Pyrimethamine Significantly Lowers CSF/SOD1 in ALS Patients With *SOD1* Mutations

Running title: Pyrimethamine Lowers CSF SOD1 in FALS

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Abstract

Background: Cu/Zn superoxide dismutase (SOD1) reduction prolongs survival in SOD1-transgenic animal models. Pyrimethamine produces dose dependent SOD1 reduction in cell culture systems. A previous phase-1 trial showed pyrimethamine lowers SOD1 levels in leucocytes in patients with *SOD1* mutations. This study investigated whether pyrimethamine lowered SOD1 levels in the cerebrospinal fluid (CSF) in patients carrying *SOD1* mutations linked to ALS (fALS/SOD1).

Methods and Study Design: Multicenter (5 sites), open-label, 9-month duration, dose-ranging, to determine safety and efficacy of pyrimethamine to lower SOD1 levels in the CSF of FALS/SOD1. All participants underwent 3 lumbar punctures, blood draw, clinical assessment of strength, motor function, quality of life, and adverse effects assessments. SOD1 levels were measured in erythrocytes and CSF. Pyrimethamine was measured in plasma and CSF. Appel ALS, ALSFRS-R and single item McGill Quality of Life (SIS-MQoL) were measured at screening, visit 6 and 9.

Results: We enrolled 32 patients; 24 completed 6 visits (18 weeks) and 21 completed all study visits. A linear mixed effects model showed a significant reduction in CSF SOD1 at visit 6 ($p<0.001$) with a mean reduction of 13.5% (95% CI: 8.4, 18.5) and at visit 9 ($p<0.001$) with a mean reduction of 10.5% (95% CI: 5.2, 15.8).

Interpretation: Pyrimethamine is safe and well tolerated in ALS. Pyrimethamine is capable of producing a significant reduction in total CSF SOD1 protein content in patients with ALS caused by different *SOD1* mutations. Further long-term studies are warranted to assess clinical efficacy.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a relentlessly progressive neurodegenerative disease of upper and lower motor neurons causing progressive weakness of limbs, swallowing and breathing resulting in death within 3-5 years (1). The cause is uncertain in most patients but in approximately 10% of patients, the disease is familial (2). Since 1993, mutations in over 36 genes have been associated with causing ALS(3). Mutations in the cytoplasmic free radical scavenging enzyme Cu/Zn superoxide dismutase (SOD1) accounts for 3-23% of familial cases (fALS) and 1-3% of sporadic cases (2-4). Transgenic mice overexpressing mutant human SOD1 develop a progressive motor neuron degenerative disease mimicking human ALS, while knock out of the murine SOD1 gene does not result in a similar phenotype (5). These findings combined with the observation that there is no relationship between the level of SOD1 activity and patient prognosis suggest that there is a toxic gain of function for the SOD1 mutant molecule with a predilection for the motor system (3). Reducing the content of mutant SOD1 (mSOD1) attenuates disease progression proportionate to the suppression of mutant protein using interfering RNA (RNAi) (6). Collective evidence supports the hypothesis that lowering the total SOD1 protein content may be beneficial and influence the disease course in ALS. Attempts to lower SOD1 expression are currently being pursued using anti sense oligonucleotides (ASO) (7) and by increasing consumption of SOD1 by activating heat shock proteins (8) via the drug arimoclomol. Using FDA approved drugs that also have the ability to lower SOD1 content is another approach (9). Monitoring the CSF SOD1 protein level has been identified as a reliable biomarker for SOD1 reduction within the anterior horn cell in transgenic rats with *SOD1* mediated ALS(7). In humans with ALS and *SOD1* mutations, CSF SOD1 shows minimal variability and is a reliable biomarker for *SOD1* mediated fALS (10). We have previously reported that oral treatment with pyrimethamine in ALS patients with a mutation in *SOD1* resulted in a reduction of SOD1 levels in peripheral blood leucocytes and, in both patients studied, a reduction in SOD1 protein content and activity in the cerebrospinal fluid (CSF)(9). We now report a phase I/II study whose primary aim was to determine if pyrimethamine lowers SOD1 in the CSF in ALS patients with a wide variety of *SOD1* mutations and over a longer period of time and a secondary aim to establish safety and tolerability.

Methods

The Institutional Review Board at Weill Cornell Medicine approved this study, followed by approval by the relevant institutional or national Ethical Review Boards at the participating institutions in the USA, Italy, Germany and Sweden, following FDA and EMA regulations and adhering to the Principles of the Declaration of Helsinki (WMA, 1964). The study was registered at www.clinicaltrials.gov as NCT01083667. This was a single arm open-label study with the primary endpoint to determine if oral medication with pyrimethamine results in a reduction of CSF SOD1 levels in ALS patients with different types of *SOD1* mutations. Based on our earlier study (9) the target dose was set at 75 mg per day supplemented with 10 mg of leucovorin. However, different dosing was achieved due to reductions required to maintain tolerability. We enrolled 32 patients (Table 1).

