rats inhibits the formation of ubiquitinated inclusions in dopaminergic neurons subjected to pro-inflammatory molecules.[65,66] PPX protects dopamine neurons exposed to hypoxic damage,[67] methamphetamine poisoning, 1-methyl-4- phenyl-1,2,3,6-tetrahydro pyridine toxicity,[68] and oxidative stress.[68,69] PPX scavenges free radicals in in vivo and in vitro models.[63] In humans, 12 ALS patients showed reduced measures of free radical production after receiving PPX in 6 mg/day doses.[70]

Dexpramipexole (RPPX; KNS-760704 [Knopp Neurosciences, Pittsburgh, PA]; (6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine), the R(+) enantiomer of PPX, was chosen for study in ALS because of greater tolerability at high doses than PPX.[32,71] The in-vivo neuroprotective effects of PPX require higher doses than can be tolerated in humans because of its high affinity for dopamine receptors, leading to dose-limiting side effects such as hypotension and hallucinations; side effects that have prevented the use of PPX as a neuroprotective agent in humans.

RPPX has a melting point between 285°C and 287°C, and is highly water soluble ($>$600 mg/mL).[71] It is stable in solution in water and physiological buffer solutions, and is not hygroscopic. RPPX is moderately bound to human plasma proteins (40.3%). Entry into the CNS is efficient with a brain-to-plasma ratio from 5 to 15, depending on species and dose. The product is rapidly and essentially completely absorbed when dosed orally and its half-life ranges from 3–8 hours across species.

RPPX has similar neuroprotective properties to PPX, but lacks dopaminergic effects. When RPPX has more than 99% chiral and enantiomeric purity, it is essentially devoid of dopamine agonist side effects; the affinity of RPPX for D2, D3, and D4 receptors is less than 1000-fold lower than PPX. Thus, the major advantage of RPPX for neurodegenerative disorders is a lower affinity for dopaminergic receptors than PPX, allowing dose escalation into the ranges necessary for reaching a neuroprotective effect, while having greater tolerability. For this reason, RPPX was chosen instead of PPX as a potentially promising drug for trials in ALS.[32,71]

Like PPX, dexpramipexole reduces free radical production and neuronal cell death in models of oxidative stress,[72,73] and reduces apoptosis.[74–76] Dexpramipexole appears to exert neuroprotective effects by acting directly on mitochondria to stabilize ion conductances and maintain the gradients needed for ATP production.[77] In several cell culture

experiments, including neurons, dexpramipexole normalized the metabolism of injured cells and was protective against proteasome inhibition, apparently through maintenance of oxidative phosphorylation in the mitochondria.[77] In models of parkinsonism, cells incubated with RPPX showed reduced markers of oxidative stress and were less susceptible to excitotoxicity.[71] In transgenic ALS mice, 100 mg/day of RPPX extended survival by approximately 5.6% and slowed motor decline compared to controls ($P = 0.011$).[75]

Dexpramipexole in ALS

Acute and chronic toxicology studies performed in animals were the preliminary steps in testing RPPX for human ALS.[71] The no-observed adverse-effect-level (NOAEL) in rats of 100 mg/kg for males and ≥300 mg/kg in females after 6 months of dosing provided approximately 7 and 25 times the highest dose planned in clinical studies (300 mg), respectively. In minipigs, the NOAEL of 50 mg/kg after 9 months of dosing provided 10 to 15 times the 300 mg human dose.[71,74,76]

The first clinical trials (Table 3) showed that RPPX was initially safe and well tolerated in humans.[38] Two Phase I randomized, placebo-controlled, double blind safety trials were conducted in 54 healthy subjects.[38] The safety and pharmacokinetic profile of PPX was evaluated in two trials that assessed a single dose of up to 300 mg or multiple doses twice daily over 4.5 days. The trials were conducted sequentially. In the first study, subjects were enrolled to receive 50 mg, 150 mg, 300 mg, or placebo. In the second study, patients received twice-daily doses of up to 300 mg/day or placebo. In both trials, pharmacokinetic profiles showed rapid absorption, with linear pharmacokinetics, and serum

half-life of 6.4–8.1 hours. Almost all the drug was renally eliminated and food did not affect the absorption or elimination. No serious adverse events occurred in either study. Dizziness and headache were the most frequently reported adverse events overall. There were no dose effects on vital signs, electrocardiogram, or laboratory data. Together, these studies showed that much higher dosages of RPPX could be tolerated by people than is the case for PPX.[38]

A series of Phase II trials were designed for the next step of testing safety and obtaining early indications of possible efficacy in ALS patients. First, a 9-month 'futility' trial in 30 ALS patients treated with 30 mg/day for 6 months following a 3-month lead-in phase compared pre- and posttreatment rates of progression.[78] The drug was reported as safe and well tolerated. The trial had power to detect a 40% difference in decline; there was a nonsignificant 13% reduction in the decline in slope of the ALSFRS-R scale. Patients who completed the entire 9 month trial had a reduction in slope that reached 16% (also nonsignificant).[78]

A dose escalation study was then performed in a sample of 10 patients with definite or probable ALS.[78] The initial dosage was 30 mg/day with a weekly twofold increase until reaching 300 mg/day for 2 weeks. The dose escalation was safe and well tolerated without any dopaminergic side effects. This trial was extended to a comparison of two dosages: 30 mg/day vs 60 mg/day over 6 months, with continued report of good safety. The slope of progression of the ALSFRS-R was nonsignificantly lower in the 60 mg/day group compared to the 30 mg/day group.[78]

