THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS (TAME)

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University of Kansas Medical Center

Research Protocol Involving Human Subjects

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I. Purpose, Background and Rationale

A. Aim and Hypotheses

Neuroprotection would be noticeable with respect to reduced neurofilament levels. Neurofilament light chain (NF-L), neurofilament heavy chain (pNFH) are beneficial for diagnosis of ALS and increase at time of symptom onset, and levels of these biomarkers remain relatively constant in the blood and CSF over time during disease progression. Chitinase-1 (Chit-1) and TNF-α are biomarkers for inflammation that are also increased in ALS patients and can be detected in the blood of ALS patients. There is data showing that across a small number of patients the level of NF-L or pNFH remains constant in the blood and CSF (1); so the ability to compare the changes seen in ALS patients treated with Memantine to a control group will be valuable data to prove or refute this observation.

Aim 1: The overall challenge is to determine if changes in the levels of specific biomarkers found in blood predict and correlate to the response of high dose Memantine treatment and if this correlates to disease progression. We believe that measurement of specific neuronal proteins and inflammatory proteins in the blood may serve as biomarkers for ALS disease progression. If this hypothesis is correct, then altering the levels of these biomarkers via a drug that modulates axonal/neuronal injury or death may serve as an early indicator of effective therapy.

Aim 2: To validate the data from the pilot clinical trial, which suggested that there was a 40% decrease in the disease progression of patients with ALS treated with Memantine. If this study is

also positive, we will have sufficient evidence to move forward with a larger placebo controlled double-blind trial of Memantine at 20 mg in ALS compared to the natural history of ALS. Obviously, the finding of any medication, in addition to Riluzole and/or Radicava, that slows the progression of the universally fatal disease would serve a tremendous unmet need for this orphan disease.

Aim 3: Finally, by using standardized, reliable tools such as the ALS-CBS and the NPI-Q, we will be able to demonstrate if Memantine can improve the neuropsychiatric changes seen in patients with ALS.

B. Background and Significance

Amyotrophic Lateral Sclerosis (ALS) is a devastating progressive neurodegenerative disease. ALS affects people of all ages and both sexes with a reported annual incidence of 1 in 100,000. There are an estimated 30,000 Americans who suffer from ALS at any given time. With no effective therapy, the average lifespan for patients is 2-5 years (2). At present the only FDA approved drug to treat ALS is Riluzole, a drug with several modes of action including NMDA receptor antagonism and Radicava, a drug thought to act as a free radical scavenger and prevent oxidative stress damage to neurons. However, the benefits of Riluzole are marginal, with the pivotal studies showing that Riluzole prolonged life by only three months and did not significantly delay the loss of functional milestones. Radicava may slow disease progression in a subset of patients with a particular clinical presentation, but more clinical trial data is required to evaluate Radicava's effectiveness in all ALS patients. Thus, a more effective therapy is desperately needed for this universally fatal disease.

Although the most visible symptom in (ALS) **i**s progressive weakness and loss of muscle, approximately half of all people with ALS also exhibit some symptoms of cognitive impairment and associated behavioral symptoms (*frontotemporal dysfunction*) (3). FTD is caused by *frontotemporal lobar degeneration* (FTLD), a type of progressive deterioration in the frontal and temporal lobes of the brain, which is responsible for cognitive function. While the neuromuscular deficits associated with ALS have been well characterized, screening methods for detecting behavioral and cognitive impairment have been very limited. In addition, few studies

have really examined ways to try to improve these neuropsychiatric effects which often have major impacts upon a patient and their family's quality of life.

We propose that Memantine, a noncompetitive NMDA receptor antagonist, which is FDA approved for Alzheimer's Disease, will provide significantly enhanced therapeutic benefits to ALS patients. We believe that our proposed study will advance the understanding of ALS and potentially lead to the development of a novel combination therapy which would slow down disease progression as well as benefit ALS patients who illustrate characteristics of frontotemporal dysfunction or frontotemporal dementia (FTD). Specifically, we believe that blocking the NMDA pathway can slow down the loss of neuronal degeneration in ALS. In addition, we have preliminary data that will be validated in this proposed study suggesting that there are specific biomarkers in the blood of patients with ALS that correlates with the efficacy of this therapeutic treatment.

The role of glutamate mediated excitotoxicity in ALS. While 90% of the cases of ALS occur sporadically, 10% of patients with ALS have a family history of the disease. One gene, which accounts for 20% of the familial ALS (fALS) cases, is superoxide dismutase-1 (SOD-1) (4). This discovery suggested that excess free radicals and excitotoxicity may play a key role in the disease (5). In addition, recent evidence suggests that glial cells in the spinal cord of ALS patients may contain a defective Excitatory Amino Acid Transporter (EAAT-2) (6). These glial cells function to buffer the amount of glutamate that is present in the neuronal synapse via EAAT-2 protein function. This finding provides additional evidence that excess glutaminergic stimulation may play a key role in the pathogenesis of the disease.

Nakamura et al demonstrated that overactivation of N-methyl-D-aspartate (NMDA)-type glutamate receptors accounts, at least in part, for excitotoxic neuronal damage, potentially contributing to a wide range of acute and chronic neurologic disorders. It was proposed that generation of excessive nitric oxide (NO) and reactive oxygen species (ROS) can mediate excitotoxicity in part by triggering protein misfolding. S-Nitrosylation, which is a covalent reaction of a NO group with a cysteine thiol, represents one such mechanism that can contribute to NO-induced neurotoxicity (7). The ubiquitin-proteasome system (UPS), in conjunction with molecular chaperones, can prevent accumulation of aberrantly-folded proteins. For example,

protein disulfide isomerase (PDI) can provide neuroprotection from misfolded proteins or endoplasmic reticulum stress through its molecular chaperone and thiol-disulfide oxidoreductase activities. They showed that inhibition of excessive NMDA receptor activity by Memantine, via a mechanism of noncompetitive open-channel blockade, can ameliorate excessive production of NO, protein misfolding, and neurodegeneration (7). Memantine has also been shown to prevent monocyte/microglia activation and reduce TNFα levels, indicating that Memantine may reduce peripheral and central inflammation and inflammatory pathways (8).