Inclusion criteria were: the presence of objective weakness in at least one neural segment and a pathogenic mutation in *SOD1* (El Escorial Definite ALS revised (11)); age 18 years or older; capable of providing written informed consent and complying with trial procedures; not taking riluzole or on a stable dose for 30 days or more not taking coenzyme Q10 or on a stable dose and brand for 30 days or more. Subjects were excluded if there was a history of malabsorption syndrome; exposure to any other agents considered a therapeutic target for ALS within 30 days of entry into this study; women who are pregnant or planning on becoming pregnant; women who are breastfeeding; alcoholism; taking phenytoin or other medications that may interfere with folate levels, seizures, megaloblastic anemia, folate deficiency, cardiac rhythm disorders, impaired renal and liver function, tracheostomy or mechanical ventilation; use of any of the following medications: cytosine arabinoside; methotrexate; daunorubicin; sulfonamides; zidovudine; lorazepam; warfarin; sulfamethoxazole; trimethoprim; and lithium.

There were 10 visits: screen/baseline, weeks 3, 6, 9, 12, 15, 18, 24, 30 and 36. Visits at weeks 0, 18, and 36 were critical visits for data acquisition. At all visits, weight, vital signs and concomitant medication screen combined with adverse effect assessment occurred. Blood for SOD1 and pyrimethamine levels was obtained. At weeks 0, 18 and 36 a lumbar puncture was performed. At weeks 0, 6,18 and 36 we measured the ALS Functional Rating Scale, revised (ALSFRSr), Appel ALS Score (AALS), and the single item McGill quality of life score (MQOL-SIS). The ALSFRSr score is a questionnaire based assessment of motor function that has been validated in natural history studies of ALS and therapeutic trials (12). The AALS score is an objective measure of global motor function that has been validated in natural history studies and therapeutic trials (13). The MQOL-SIS is a single question in which the patient rates their overall quality of life on a scale from 1-10 (10 being the best possible and 0 being the worst possible) for the past 48 hours. MQOL-SIS has good correlation with ALS QOL (14).

Summary of Dose Escalation and Algorithm for Reduction: Pyrimethamine was supplied in 25 mg tablets (Core Pharma, LLC). The target dose was 75 mg based on our experience in the first study of pyrimethamine (9) where 100 mg was poorly tolerated but 75 mg was deemed to be a dose that most patients could tolerate over an extended period of time. The escalation of dose was as follows: at baseline, patients started taking a 25 mg tablet daily together with 5 mg leucovorin twice daily. The leucovorin remained at the same dose for the duration of the study. Pyrimethamine dose increased to 37.5 mg at 3 weeks; 50 mg 6 weeks, 62.5 mg 9 weeks and 75 mg at 12 weeks. Patients remained at 75 mg for the duration of the study (36 weeks) if tolerated.

Adverse effects were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A defined algorithm existed to limit dose escalation or dose reduction. Escalation was stopped if any of the following symptoms achieved a grade 2 or above level: nausea, diarrhea, vomiting, glossitis, heart rate change (>130 bpm or > 50% over baseline on two separate measurements).

Reduction of dose occurred as follows: 75 mg to 50 mg; 50 to 37.5; 37.5 to 25. Reduction was mandated if either one grade 3 or two or more grade 2 of the adverse effects were identified, including: persistent nausea, persistent diarrhea, persistent vomiting, persistent heart rate change, and persistent anemia (> 1 study visit). Once dose reduction occurred or escalation was stopped, there were no further attempts to change the dose of medication until the end of the study.

Collection of blood
Venous blood samples were collected in vacuum tubes containing EDTA as anticoagulant. They were centrifuged at 1500xg for 15 min and were separated into plasma, buffy coat and erythrocytes. The samples were stored at -80° C until analysis.

Collection of CSF
Lumbar puncture to obtain cerebrospinal fluid (CSF) was performed in all patients at baseline, visit 6 (18 weeks) and visit 9 (36 weeks). The samples were stored at -80° C until analysis. Specimens from tube #3 were sent for SOD1 analysis.

SOD1 enzymatic activity in erythrocytes
The SOD1 enzymatic activity was analysed in erythrocytes by the direct spectrophotometric method using potassium superoxide as previously described (15, 16). The activity was related to the content of hemoglobin in the erythrocyte lysates. There is a strong correlation between amounts of SOD1 protein measured by ELISA and the SOD1 enzymatic activity (16).

SOD1 protein in CSF
For analysis of SOD1 protein, an ELISA based on rabbit and goat anti-native human SOD1 antibodies was used (16). The rabbit antibody was used as a primary antibody and the goat antibody as the secondary antibody. The ELISA was standardized with a human hemolysate, with the SOD1 content calibrated against pure human SOD1, the concentration of which was determined by quantitative amino acid analysis (17).

