

# Adjuvant Strategies for Managing ALS

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive loss of upper and lower motoneurons, typically culminating in death owing to respiratory failure after several years. Factors such as excitotoxicity, endoplasmic reticulum stress, oxidative stress, neuroinflammation, improper RNA editing, and cytoplasmic aggregation of RNA-binding proteins are suspected to play a pathogenic role in this syndrome. Nonetheless, the pathogenesis of the sporadic form of ALS – constituting about 80% of all ALS cases - remains murky, in large part because there is no adequate rodent model for this disorder. Considerable work has been done with transgenic mice expressing mutant forms of SOD1 encountered in rare familial forms of ALS, but mutations of SOD1 are encountered in only about 2% of patients with ALS, and there is reasonable doubt that SOD1-linked ALS is substantially homologous to sporadic ALS in its pathogenesis.<sup>1</sup>

To date, the drug riluzole, which suppresses excitotoxicity by inhibiting the release or activity of glutamate, is the only clinically-proven treatment for ALS; sadly, its efficacy prolongs survival by no more than several months.<sup>2</sup> There is reason to hope that the heat shock protein-inducer drug arimoclolomol, currently in clinical evaluation, will prove useful, as there is considerable evidence that endoplasmic reticulum stress and the unfolded protein response play a key role in the pathogenesis of ALS;<sup>3</sup> moreover, this agent has life-prolonging activity in transgenic mouse models of familial ALS.<sup>4-6</sup>

There will however remain a need for additional strategies that can retard the progression of this devastating syndrome. An overview of current evidence suggests that several other low-risk approaches may have merit as adjuvant measures:

**Inosine and Other Antioxidants** – Several recent epidemiological studies indicate that serum urate levels of men with ALS tend to be lower than those of matched controls.<sup>7-10</sup> Moreover, a recent longitudinal study concludes that, in male ALS patients, their rate of downhill progression tends to correlate inversely with their urate levels.<sup>9</sup> (Prospective studies examining the impact of urate levels on *risk* for ALS have not yet been done.) Notably, increased urate levels have been linked to decreased risk and/or improved prognosis in Parkinson's disease, Huntington's disease, and multiple sclerosis, as well as to a slower rate of cognitive decline in minimal cognitive impairment.<sup>11-15</sup> It is suspected that these findings reflect the fact that urate is a potent physiological scavenger of peroxynitrite-derived radicals, which play a pathogenic role in various neurodegenerative disorders.<sup>16</sup> The recent findings on ALS and urate may therefore be viewed as suggestive evidence that peroxynitrite likewise plays a pathogenic role in ALS – consistent with histological studies demonstrating 3-nitrotyrosine immunoreactivity in motor neurons of patients with ALS.<sup>17, 18</sup> If so, supplemental inosine, a clinically documented strategy for boosting blood urate levels, may be of some benefit in this disorder, as suggested by Keizman and colleagues.<sup>7</sup> Pilot trials with supplemental inosine in multiple sclerosis have been reported; doses of 1-3 g inosine daily, carefully titrated to insure that serum urate remains at a high but safe level (6-9 mg/dl), appear to be well tolerated, save for increased risk for urate renal stones.<sup>19</sup> This latter risk presumably could be minimized by keeping urine alkaline, as acidity reduces urate solubility; hence, inosine could be ingested in conjunction with ample doses of sodium bicarbonate.

Although inosine has not been tested in transgenic mouse models of ALS – urate has a pro-oxidative toxic effect in rodents not seen in humans<sup>20, 21</sup> – a copper-based antioxidant drug capable of suppressing tyrosine nitration has been shown to delay onset of paralysis and prolong survival in low-expression SOD1G93A mice; this agent also lessens cytoplasmic accumulation of truncated TDP-43 in spinal cord motoneurons – a characteristic feature of sporadic ALS.<sup>22</sup> Intriguingly, high-dose folic acid – which has peroxynitrite scavenging activity in cells which accumulate it<sup>23-25</sup> – is reported to have a similar, if less substantial, protective effect on these mice.<sup>26</sup> However, it is unclear whether high-dose folate can notably increase folate concentrations in spinal neurons, and it seems unlikely to do so in brain parenchyma owing to the blood-brain barrier.<sup>25</sup>

If peroxynitrite does indeed contribute to the pathogenesis of ALS, measures which blunt peroxynitrite generation by suppressing superoxide production presumably also would be of some value. Studies with transgenic mutant SOD1 mice suggest that NADPH oxidase activation may accelerate motoneuron death in ALS; concurrent knockout of Nox2 is associated with increased survival in these mice - albeit treatment with apocynin has yielded inconsistent results.<sup>27-30</sup> There is recent evidence that phycocyanobilin (PhyCB), a chromophore richly supplied by spirulina and other cyanobacteria, can mimic the physiological activity of bilirubin as an inhibitor of certain NADPH oxidase complexes.<sup>31, 32</sup> Moreover, rodent studies imply that orally administered PhyCB can pass through the blood-brain barrier to exert anti-inflammatory effects.<sup>33-35</sup> Hence, ingestion of adequate amounts of spirulina may have the potential to inhibit peroxynitrite production in ALS.