C. Rationale

Development and rationale for selection of biomarkers for this study

We propose that throughout the course of ALS, proteins are released from injured/dying axons and cell bodies and accumulate in the CSF for elimination from the CNS via the bloodstream. Proteins originating from motor neurons may serve as prognostic indicators for the rate of disease progression, or as biomarkers of neuronal injury which could be used to monitor the therapeutic efficacy of drugs that reduce motor neuron injury and/or cell death. Of these proteins, cytoskeletal proteins including pNFH and NF-L have been shown to be elevated in the blood and CSF of neurodegenerative diseases and proposed as biomarkers for ALS (1,2,9-12). Protein aggregates and inclusions have been observed in spinal cord motor neurons of ALS patients as well as other neurodegenerative diseases (13). Recently published data by Deng et al furthered the importance of these findings with the identification of genetic abnormalities in UBQLN2 in patients with X-linked adult onset ALS and ALS/Dementia. UBQLN2 mutations led to an impairment of degradation of ubiquitinated proteins. This could potentially explain a common pathway leading to abnormal protein aggregation and neurodegeneration (14). These findings have suggested that being able to follow specific protein biomarkers in the blood and CSF could be a valuable diagnostic and prognostic marker for ALS and ALS disease progression.

Prior studies have shown significantly increased levels of pNF-H and NF-L in the blood and CSF of ALS patients when compared to healthy control or neurologic disease subjects (15). Dr. Bowser's lab has measured blood and CSF levels of pNF-H in sporadic ALS patients (SALS),

familial ALS patients (FALS), age-matched healthy controls (HC), neurologic disease controls lacking motor involvement (DC), and primary lateral sclerosis subjects (Disease mimics). Example results are shown in **Figure 1**. They observed significantly increased pNFH levels in the CSF of SALS and FALS patients when compared to control groups, including disease mimics.

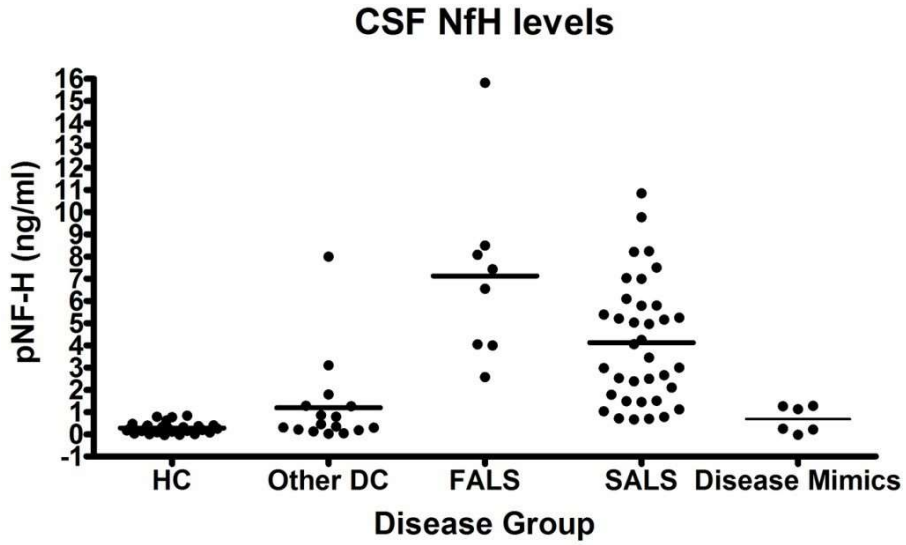

Figure 1: pNFH Levels in CSF *CSF from a total of 104 subjects was analyzed for pNFH by ELISA. All samples were run in triplicate and average value for each shown in ng/ml.*

The Bowser lab has also observed significantly increased levels of pNFH and NF-L in the blood of ALS patients when compared to healthy controls and disease mimics, including pure upper motor neuron disease and pure lower motor neuron disease (**Figure 2**). These results demonstrate the ability to detect a biomarker of neurodegeneration (neurofilament) in the blood of ALS patients.

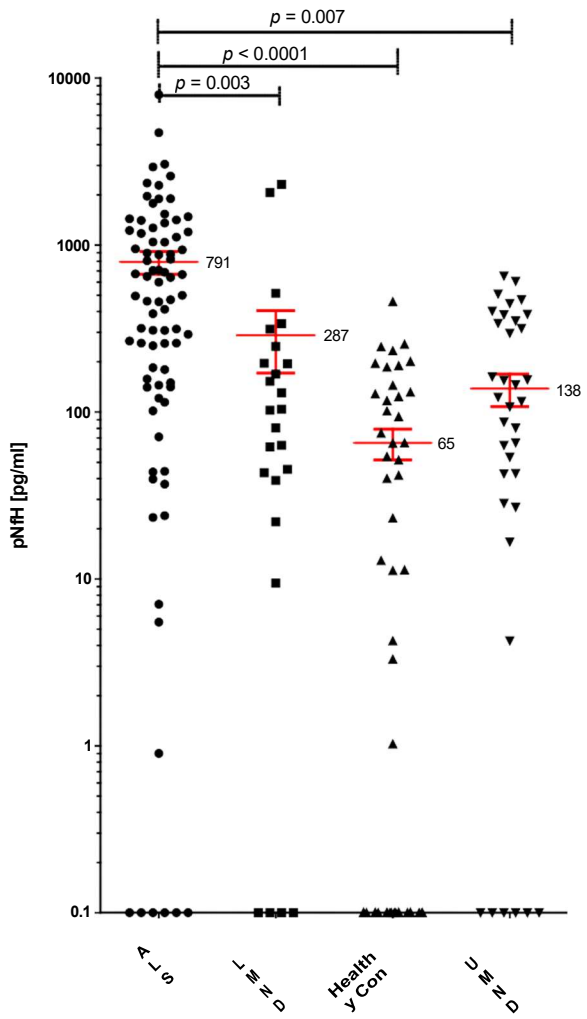

Figure 2: pNFH levels in blood plasma. Plasma from 87 ALS patients, 50 healthy controls, 44 pure upper motor neuron disease (UMND) and 25 pure lower motor neuron disease ((LMND) disease mimics were used to measure pNFH. The level of pNFH in the plasma of ALS patients was significantly increased versus all other subject groups.

CSF levels of pNFH for 65 ALS subjects were also examined to see if there was a correlation between levels of pNF-H and disease survival. These data indicated a clear correlation (Pearson test R= -0.485, *p*= 0.016) between pNFH levels and days from symptom onset to death (**Figure 3**). This result suggests that pNF-H levels in the CSF are a candidate prognostic biomarker, with higher CSF levels corresponding to more rapid disease progression. Similar results were obtained when pNF-H levels were correlated to the rate of clinical disease progression as determined using the ALS Functional Rating Scale - Revised (ALSFRS-R) (Data not shown).