Measurement of pyrimethamine in plasma and CSF
A liquid chromatography/mass spectroscopy method was used for analysis of pyrimethamine in both plasma and CSF. Protein precipitation method was used for sample extraction with a sample size of 50μL (Primera Analytical Solutions Corp., Princeton, NJ).

Statistical Methods

Based on previous data, the mean ± SD baseline SOD1 content was estimated to be 112 ± 30 ng/cc (9). It was determined that enrollment of 34 patients with ALS and mutations in *SOD1* would provide 80% power at a two-sided alpha level of 0.025 to detect a 15% reduction in SOD1 content from baseline at 18 weeks and/or 36 weeks post study commencement. A total of 40 patients were to be enrolled to account for potential drop

outs. However, the trial was terminated after the enrollment of 32 patients due to a sudden and exorbitant increase in the cost of pyrimethamine(18).

Continuous variables are presented as means with standard deviations or medians with 1st and 3rd quartiles. Kaplan-Meier analysis was used to estimate the median, 1st quartile, and 3rd quartile duration of disease. Categorical variables are presented as counts and percentages. Continuous baseline variables were compared between patients with complete vs. incomplete followup data using two-sample *t*-tests or Wilcoxon rank-sum tests, depending upon the distribution of the data. Categorical baseline variables were compared between patients with complete vs. incomplete followup data using χ^2 or Fisher's exact tests, as appropriate. The change in continuous outcomes from baseline across repeated measurements was modeled using linear mixed effects modeling with a Gaussian random intercept, producing equivalent point estimates to a generalized estimating equations approach with a compound symmetric correlation structure. Given the limited sample size, each model contained timepoint as the sole predictor (treated as a fixed effect). A compound symmetric correlation structure was employed because the models would not converge with more complex correlation structures. As a sensitivity analysis, the mean change in continuous outcomes over time was calculated after multiple imputation of missing values. Specifically, age, sex, and baseline ALSFRSr, AALS, and MQOL-SIS scores were used to to create 10 imputed datasets. Results were combined to obtain pooled mixed effects model estimates of the change in continuous outcomes from baseline to 6, 18, and 36 weeks. All statistical hypothesis tests were two-sided, with $P < 0.05$ considered statistically significant. Statistical analyses were performed with SAS Version 9.3 (SAS Institute, Cary, NC) and the ICE package implemented in STATA version 14 (Stata Corporation, College Station, TX).

Results

After signing informed consent, 32 patients were screened, 16 men and 16 women whose symptoms started at a mean (range) age of 47.0 years (19-67). All patients had confirmed mutations in *SOD1*, 21 were unique and 3 were novel (H80Y, V119F, E133K; Table 1). At baseline, the mean AALS score was 65.6 (34-115). The mean ALSFRSr score was 40.1 (27-47). 22 patients were on a stable dose of riluzole at entry and continued throughout the study; 8 were riluzole free.

Of the 32 patients enrolled, 8 dropped out before the scheduled 2nd lumbar puncture; 6 due to disease progression; 2 due to inability to tolerate medication. Therefore, 24 samples were available to analyze at week 18 (Fig 1). In the final 18 weeks of the study, two additional patients dropped out (leaving 22 potential CSF samples for analysis). However, one patient declined the last LP and AALS score, leaving 21 patients with 3 longitudinal CSF, blood and clinical assessments.

The results from all measures obtained during the study, expressed as change from baseline, are presented in Table 2. The primary aim of the study was to determine if patients with ALS associated with an *SOD1* mutation taking pyrimethamine showed a

reduction in the level of CSF SOD1. The mean reduction of CSF SOD1 in all patients at 18 and 36 weeks was 13.5% (raw mean reduction [95% CI]: 8.8ng/ml [5.5, 12.1], $p<0.001$) and 10.5% (6.8ng/ml [3.4, 10.3], $p<0.001$) (Table 2). Achieving a significant reduction in CSF SOD1, an accepted biomarker for SOD1 associated fALS over the 9-month study period fulfilled the primary aim of this protocol. Peripheral blood SOD1 did not change during the study. Pyrimethamine levels in the CSF approximated 10% that seen in the plasma. During the final 18 weeks of the study, the mean dose of pyrimethamine declined as did CSF SOD1 levels suggesting a dose response effect (Fig 2).

Of the 32 patients enrolled 24 completed 18 weeks of treatment with two lumbar punctures. The change in CSF SOD1 from baseline in each patient per mutation, undergoing two lumbar punctures 4.5 months apart (18 weeks) is shown in Figure 3. There is substantial variation between patients and between patients with the same mutation. Nine patients (39%) showed a reduction of 20% or more from baseline.

Of the 22 patients completing the study (36 weeks of treatment) 13 were able to continue at the protocol-directed 75 mg. One patient refused the last LP leaving 12 patients with CSF to analyze. In 9 (75%) SOD1 reduction exceeded 14% (14.3 to 24.3%). In one, a woman heterozygous for A4V, the visit 9 (36 week) SOD1 level increased by more than 20% from baseline and more than 40% from the SOD1 measurement at week 18. Excluding that patient, protocol compliant patients showed a mean decrease of 15.6% at 36 weeks.