To the extent that damaged mitochondria may contribute to superoxide production in ALS, the carotenoid astaxanthin may be of some value, as it provides efficient antioxidant protection to the mitochondrial inner membrane, helping to prevent up-regulation of mitochondrial superoxide generation.<sup>36-39</sup> Since glutathione acts physiologically to prevent peroxynitrite-mediated nitration of tyrosine, agents such as N-acetylcysteine or L-cystine, which promote tissue glutathione synthesis by supplying the rate-limiting substrate cysteine, may also be useful.<sup>40-42</sup> And phase 2 inductive agents, or the hormone melatonin, have potential for suppressing peroxynitrite production by boosting the expression of superoxide dismutase, while also up-regulating glutathione synthesis.<sup>43-46</sup> It may be noted that lipoic acid, EGCG (both phase 2 inducers), and N-acetylcysteine are reported to have modest efficacy in transgenic mouse models of SOD1-linked ALS.<sup>47-49</sup> Moreover, intrarectal melatonin (300 mg daily) has been found to suppress systemic markers of oxidative stress in patients with ALS, and to be well tolerated.<sup>50</sup>

**Tolovax** – Neuroinflammation mediated by glial cells and T cells in the motor cortex and ventral spinal column is suspected to play a cofactor role in clinical ALS and in SOD1-linked mouse models of this disorder.<sup>51</sup> (Activated glial cells could, for example, be a source of peroxynitrite.) Repeated intraperitoneal administration of heat shock protein 70 has been shown to prolong lifespan in G93A SOD1 transgenic mice.<sup>52, 53</sup> There is suggestive evidence that Treg cells which recognize epitopes derived from various heat shock proteins play a physiological role in controlling inflammation, likely because they exert an anti-inflammatory effect on macrophages and dendritic cells in inflamed tissues.<sup>54, 55</sup> Moreover, vaccination with various heat shock proteins has the potential to boost the levels and activity of these protective Treg cells. Al-Harbi and Haines have developed a simple strategy (“Tolovax”) entailing the repeated subcutaneous administration of autogenous or allogeneic monocytes which have been subjected to 30 minute heat shock (42 degrees C) *ex vivo* and then incubated at physiological temperature for a further 48 hours prior to injection; in effect, this vaccinates the patient with a gemisch

of heat shock proteins, rather than a single protein.<sup>56</sup> The Tolovax strategy has been tested in Kuwait on a pilot basis in patients with a wide range of autoimmune and inflammatory disorders, allegedly with very encouraging clinical results. Al-Harbi has suggested that this strategy be employed in ALS, and has already treated a handful of patients in this way.<sup>56</sup>

Recent reports indicate that Treg levels may be subnormal in ALS patients, and that Treg levels correlate inversely with survival.<sup>57-59</sup> If the Tregs which target hsp-derived epitopes are likewise deficient, this would argue for a trial of the Tolovax strategy. Moreover, there is reason to suspect that PhyCB may mimic the activity of biliverdin/bilirubin in promotion of Treg induction.<sup>60, 61</sup> Hence, PhyCB might have utility in ALS both for its antioxidant activity and an anti-inflammatory Treg-inducing effect.

**Caffeine** – Heavy regular consumption of coffee has been linked to decreased risk for Parkinson’s disease and Alzheimer’s, likely because caffeine acts to counter neuroinflammation and excitotoxicity through its inhibitory effect on A2A adenosine receptors.<sup>62-68</sup> The possible impact of coffee on risk for ALS has received little attention, but a recent case-control study has found that ALS patients were less likely to drink coffee than were several groups of matched controls.<sup>69</sup> No study to date has examined the impact of caffeine ingestion on ALS prognosis; nonetheless, it might be prudent to encourage coffee consumption in ALS patients who tolerate this beverage.

**Overview** – It should be acknowledged that there is no clear evidence that any of the strategies suggested above would strike to the core of ALS. Arguably, neuroinflammation may be a reaction to the primary motoneuron damage that further exacerbates and accelerates this damage, but is not crucial to the further progression of motoneuron loss. With respect to low urate levels observed in ALS patients, there so far are no prospective studies assessing whether urate can predict risk for ALS. It is notable, however, that the relatively low urate levels seen in most ALS patients do not appear to decline during progression of the disease; hence, it is reasonable to suspect that these low urate levels may have predated onset of the syndrome. If so, this may mean that peroxynitrite plays a role in triggering sporadic ALS, and that measures which suppress the production of peroxynitrite, or which alleviate its disruptive impact, may be useful for primary prevention of this disorder. Inosine supplementation may not usually be practical for primary prevention, since dose must be titrated to avoid hyperuricemia and urine alkalinization is desirable to minimize renal stone risk. But other antioxidant measures suggested here, as well as caffeine, are appropriate components of preventive health programs, and hence may have more practical potential for ALS prevention.

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