These results confirm a prior study that also demonstrated higher CSF levels of pNFH in patients with a more rapid disease progression (15). Rate of disease progression was defined in both studies by the rate of decline in clinical disease parameters (ALSFRS-R measures), with greater than 1.0 unit per month defined as rapid disease progression and less than 1.0 unit per month as slow disease progression. Therefore, blood and CSF levels of pNFH and NF-L have been verified by multiple laboratories as a biomarker for ALS disease progression.

We propose that therapies to reduce axonal injury would reduce pNFH and/or NF-L levels in the blood and correlate to a reduction in neuronal cell death and increased patient survival. The prior data from a phase II trial for Memantine in ALS patients indicated that reductions in CSF tau levels correlated to slowing of disease progression (2). Sussmuth et al demonstrated that elevated levels of CSF tau were found in patients with ALS (1); however, other studies have not found a strong correlation (16). Therefore, we will not examine tau levels in our current clinical trial.

We feel there is a significant body of literature to support the fact that pNF-H, NF-L,, chitinase-1 (Chit-1) and TNF-α may be sensitive biomarkers for neurodegeneration and activation of glial cells and inflammation during ALS. Studies related to neurofilament were noted above. Cereda and colleagues demonstrated that ALS patients exhibit elevated levels of TNF-α and soluble TNR receptor in the plasma (17). Recent studies have indicated that the chitinase Chit-1 is elevated in the CSF of ALS patients and denotes microglial activation in the spinal cord (18). We also detect significantly increased levels of Chit-1 in the plasma of ALS patients when compared to controls (**Figure 4**).

More importantly, we believe based on the open label trial and other previously mentioned studies, that the blood levels of these biomarkers may predict rate of disease progression and monitor effectiveness of Memantine during our upcoming clinical trial. This would offer a number of potential benefits if found to be true. It would allow for a better stratification in future clinical trials as equal number of fast and slow progressors could be randomized into each arm of future studies. This may negate a potential bias against an effective drug if too many rapid progressors are randomized into the active arm. We also believe that if an effective therapy for ALS is found that there may be observed changes in the levels of the blood biomarkers and that these changes may occur as early as four to six months. Therefore, we propose to study the levels and ratios of these

biomarkers at screening and at multiple times during the trial to see if response to therapy can be correlated with rate of disease progression as our preliminary work suggested.

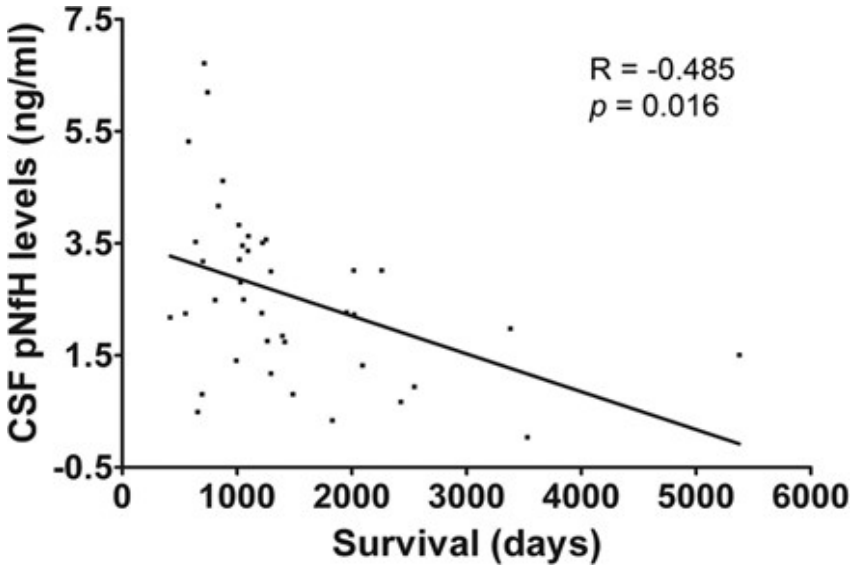

Figure 3: pNFH levels in CSF correlate to patient survival and rate of disease progression

An inverse correlation was seen between CSF pNFH levels and survival from symptom onset (days). Pearson correlation test (R = -0.485, p = 0.016).

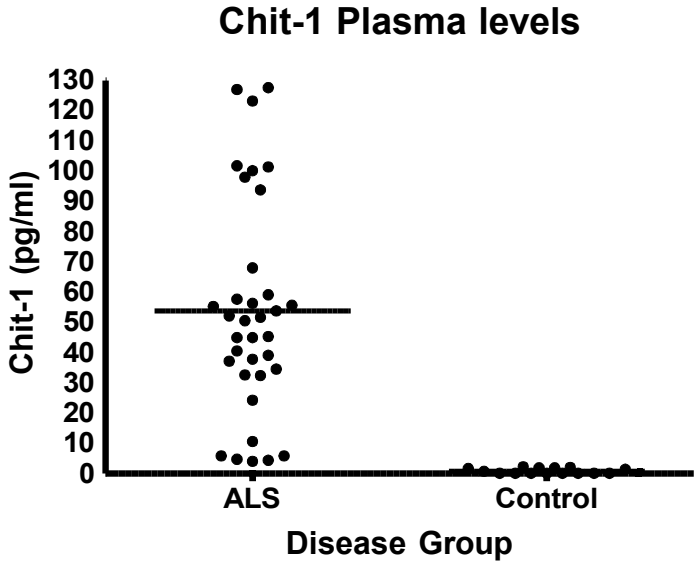

Figure 4: Plasma levels of Chit-1 in ALS. Chit-1 plasma from 35 ALS and 15 age-matched healthy controls. We detected significantly increased levels of Chit-1 in ALS versus control subjects ($p < 0.001$).

The potential role of Memantine in treating patients with ALS

We believe that there are multiple lines of evidence to support the use of Memantine in the treatment of ALS.

1) Memantine is a non-competitive NMDA receptor antagonist that may reduce the effects of glutamate mediated excitotoxicity (19).

2) Memantine can inhibit and reverse the abnormal hyperphosphorylation of tau (19). Tau hyperphosphorylation leads to protein aggregation and sequestration of other cytoskeletal proteins including microtubule associated protein 1 (MAP-1) and MAP-2. Further, Memantine has been shown to block the disassembly of microtubules which follows the hyperphosphorylation of Tau (20).

3) Memantine has been shown to prolong survival in a mutant SOD1 transgenic mouse model of ALS. The data demonstrated that mutant SOD1 transgenic mice survived longer when treated with Memantine than placebo controls (p=0.032) (21).