CSF SOD1 normal variation is minimal. One study showed that CSF SOD1 varies by ±7.1% on repeated analysis (10). Moreover, it appears that CSF SOD1 content does not decrease over time in ALS patients not treated with pyrimethamine. For comparison, we analyzed CSF SOD1 in 12 untreated ALS patients without *SOD1* mutations that had lumbar punctures on 2 occasions. The SOD1content increased by an average of 2.8% over a mean interval of 13.8 months (from 98.3 ± 33.5 to 102.9 ± 35.7 ng/ml).

There were 11 dropouts (34%). We compared baseline characteristics between patients who did and did not complete the study (Table 3A) and performed multiple imputation analyses on all measures (Table 3B). Point estimates from multiple imputation and available case analyses were fairly similar for most outcomes and time points. However, multiple imputation analysis did show a less pronounced decrease in CSF SOD1 levels between baseline and 36 weeks compared to complete case analysis (9.0% vs. 10.5%, respectively) and a greater increase in AALS score over the same time period (29 vs. 18, respectively). Comparing baseline features between patients that completed relative to those that dropped out showed those that did not complete the 36 week protocol were often women with greater burden of disease (admission AALS/ALSFRSr in fully protocol compliant 53/39 vs 76/42 in dropouts). There is no change in self perceived quality of life between baseline and completion.

Does pyrimethamine slow progression of FALS?

Although the primary aim of the study was to determine if pyrimethamine significantly lowers CSF SOD1 levels, we found patients on pyrimethamine may have progressed slower than expected based on data from historical studies of mutant *SOD1* patients and placebo groups from other therapeutic trials. The mean change from baseline showed a significant progression using both AALS and ALSFRSr measures at 18 and 36 weeks (Table 2), but the rate of change was 2ppm and -0.7ppm respectively for all patients. Of note, disease progression in patients with A4V/A4T mutations needs to be considered separately because of marked differences in progression and survival (19). In one study, survival in patients with non-A4V mutations showed a mean disease duration to be 6.6 +/- 7 years compared to 1.4+/-0.7 years in A4V and 0.8 +/- 0.1 months in A4T patients(19).

Of our non-A4V patients, mean progression was 0.52 ppm on the ALSFRSr scale and 0.92 ppm on the AALS score (Fig 4 A,B). In comparison placebo and historical controls from non FALS patients show average ALSFRSr/AALS change to be 1.18 +/- 0.85 and 4.70 ppm respectively (20, 21)

We had 4 patients that showed no change in ALSFRSr or AALS over the 9-month period (i.e. non-progressors) The mutations were H46R, I113T, L144F, A95T. All of these mutations are known to produce a progressive motor neuron disorder with either a protracted or a variable time course (22, 23).

Progression is more uniform in rapidly progressive mutations, such as A4V and A4T. Our population included 3 patients with A4V (2; numbers 2,3 Table 1) and A4T (1; Number 1, Table 1) who were fully compliant with the protocol and completed the entire 36-week treatment period with ALSFRSr, AALS scores and CSF SOD1 values (three other A4V patients dropped out because of disease progression). For the first 18 weeks, the rate of change of AALS/ALSFRSr was 2.5/1.0 ppm for the patients with A4V and 4/2ppm for the patient with A4T; the last 4 months showed an increase to an average of 12/4.35ppm for A4V and 6.25/1.5 ppm for A4T (Fig 5 A,B). The median [Q1, Q3] duration of disease for the 5 A4V patients was 20 [16, 22] months and the duration of disease for the A4T patient was 24 months.

There was a proportionate reduction in SOD1 levels in all 3 compliant A4V/T subjects during the first 18 weeks of the study (mean 16.6 ng/ml). One A4V patient showed a reduction in SOD1 content of 20% during the first 4.5 months. However, during the last 4.5 months of the study, SOD1 content increased 47%. This subject had the shortest disease duration of 19 months and a change in AALS rate of change from 4.0 ppm during the first half to 6.25 ppm during the last 18 weeks. She expired from disease related respiratory failure 2 months after completing the study. The 2nd A4V patient showed a slower but progressive decline in SOD1 content and he lived 22 months, i.e. almost double the mean survival time for patients with A4V (19). The patient with A4T, often more aggressive that A4V lived the longest at 24 months from symptom onset. Clearly no conclusions can be drawn from these three A4V/T patients but these observations seem to suggest a slowing of progression in this most aggressive of familial diseases.