Overall, these open-label trials enrolled a total sample of approximately 40 patients who received single and multiple doses of RPPX. Safety analyses, the most useful data from

Table 3 Trials of dexpramipexole in ALS

Design	Sample size	Primary endpoints	Secondary endpoints	Outcome
Phase I randomized controlled trials (single dose, then multidose)	54	Safety and pharmacokinetics		Safe, well-tolerated, linear PK
Open-label trial of 30 mg/day for 6 months	30	Safety	Change in slope of ALSFRS-R pre to post-treatment	Safe and well tolerated at this dose; no significant change in ALSFRS-R
Open-label dose escalation trial up to 300 mg/day	10	Safety		Safe and well tolerated at high doses
Open-label extension of 30 mg vs 60 mg/day	10 (?)	Safety	Difference in decline of ALSFRS-R	Safe and well tolerated; no significant difference in slopes of ALSFRS-R
Phase II randomized controlled trial of three doses for 12 weeks	102	Safety and pharmacokinetics		Safe and well tolerated; linear pharmacokinetics
Phase II trial of 50 or 300 mg/day	92	Safety	ALSFRS-R and survival	Safe; improvement in a joint ALSFRS-R-mortality outcome
Phase III trial of 300 mg/day	800	ALSFRS-R – Survival joint endpoint	Other functional measures and safety	Ongoing

Abbreviation: ALSFRS-R, revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale.

small open-label trials, in which placebo effect and other confounders cannot be controlled for and which has low power to detect efficacy, showed that the drug was safe and well tolerated in doses thought high enough to have neuro-protective effects in humans. The drug did not significantly modify the slope of ALSFRS-R nor the forced vital capacity, but there was inadequate power to detect changes in these secondary outcomes.[78]

A subsequent double-blind, placebo-controlled Phase II trial also assessed the safety and tolerability in ALS, with examination of early efficacy as measured by function and mortality.[79] The trial had two parts: the first randomized 102 patients to three treatment groups (RPPX 50 mg/day, RPPX 150 mg/day, and RPPX 300 mg/day, in two daily doses) and a placebo group. After a 12-week treatment period, 97 remaining subjects underwent a 4-week washout period before the second part of the trial in which 92 patients were randomized to two double-blind treated groups, 50 mg/day or 300 mg/day, for an additional 24 weeks. Among the 10 patients who dropped out between part 1 and part 2 of the trial, three died from ALS and the others discontinued treatment. Seventy-one patients completed both parts of the trial with an equal number in the treated groups of the second part. Overall RPPX was safe and well tolerated. Adverse effects were modest with only dizziness and reversible neutropenia occurring in the higher dosage sample, causing two patients to discontinue therapy. Pharmacokinetic analyses showed linear plasma pharmacokinetics and an elimination half-life of 6.7–8.2 hours. There were no dose-related changes in vital signs, electrocardiography, or laboratory data. There was no interaction between riluzole and RPPX no matter the dosage. The first part of the study showed a nonsignificant reduction of decline in the slope of the ALSFRS-R score that was more apparent at higher doses. Nonresponders to RPPX who were defined by a drop of 6 points or more of the ALSFRS-R score during the 12 weeks of the first phase were inversely proportional in number to the dosage of RPPX with a significant logistic regression between the four groups. The functional impact of RPPX was greater on the fine motor items of the ALSFRS-R scale. There was no effect on respiratory parameters. In part 2, there were 12 deaths (nine in the 50 mg group and three in the 300 mg group) and a total of 17 serious adverse events, most considered unrelated to the medication. The second part of the trial showed a nonsignificant reduction of the rate of the decline of the ALSFRS-R scores for the 300 mg/day group compared to the lower dose group ($P = 0.17$) and a reduction of 68% in the hazard of mortality in the higher dosage group

(Log-rank test, $P = 0.07$).[79] A joint-rank test, performed as a prespecified sensitivity analysis, showed a significant benefit in the combined outcome of change in the ALSFRS-R and mortality ($P = 0.046$).

Based on these results, a Phase III trial of RPPX in ALS is ongoing. The objective is to determine whether 150 mg twice daily of RPPX is effective in ALS compared to placebo. Up to 900 patients (with El Escorial possible, laboratory-supported probable, probable or definite ALS) have been enrolled in this randomized, double-blind, placebo-controlled, multicenter trial at sites in the United States, Canada, Australia, and Europe. The primary outcome measure is the effect on the joint mortality-function endpoint used in the Phase II trial. The study is funded by Biogen idec (Weston, MA) and results should be available in 2013.

Conclusions

Pramipexole and its R(+) enantiomer, dexpramipexole (RPPX), appear to exert meaningful neuroprotective properties through actions involving mitochondria. RPPX was selected for study in ALS because of greater tolerability at high doses than PPX due to lower affinity for dopamine receptors. Studies in models of neurodegeneration show that RPPX reduces oxidative stress, excitotoxicity, and apoptosis. Animal studies suggest an effect on survival in ALS, and early phase human trials have indicated adequate safety and tolerability at doses that provide neuroprotection in animals. It is too soon to reach conclusions about efficacy, but RPPX has undergone thorough groundwork investigation: the scientific justification for study in ALS has been established in preclinical studies, and dose selection has been accomplished in early phase human trials. The investigators can be confident that they are proceeding with a drug that is ready for efficacy testing. The Phase III trial was designed with good power to detect a realistic change in an interesting endpoint. This trial should determine clearly what the effect of RPPX is in ALS, and in the process will show whether meaningfully effective treatments can be identified in ALS before the causes of this still mysterious disease are known.

Disclosure

The authors report no conflicts of interest in this work.

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