4) Memantine has been shown to protect neurons against NMDA or glutamate induced toxicity in vitro (20).

5) Memantine has validated significant benefits on cognitive function in other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (22, 23).

Potential benefits of this study

The data that was presented in the preliminary data results section suggests that Memantine at 10 mg BID, when used in conjunction with Riluzole, lowered the levels of tau and pNF-H in the CSF of patients with ALS. This proposed study will explore the potential benefits of a higher dose of Memantine, 20 mg BID, in slowing disease progression. In addition, this study will be testing the cognitive and behavioral impairments in ALS patients. Two psychological batteries

will be administered over the course of the study to determine the incidence of frontal temporal dementia and cognitive dysfunction and the effects of Memantine treatment.

One of the limitations to the development of novel therapeutic agents is the lack of an objective rapid outcome measure which would facilitate drug testing. In collaboration with Dr. Robert Bowser, we have been investigating several potential ALS biomarkers, including pNFH, NF-L, Chit-1 and TNF-α. Previous data indicates that there are elevated levels of tau and phosphorylated pNF-H in the CSF of ALS patients as compared to controls (9, 15, 24). Dr. Bowser has also performed longitudinal studies in ALS patients to demonstrate that levels of blood and CSF tau and pNF-H increase during disease progression and are correlated to clinical parameters of disease progression (25). Further, Dr Bowser has demonstrated that the ratio of pNFH to C3 is a sensitive and specific assay for ALS (1). In addition to determining the effects of Memantine on disease progression, we also wanted to assess the effects it may have on ALS patients who illustrate cognitive impairment and signs of frontotemporal dysfunction. There is no known therapy to improve the cognitive changes in patients with ALS and these changes are seen in as many as 50% of ALS patients.

Despite a lack of success, the development of potential new drug therapies for Amyotrophic Lateral Sclerosis (ALS) has proceeded down several different paths. Basic science discoveries are in the process of being translated into potentially new therapeutic strategies. The possibility of a connection between ALS and cognition has been considered and studied for years; however, ALS clinics and physicians rarely test for cognitive and behavioral changes. One of the reasons that the cognitive underpinnings of ALS have not been investigated until recently is the inability of researchers to reliably and accurately test patients because of the length and the inconsistently of the testing. This lack of testing has led several ALS patients undiagnosed and untreated. Recently studies have shown the specificity and the sensitivity of the ALS Cognitive Behavioral Screen (ALS-CBS), to be 80% and 88% respectively, and can be administered in 5 minutes, which could be extremely beneficial and utilized easily in all ALS clinics. Currently, due to the lack of testing and diagnosis, there are no treatments for ALS patients who suffer from behavioral and cognitive impairment which often have profound effects on the quality of life of the patients and their caregivers.

This proposal is a collaboration between well experienced Neurologists and clinical research scientists. Dr. Richard J. Barohn, (PI) and Dr. Todd Levine (co-PI) have over 26 years of combined experience conducting ALS research trials. Since this is a multi-centered clinical trial, approximately 10 centers in the United States were chosen based on their ALS research experience. These sites are active ALS centers who have decades of ALS research experience and were willing to participate in this trial. Drs Barohn and Levine have partnered with the Western Amyotrophic Lateral Sclerosis Study Group (WALS) to be the coordinating center because of their extensive experience as a coordinating and data collection center.

Dr. Robert Bowser at the Barrow Neurological Institute will be conducting the blood protein biomarker analysis. He has been studying biomarkers in ALS for over 15 years and published the first biomarker panel with predictive value for ALS (26). He has extensive experience measuring each of the biomarkers utilized in the clinical trial and established the standard operating procedures used for the collection, processing and storage of blood samples collected for biomarker analysis. Dr. Dan Moore at California Pacific Medical Center in San Francisco will provide biostatistical support for this study. He has been a biostatistician at California Pacific Medical Center since 1994 and has been doing biostatistics for ALS trials since 1996.

We feel this collaboration brings together leaders in the basic science and clinical research fields in ALS.

Results of Open Label Trial

Memantine, an FDA approved agent for the treatment of Alzheimer's disease, has been thought to reduce the effects of glutamate mediated excitotoxicity (19). It may also inhibit and reverse the abnormal hyperphosphorylation of tau, which leads to its sequestration in neuronal intracellular inclusions (20). In addition, Memantine has been shown to prolong survival in a transgenic mouse model of amyotrophic lateral sclerosis (21). Based on the potential for Memantine to alter the course of the disease in ALS patients, Phoenix Neurological Associates conducted a 20 patient, open label pilot trial of Memantine given at 10 mg bid, in patients taking Riluzole. The data suggested that Memantine might have significant clinical benefit in patients with ALS.

19 patients completed more than 3 months of therapy, 16 patients completed 6 months of therapy and 12 patients completed the entire 18 months of the study. Patients were evaluated based on the number of points lost per month on the ALS Functional Rating Scale - Revised (ALSFRS-R). The ALSFRS-R is a validated measure of ALS disease progression and is a predictor of survival that is typically used as an accepted outcome measure in ALS clinical trials (27, 28). As this was an open-label trial, patients were compared to historical controls and a subset of patients were compared to their pre-study rate of progression. Numerous placebo-controlled studies in ALS have shown that patients on Riluzole, or on no therapy, lose on average -0.9 ALSFRS-R points per month (29).

A linear mixed effects model was used to compare rates of decline for the 20 patients taking Memantine with 415 placebo patients from previous clinical trials. This natural history model (from 8 previous clinical trials) estimated a slope of -0.89 (95% CI -0.95 to -0.83) for placebo and -0.55 (95% CI -0.95 to -0.15) for Memantine treated patients (two-sided p=0.10 for the treatment effect). This represents a 38% reduction in slope in this small sample.

In addition, 12 of these patients were followed in clinic before beginning the pilot trial. Therefore, PNA had an established baseline rate of disease progression for these patients, which was -1.07 ALSFRS-R points per month **(Figure 5).** Since study conclusion, 8 patients have remained on Memantine. These patients have averaged 28 months on Memantine treatment and have progressed at only -.48 points per month. Thus, compared to their own baseline disease progression or to placebo controls, patients who began Memantine therapy showed a marked drop in their rate of disease progression.