Adverse Events

There were 30 unique symptoms classified as adverse effects, 77 in total. Among the most common, headache (n=13/32 patients; most associated with lumbar puncture), nausea (14/32), diarrhea (6/32), pain (5/32), falls, upper respiratory tract infections, shortness of breath/aspiration pneumonia and fatigue (4/32), weight loss (3/32), decreased appetite and rash (2/32), nightmares, lightheadedness, hunger, dysgeusia (1/32). Seven patients had grade 3 adverse effects encompassing headache (n=3), aspiration pneumonia, weight loss, shortness of breath, UTI. One patient had shortness of breath deemed to be grade 4. There was no increase in adverse events in the patients taking riluzole relative to those not taking that medication.

Quality of Life (MQOL)

The mean score from the MQOL SIS showed no significant change during the duration of the study (Table 3). However, when individual patients with more aggressive mutations, such as A4V and A4T were measured, there was a decline in MQOL-SIS.

Discussion

To our knowledge, this is the first study in humans with ALS that targeted and achieved a significant reduction of an ALS biomarker in the CSF: the content of SOD1 (10). The degree of reduction varied among patients, but, on average, the reduction of 13.5% relative to baseline was statistically significant with the greatest reduction noted being 46%. There was also a dose response observed with respect to mean dose of pyrimethamine relative to mean reduction of SOD1 CSF content.

In humans, it is unknown if lowering of CSF SOD1 attenuates disease progression and if it does, what the critical threshold is, if any. We originally targeted 15% based on our observations in our first study. However, subsequent studies showed that normal variation in CSF does not exceed 7.1% (10). Total CSF SOD1 content includes both wild type and mutant proteins. Owing to widely variable stabilities, the mutant SOD1s are degraded in the CNS at variable rates. Depending on the mutation, the mutant SOD1 in the CNS have, in earlier studies, been found to vary from nearly 100% (for stable mutants) to around 0.5% (highly unstable truncating mutations) of the amounts of wild type SOD1 in heterozygous mutant *SOD1* patients(24, 25). This is reflected in our highly variable amounts of SOD1 in CSF from our patient population, which overall are lower than those found in the time-course study in ALS patients without *SOD1* mutations. Therefore, a small reduction in total SOD1 may cause a large or minimal change in mutant SOD1. The technology to reliably measure the relative composition of wild type and mutant SOD1 in the CSF is not yet available.

Our findings suggest that there may be slowing of progression using historical controls. However, patients with fALS may have a more variable course than sporadic disease, sometimes unusually long. Therefore drawing any conclusion about pyrimethamine's impact on disease progression from these studies would be incorrect and misleading. Our findings should only serve as a basis for further studies sufficiently powered to determine

if pyrimethamine will change rate of progression.

The mechanism behind the lowering of the SOD1 content by pyrimethamine is not known. If the synthesis of the protein is reduced it is likely that the wild type and mutant SOD1s are affected to equal extents. However, our results show excellent CNS penetration. Pyrimethamine is one of the lipophilic anti-folates (26). More than 90% of pyrimethamine is (27) is bound to plasma proteins suggesting there is 10% free pyrimethamine in the plasma. This equals the 10% of plasma levels we found in the CSF. The proportion of pyrimethamine bound to protein in the CSF should be negligible given the very low CSF protein content.

A single study found that pyrimethamine has no impact on SOD1 levels when administered to cell cultures or homogenates of liver, spinal cord and brain of wild type mice (28). Both our previous study and the present study find that there are no significant changes in SOD1 content in erythrocytes. However, our previous work showed a reduction in leucocytes and now in CSF, a very different tissue from the ones tested by Wright et al. (28). Perhaps of importance, different methodologies to assay SOD1 were used by Wright et al. and us.

In our first study, pyrimethamine was poorly tolerated at higher doses, especially at the 100 mg dose causing multiple adjustments in dose (9). We therefore set 75 mg as the maximum dose and established strict criteria for dose reduction after which the tolerated dose would not be altered. This, allowed us to discern a possible dose impact on CSF SOD1 levels, which we did observe. Therefore, it would be important to try to achieve the target of 75 mg per day. When factors that impacted dose reduction were reviewed, the severity of illness at entry seemed to be the best predictor of successful escalation to and maintenance of the maximum dose. In future studies, a maximum level of disease severity prior to entry should be established

The McGill Quality of Life Single Item Scale (MQOL-SIS) is a validated scale found to be useful in patients with ALS (14, 29). Our results show that over a 9-month period, there is no difference in MQoL-SIS, and in fact, in some patients, there was a self-perceived improvement in QoL. Of import, there is no significant decrease in QOL over the 9 month study. However, in rapidly progressive forms (A4V/A4T), weakness and QoL declines as observed by others (30).