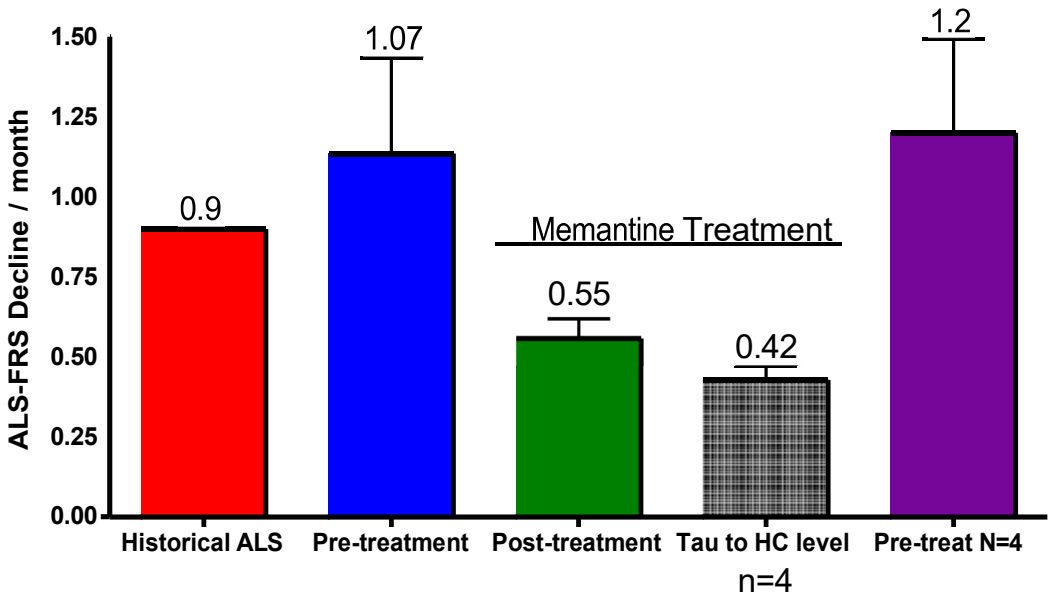

Figure 5. Illustrates historical ALS control patients on Riluzole lose on average, -0.9 pts per month, whereas the patients that were analyzed from the pilot trial lost an average of -0.55. Patients whose tau levels corrected to within the normal range lost -0.42 points per month. This group of 4 patients was losing -1.2 points a month prior to treatment.

Biomarker Studies The patient's CSF, at baseline, 6, and 12 months of Memantine therapy, was analyzed. Compared to healthy control subjects, the levels of tau and phosphorylated neurofilament heavy chain (pNF-H) at baseline were significantly higher in the patients with ALS (p=0.038) **(Figure 6).** It was previously determined that the levels of CSF tau and pNF-H increase over time in untreated ALS patients, perhaps reflecting ongoing neuronal death **(Figure 7).** In contrast, ALS patients treated with Memantine showed a decrease in average CSF tau levels over 12 months of therapy (p=0.04) **(Figure 8),** while healthy untreated control subjects do not exhibit a change in CSF tau levels over time.

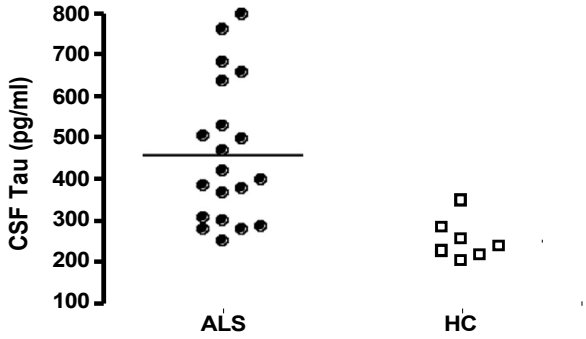

Figure 6. Compared to healthy control subjects, the levels of tau at baseline were significantly higher in patients with ALS enrolled in our pilot trial.

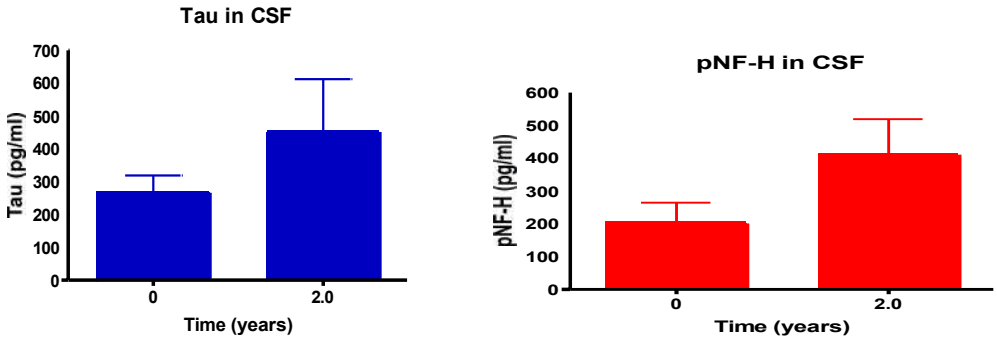

Figure 7. ALS patients who were not treated with Memantine show an increase in their levels of tau and pNF-H over two year

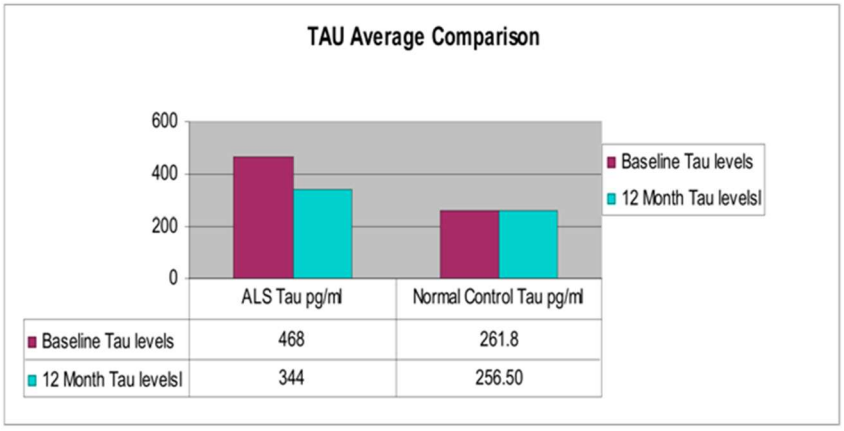

Figure 8. There is a decline in tau levels in ALS patients treated with 12 months of Memantine compared to healthy (normal) controls.

Correlation between levels of biomarkers and disease progression

The results from our CSF biomarker studies suggest a correlation between levels of these cytoskeletal proteins and disease progression. Patients who progressed faster than -0.5 ALSFRS-R points per month had an average CSF tau level of 574 pg/ml, compared to an average level of 298 pg/ml in patients who progressed slower than -0.5 ALSFRS points per month (p=0.005) **(Figure 9).** Levels of CSF pNFH showed a similar correlation to rate of clinical disease progression **(Figure 10)**.