This is the largest prospective clinical and biological investigation of patients with FALS in the literature to date. There are, however, limitations to this study. We admitted all patients with varying levels of disease severity, and patients with higher disease burden were more likely to drop out. Our multiple imputation results suggest the possibility that results from the available case analysis were biased towards a greater average decrease in CSF SOD1 or lesser average worsening in disease than would have been observed had these sicker patients remained in the trial. Future power analyses should likely target levels exceeding 7% change in CSF content We are not certain that patients willing to undergo 3-4 lumbar punctures might in some way be different from those not willing to

have invasive procedures. We also did not study patients with sporadic ALS in whom misfolded wild type SOD1 may act like the mutant protein justifying use of potential SOD1 reducing therapies in this disease(31) (32) (33). Despite these limitations we showed for the first time in humans, a pharmacologic agent caused a significant reduction in CSF SOD1 in patients with ALS and *SOD1* mutations. Although no comment can be made with confidence regarding impact on disease progression, slowing relative to historical controls may be present and further study regarding impact on disease progression and its relation to SOD1 reduction is needed.

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Author Contributions

Study concept and design: DJL, MS, SHA, PMA, SM, VS, AL, SAD
Data acquisition and analysis: DJL, VS, AL, JW, SAD, CM, AD, LM, SM, UW.
Drafting of manuscript and tables: DJL, PMA, SM, VS, AL

Potential Conflicts of Interest

No authors have any real or perceived conflict of interest regarding the material presented in this study.

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Figure Legends

Figure 1: Baseline values of CSF SOD1 protein content according to mutation in each subject with two lumbar punctures.

Figure 2. Dose dependent change in CSF SOD1 content in patients with ALS at (A) visit 6 (18 weeks) and (B) visit 9 (36 weeks)

Figure 3. Percent change from baseline in CSF SOD1 content at 18 weeks of treatment according to mutation.

Figure 4. Rate of change expressed as points per month (ppm) in AALS score (A) and ALSFRSr (B) over 9 months (36 weeks) in non-A4V patients. Horizontal line represents change of controls in cited studies.

Figure 5. Rate of change expressed as points per month (ppm) in AALS score (A) and ALSFRSr (B) over 9 months (36 weeks) in 3 A4V /A4T patients.

Tables

Number	Mutation	Gender	Age Sx Onset	Site of Onset	AALS Entry
1	A4T	Male	50	LE	37
2	A4V	Male	51	LE	37
3	A4V	Female	36	LE	42
4	A4V	Female	49	LE	93
5	A4V	Male	40	LE,B	90
6	A4V	Female	49	LE,L	111
7	L8Q	Female	33	UE	82
8	H46R	Female	38	LE	37
9	H46R	Male	37	LE	72
10	H80Y#	Male	51	UE	109
11	L84F	Female	36	LE	100
12	L84F	Male	32	LE	46
13	N86K	male	53	UE	58
14	D90A (heterozygous)	Male	55	LE	53
15	D90A (heterozygous)	Male	48	UE	52
16	G93A	Female	37	LE	57
17	G93D	Female	19	LE,R	115
18	A95T	Female	45	LE,L	61
19	E100G	Male	57	LE	79
20	D109Y	Female	59	LE,L	45
21	I113T	Male	67	LE	50
22	I113T	Female	61	UE	63
23	L117V (homozygous)	Male	44	LE,L	91
24	R116G	male	54	LE	72
25	R116G	female	48	LE	76
26	V119F#	Male	53	BU	51
27	G127Gfs*7	Female	21	LE	36
28	G127Gfs*7	Female	48	UE	34
29	E133K#	male	62	LE	59
30	G148C	Female	61	LE	70
31	L144F	Male	58	LE,R	48
32	L144F	Female	53	LE,L	84

Table 1. List of mutations and clinical features; #Novel mutation. LE=lower extremities; UE = upper extremities

	n	Mean change from baseline (95% CI)	P value
SOD 1 CSF			
18 weeks	24	-8.8 (-12.1, -5.5); -13.5% (-18.5, -8.4)	< 0.001
36 weeks	21	-6.8 (-10.3, -3.4); -10.5% (-15.8, -5.2)	< 0.001
Pyrimethamine CSF			
18 weeks	24	273 (242, 304)	< 0.001
36 weeks	22	240 (208, 272)	< 0.001
SOD1 peripheral blood (PRBC)			
6 weeks	15	-0.3 (-2, 1.5)	0.773
18 weeks	14	0.6 (-1.2, 2.4)	0.501
36 weeks	14	0.3 (-1.4, 2.1)	0.707
Pyrimethamine blood (plasma)			
6 weeks	20	1935 (1534, 2336)	< 0.001
18 weeks	23	2851 (2466, 3237)	< 0.001
36 weeks	22	2435 (2045, 2825)	< 0.001
AALS			
6 weeks	24	2 (-3, 8)	0.410
18 weeks	23	8 (2, 14)	0.008
36 weeks	22	18 (12, 24)	< 0.001
ALSFRSr			
6 weeks	24	-2 (-4, 1)	0.192
18 weeks	23	-4 (-6, -1)	0.004
36 weeks	24	-7 (-9, -4)	< 0.001
MQOL-SIS			
6 weeks	23	0 (-1, 1)	0.782
18 weeks	19	-1 (-2, 0)	0.157
36 weeks	23	0 (-1, 0)	0.411