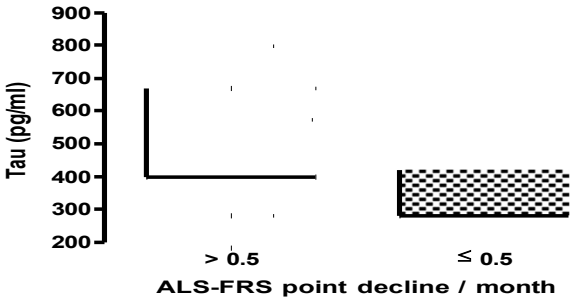

Figure 9. Patients with elevated levels of CSF tau at baseline predicted a more rapid decline based on change in ALSFRS-R scores (p=0.005)

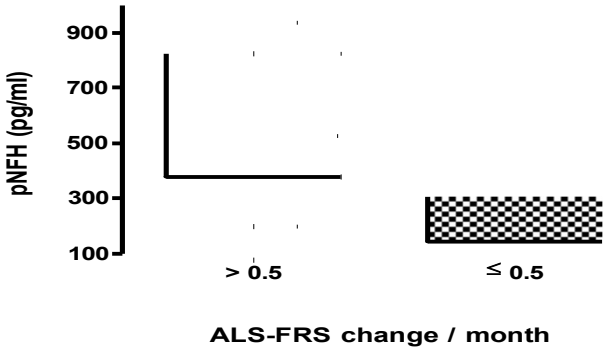

Figure 10. Elevated levels of pNFH in the CSF at baseline was associated with a more rapid progression of disease as evidenced by change in ALSFRS-R scores per month (p=0.002).

After 12 months of treatment with Memantine, patients showed a 27% decline in CSF tau (p=0.04). (**Figure 8)** This contrasts with data from 10 untreated ALS patients that exhibited a

69% increase in median CSF tau levels over 18 months from 266 pg/ml to 451 pg/ml (**Figure 7).** Healthy control subjects showed no change in CSF tau levels over 12 months of follow up **(Figure 8).** Perhaps most intriguing was the finding that 27% of the patients corrected their CSF tau levels to that detected in healthy controls during the course of the therapy with Memantine and these patients showed a marked slowing in the progression of their disease to -0.42 ALSFRS-R points per month **(Figure 5)**. These same four patients were followed in PNA clinic before enrollment in the trial and were losing on average -1.2 ALSFRS-R points per month prior to therapy.

Conclusions from Open Label Study

The results from this trial suggest that the Memantine may slow down the progression of ALS. Another phase II study with Memantine was completed by a group in Portugal who studied 60 patients in a blinded placebo-controlled study which did not illustrate benefit in slowing disease progression. This study; however, did not analyze CSF and due to its small size the power only 80% to detect a 50% decline (30). Despite these conflicting results we believe the pilot trial data and the scientific background behind Memantine indicate that a placebo-controlled trial using higher doses of Memantine should be undertaken in order to evaluate the possibility that Memantine may have a positive effect on this universally fatal disease.

The data from the open label trial suggests that ALS patients could be stratified based on the level of tau and pNFH in their CSF. We will also examine whether the pNFH/C3 ratio can be correlated with disease progression. Patients' CSF could be analyzed at baseline and this could be used to stratify them into treatment arms with an equal number of slow and fast progressors in each arm. If a robust diagnostic biomarker for ALS was found, it would accelerate and simplify the investigative process and result in the earlier implementation of appropriate therapies.

Despite extensive research into new therapeutic modalities for ALS, the disease remains universally fatal with an average life expectancy of 2-5 years. Data presented in the preliminary results section suggests that patients with ALS treated with Memantine at 10 mg BID and Riluzole demonstrated significant slowing of their disease progression compared to their baseline rate of progression while on Riluzole alone. A concurrent phase II study performed by Dr. Carvalho; however, found no effect in a randomized placebo-controlled study of 60 patients. Nor did the Carvalho study examine the potential that a higher dose of Memantine might be effective (30). We believe that both trials established that 10

mg BID is a safe and tolerated dose in ALS patients. There are numerous studies of Memantine in other disease states at doses up to 60 mg a day that show good tolerability (31-34). The majority of side effects that were seen were dizziness, headache, fatigue and nausea. Therefore, this study will test whether a higher dose of Memantine, 20 mg BID would have a more significant slowing on disease progression in a randomized placebo-controlled Phase IIB trial. We feel the scientific background of Memantine with numerous proposed mechanism that could benefit ALS patients, and the results of our open label pilot trial, warrant studying Memantine at a higher dose to see if an effect can be demonstrated in a placebo-controlled trial.

The dose of 20 mg BID of Memantine has been studied in major depressive disorder, tension headache, bipolar disorder, nystagmus, diabetic neuropathy, and tremor. These studies found that dizziness, headache, fatigue, and nausea were the most common side effects. There were no clear drug related serious side effects seen in these studies to raise significant concern about using such a dose in a study of ALS patients. Discontinuation rates tended to be higher in patients on active drug and dizziness was the most common adverse event seen occurring in 20-25 percent of patients. These studies indicate that Memantine has been studied and shown to be safe even at doses as high as 60 mg a day; therefore we feel that ALS patients will tolerate 40 mg a day; however as stated in the tapering section of this proposal, patients will have options to down titrate if necessary.

The primary outcome measure will be disease progression as measured by the number of points lost on the ALS Functional Rating Scale- Revised (ALSFRS-R) during the 36 weeks of therapy. The patient's rate of progression on active therapy during the 36-week treatment arm will be compared to the rate of progression of the placebo arm. We will also attempt to enrich the placebo group data from historical controls from previous ALS studies. Disease progression in numerous ALS clinical trials has been measured using the ALSFRS-R which is a 12 question rating scale used to determine each participant's assessment of their capability and independence in daily activities. The ALSFRS-R can be administered with high inter-rater reliability and test-retest reliability in person or over the phone. The advantages of using such a measurement to determine disease progression are that the categories are relevant to ALS, it is a sensitive and reliable tool, and the rate of decline correlates strongly with survival (27, 28).

II. Research Plan and Design

A. Study Objectives

This study is designed to not only validate the preliminary data that Memantine can significantly slow down the course of ALS but to also determine if Memantine can improve ALS patients who illustrate characteristics of FTD. We also hope to establish the foundation for conducting future clinical trials in ALS by providing prospective blinded data for the utility of biomarkers as novel outcome measures that may more rapidly assess the efficacy of future drugs.

B. Study Type and Design

This is a phase IIB multi-centered double blind, placebo-controlled study evaluating the safety and efficacy of Memantine at 20 mg BID in patients with ALS. 90 participants will be recruited from 14 centers in the United States. Sites were chosen based on their ALS research experience and with the help of the Western Amyotrophic Lateral Sclerosis (WALS) database. The sites that we have chosen to participate are active ALS centers who have years of ALS research experience. WALS has experience as a central coordinating center and has data management personnel and a leading biostatistician. The clinical evaluators, who gather efficacy data, are highly trained and standardized. They are experienced in ALS study details and standards.

The steering committee for this study consists of Richard Barohn, MD. (PI), Todd Levine, MD (Co-PI), David Saperstein, MD (Co-I) Robert Bowser, PhD, Dan Moore, Ph.D. (Biostatistician), and Andrew Heim, CCRP (Project Manager).. The external medical monitor for this study is Constantine Farmakidis, MD. The Data and Safety Monitoring Board is comprised of Nicholas Silvestri, MD (Chair), Andrea Swenson MD, Jonathan Katz MD, Americo Fernandes MD, and Alex Karanevich PhD.

The University of Kansas Medical Center will be the Outcomes and Monitoring Center of the study. This will include the training of the study site evaluators and the validation of secondary outcome measures (FVC, ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS) with ongoing effort in monitoring quality assurance and test-re-test reliability. The University of Kansas Medical Center will provide ongoing quality assurance of all clinical monitoring duties and clinical monitoring responsibilities for the study.

C. Sample Size, Statistical Methods, and Power Calculation

Sample size

90 patients will be randomized in a 2:1 ratio to either Memantine, 20 mg BID or placebo. The active and placebo will be over encapsulated by the research pharmacy at the University of Iowa Research Pharmacy. Medication will be dispensed at 10 mg and placebo kits that are blinded to the investigator and the patient. After the patient fulfills screening criteria, data entered into the database, and their screening visit is monitored remotely, the patient will be randomized using a predetermined randomization formula. KUMC will provide the site with proper kit numbers for each randomized patient. All randomized patients will be instructed to take one capsules once a day for the first two weeks from a blinded bottle that contains the 10 mg capsules or matching placebo. At week three, patients will be instructed to take one capsule twice a day. At week five, patients will be instructed to take one capsule in the morning and 2 capsules in the evening. At week seven, patients will be instructed to take two capsules twice a day. Patients will remain on this dose for the remainder of the study; unless they cannot tolerate the dose. If this is the case, they will follow the proper dosing taper (see dosing reduction/discontinuation section).

Statistical Methods

The primary comparison for efficacy will be based on a linear mixed effects (lme) model fit to the ALSFRS-R data for the 90 patients followed over 36 weeks. The model will be used to calculate fixed effects for intercept, placebo slope (rate of decline of ALSFRS-R) and change in slope for those treated with Memantine. Random effects included are for individual patient variation in intercept, slope and random error at each time point. The test for treatment effect is based on the change in slope due to treatment, as estimated by the model. Testing for significance is two-sided at a 10% level of significance. Analysis is limited to patients who receive at least one treatment dose and have at least 2 measurements (one at baseline and one after treatment). Deaths are treated the same as drop-outs. This model weighs each patient inversely by his estimated variance so that patients with missing values receive less weight than those with complete data.

We will also attempt to enrich the placebo group with historical controls with matching patients from the historical placebo database of 715 patients added to the 30 placebo patients from this trial. The historical controls will be selected to have same entry criteria as patients in this trial. There will be no patient-by-patient matching. We will first test whether the 30 patients from this trial differ from those in the historical database (using the same model with a term added to identify source: historical or contemporary). If this test finds that the two placebo groups differ, historical control will not be added and the combined placebo vs treated test will not be conducted. If the placebo control sets do not differ, the lme model described above will be fit to the combined data.

Statistical Power

We used data from our historical placebo to estimate power for both the test that uses only patients enrolled in the trial and the test where historical placebo is added. To accomplish this we first randomly sampled 75 placebos from our database. The first 30 are assigned as placebo and their ALSFRS-R values are unchanged. The remaining 60 have their slopes reduced by a fixed percentage of their lme estimated slope and the random error is added back in to simulate real measurements. Next, we test for a treatment effect in the simulated dataset of 90. The simulated data are then combined with the remaining (unsampled) placebo and tested for difference in the placebo subgroups. If this test is not significant (at two-sided $p<0.05$), the combined data are tested for a treatment effect. This procedure was carried out 1000 times, noting the p-values from each simulation. Power was estimated by counting the number of times p-values were less than 0.1 (two-sided test).

These simulations with a treatment effect set to 40% show that with 90 patients at a 2:1 ratio of Memantine to placebo, power was 82% for the test that uses only the 90 patients simulated to be in the trial. Adding historical placebo boosted the power to over 90%.

Because there is a great deal of clinical variability among patients with ALS the major drawback to this study is its small size. A previous study looking at 60 ALS patients performed by Carvalho et al, found no significant improvement in patients randomized to 10 mg BID of Memantine compared to placebo (30). This contradicts the data from our pilot study in which patients' rate of progression was compared both to historical controls and their own baseline rate

of progression. Since it is impossible to identify if a patient is a fast or a slow progressor at randomization, small studies such as Carvalho's, or our previous pilot open label trial, can be skewed if a group of slow progressors are randomized to a placebo arm or a group of fast progressors are randomized to an active arm. We have attempted to modulate these effects somewhat by excluding patients who have had the disease for more than three years as possibly being slow progressors. In addition, we have excluded patients with significantly impaired FVC's, to try to limit the number of fast progressors. These measures are very standard in current ALS trials. Another limiting factor to both Carvalho's and our pilot study is that neither study analyzed the efficacy of Memantine at a higher dose. We feel that by using a higher dose of Memantine a more efficacious response may be seen even in a study which is relatively small. Another limitation to this study is 20mg/bid of Memantine has not been studied in ALS patients. Although 40, 50, 60 and even 80mg/d of Memantine have been studied in numerous other disease states and have been shown to be safe and tolerated, it is still unknown how this population will respond. However, we will assess carefully for safety and adverse events.