Table 2. Change from Baseline for all measures studied

	CSF SOD1 level at 36 weeks (n = 21)	No CSF SOD1 level at 36 weeks (n = 11)	P value
Age (years), mean ± SD	49.7 ± 13.0	52.7 ± 9.7	0.506
Female, n (%)	8 (38.1)	8 (72.7)	0.063
AALS, median [Q1, Q3]	53 [42, 72]	76 [61, 91]	0.066
ALSFRSr, median [Q1, Q3]	42 [38, 45]	39 [37, 43]	0.186
MQOL-SIS, median [Q1, Q3]	8 [8, 8]	7 [6, 8]	0.099

(A)

Table 3

	Mean change from baseline (95% CI)	P value
SOD 1 CSF		
18 weeks	-8.6 (-12.0, -5.3); -13.6% (-18.8, -8.3)	< 0.001
36 weeks	-5.7 (-9.8, -1.7); -9.0% (-15.4, -2.6)	0.007
Pyrimethamine CSF		
18 weeks	289 (244, 334)	< 0.001
36 weeks	258 (212, 305)	< 0.001
SOD1 peripheral blood (PRBC)		
6 weeks	-4.0 (-11.3, 3.2)	0.260
18 weeks	2.1 (-3.5, 7.7)	0.452
36 weeks	1.3 (-3.6, 6.3)	0.592
Pyrimethamine blood (plasma)		
6 weeks	1809 (1137, 2481)	< 0.001
18 weeks	2837 (2152, 3523)	< 0.001
36 weeks	2359 (1783, 2935)	< 0.001
AALS		
6 weeks	3 (-15, 21)	0.734
18 weeks	12 (-9, 33)	0.250
36 weeks	29 (-14, 72)	0.170
ALSFRSr		
6 weeks	-2 (-7, 4)	0.548
18 weeks	-5 (-13, 3)	0.188
36 weeks	-8 (-18, 3)	0.142
MQOL-SIS		
6 weeks	0 (-4, 3)	0.892
18 weeks	1 (-4, 6)	0.712
36 weeks	1 (-2, 5)	0.455

(B)

Table 3. (A) Baseline comparison between drop outs and completers. (B) Multiple imputation analysis

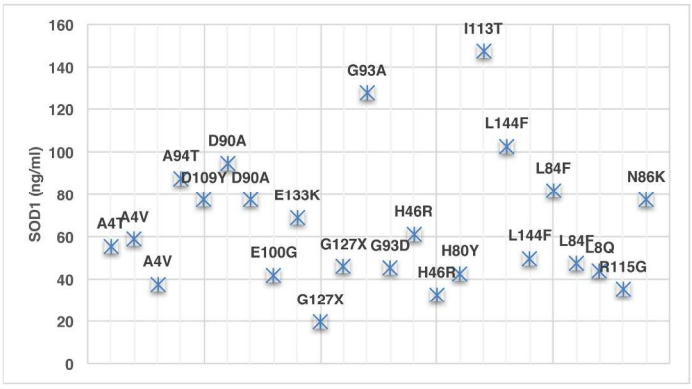

Figure 1: Baseline values of CSF SOD1 protein content according to mutation in each subject with two lumbar punctures.

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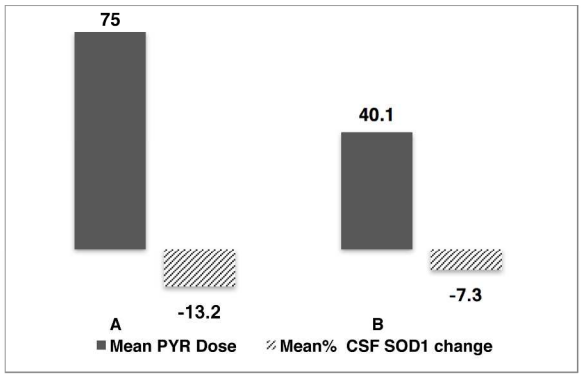

Figure 2. Dose dependent change in CSF SOD1 content in patients with ALS at (A) visit 6 (18 weeks) and (B) visit 9 (36 weeks)

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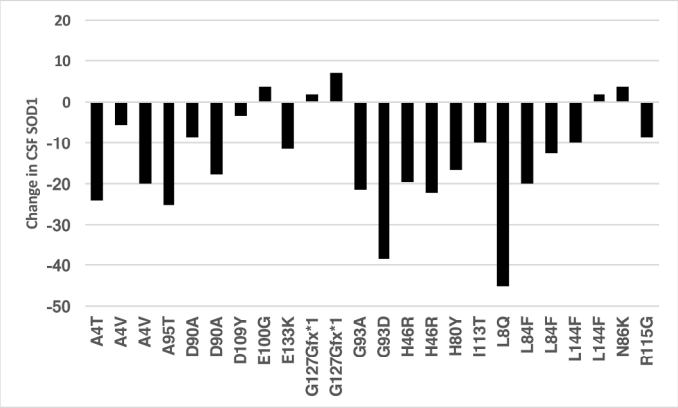

Figure 3. Percent change from baseline in CSF SOD1 content at 18 weeks of treatment according to mutation.

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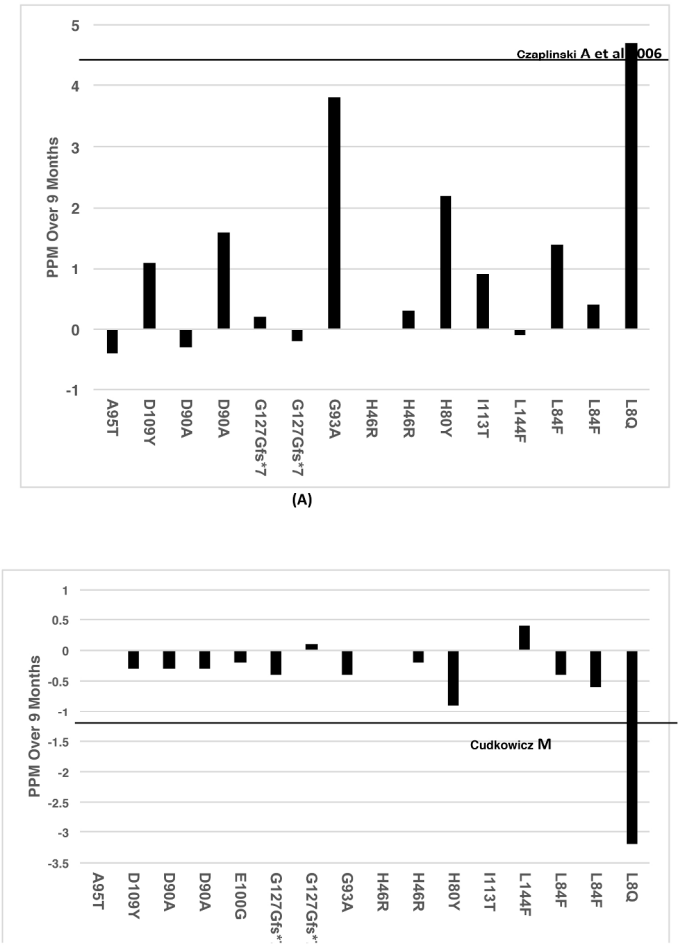

Figure 4. Rate of change expressed as points per month (ppm) in AALS score (A) and ALSFRSr (B) over 9 months (36 weeks) in non-A4V patients. Horizontal line represents change of controls in cited studies.

215x279mm (300 x 300 DPI)

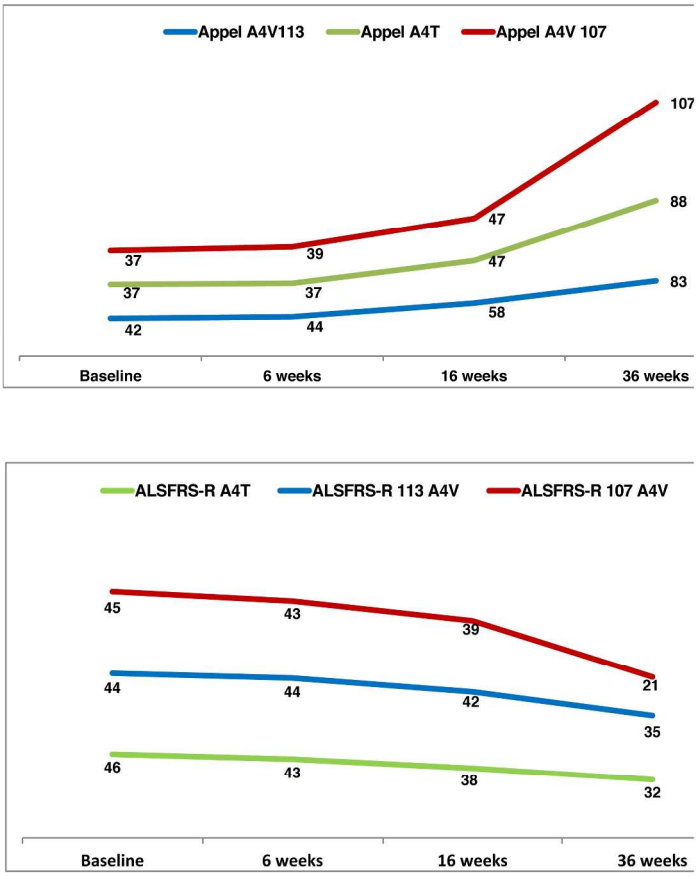

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Figure 5. Rate of change expressed as points per month (ppm) in AALS score (A) and ALSFRSr (B) over 9 months (36 weeks) in 3 A4V /A4T patients.

215x279mm (300 x 300 DPI)