To date the most validated tool for assessing outcome of ALS clinical studies remains the ALSFRS-R. This is a subjective assessment of a patient's ability to perform their activities of daily living. Unfortunately, studies looking for a change in ALSFRS-R can be limited by inter-rater reliability and the length of time it takes to see a consistent change in these clinical parameters. If there were a reliable biomarker, which could be correlated to a patient's rate of disease progression, future studies could have a more rapidly assessed objective outcome measure and could stratify an equal number of slow and fast progressors into each treatment arm. Our preliminary data have demonstrated that there are elevated levels of tau and phosphorylated neurofilament heavy chain (pNF-H) in the CSF of patients with ALS as compared to healthy controls (2) suggesting that these proteins could also be used for measuring a patient's disease progression. We have found strong correlation between blood and CSF levels of pNFH and NF-L in ALS patients, and propose that blood levels of these proteins will reflect neurodegeneration in the CNS and provide an easier biofluid for longitudinal sample collection during this next Memantine clinical trial. Recently published data by Dr Bowser have also shown a high sensitivity and specificity for the ratio of pNFH/C3 in the CSF for diagnosing ALS (35). We feel these combinations of biomarkers are markers for axonal injury and neuronal cell death. Since motor neurons have very long axons, injury to them can release pNF-H, NF-L and tau into the

CSF, and ultimately the blood. pNFH and NF-Lare quite stable and therefore can be measured in the CSF very readily and ultimately in the blood.

In the preliminary results section of this study, we present data from our open label trial of 20 patients with ALS treated with Memantine and Riluzole. This data demonstrated that there was a very strong correlation between a patient's rate of progression and levels of tau and pNF-H in the CSF. Patients who progressed faster than -0.5 ALSFRS-R points per month had an average CSF tau concentration of 574 pg/ml compared to those who progressed at less than -0.5 ALSFRS-R points per month whose CSF tau levels averaged 298 pg/ ml ($p=0.006$). Further, patients in our pilot clinical trial whose CSF tau levels corrected back to that observed in healthy controls exhibited the greatest decline in ALS disease progression, reducing the rate of decline to -0.42 ALSFRS-R points per month. Thus, Memantine may provide neuroprotection as well as decreased levels of phosphorylated cytoskeletal proteins. Therefore, measuring levels of neurofilament in the blood may provide an indirect measure of Memantine drug effects. In addition, recent studies have identified chitinase-1 (Chit-1) as a protein expressed by activated monocytes and microglia and can be measured in the CSF of ALS patients (36). The Bowser lab has discovered the Chit-1 levels can also be detected in the blood of ALS patients and levels in the CSF and blood can increase over time in ALS patients (Figure 4 + manuscript in preparation). We propose that blood levels of Chit-1 are a biomarker for peripheral and central inflammation due to monocyte/microglia activation. We will determine the ability of Memantine to reduce Chit-1 levels in the blood before and after Memantine treatment.

Blood samples for biomarker analysis will be collected at Screening, Week 4, Week 12, Week 24 and Week 36. Samples will be collected at a similar time of the day for all subjects to account for daily variability and to standardize the time from the last dose of Memantine. Standard operating procedures will be used for blood collection, processing for plasma and storage at -80C. All samples are coded in order to maintain patient confidentiality and the blinded manner of the biomarker studies. Coded samples will then be batch sent to Barrow Neurological Institute for biomarker analysis. Once the coded samples are batched sent to Barrow Neurological, Dr. Bowser will assay for pNFH, NF-L, Chit-1 and TNF-α by ELISA.

Phosphorylated neurofilament heavy chain (pNF-H) concentration in blood is determined using a human pNF-H ELISA Kit (Iron Horse Diagnostics, Inc, Phoenix, AZ) in accordance with the

manufacturer's instructions. 10 µl of plasma is diluted in 50 µl of sample diluent buffer prior to analysis. For the pNFH ELISA, the intra-assay variation is ±4.0% and the inter-assay variation is ±8.0%. Purified protein is used as the standard curve. All samples will be analyzed in triplicate within each experiment, and all experiments will be performed at least twice. NF-L levels are measured using the Simoa NF-L assay (Quanterix). Chit-1 levels in blood are determined using a commercially available ELISA kit (LifeSpan BioSciences, Inc, Seattle, WA) in accordance with the manufacturer's instructions. TNF-α levels in plasma are measured using a commercial ELISA kit (R&D Systems, Inc.) following manufacturer's instructions.

We have extensive experience in the proposed methods and have published proper sample collection procedures to maintain protein stability in human biofluids. Therefore, we do not expect any difficulties in collecting coded samples and generating the ELISA results. We anticipate replicating our preliminary findings that blood levels of pNFH and NF-L will decrease in response to Memantine treatment and with the addition of a higher dose (20 mg bid) results will show a greater reduction. We propose that the inflammatory biomarkers including Chit-1 and TNF-α will also decrease during the course of Memantine treatment.

Our previous open-label trial with Memantine employed this same methodology and we have not encountered any problems with the procedures or experimental decline in the levels of these CSF biomarkers. This data would support our hypothesis that Memantine reduces axonal or neuronal injury/damage and thereby reduces pNFH and NF-L levels detected in the blood. Chit-1 levels will be decreased due to reduced peripheral inflammation in response to Memantine. We will correlate the blood levels of each biomarker to clinical measures of ALS disease progression as determined by ALSFRS-R and compare results to placebo.

In order to achieve the third aim of**: Determining if patients who demonstrate characteristics of FTD, illustrate a slowing of behavioral decline on Memantine treatment over the course of the study** we discuss the rationale, designed a study design and discuss possible limitations.

It has been demonstrated that up to half of patients with ALS may develop cognitive impairment during the course of the disease (37) and up to 40% of ALS patients develop frontotemporal dementia (FTD) (38). Despite the relatively high chance of developing FTD, few studies have examined the potential for independent effects on the neuropsychiatric manifestations of this disease. The ALS Cognitive Behavioral Screen (ALS-CBS™) and the Neuropsychiatric

Inventory Questionnaire (NPI-Q) are two neuropsychological batteries that are validated, brief practical measures that are easily administered and only take about 5 minutes to complete. We feel that given the previous data with Memantine in slowing the progression of behavioral and cognitive decline in other neurodegenerative diseases, such as Alzheimer's, Parkinson's and Lewy Body Disease, that we should study the potential for a positive effect in patients with ALS. The short administration time along with the validity of both scales will be logical additions to this study and should be great indicators of not only the diagnosis frontotemporal dysfunction and even FTD, but also whether Memantine can have any positive effect upon the progression of these changes.

The ALS Cognitive Behavioral Screen (ALS-CBS™), which is a standardized reliable research measure, consists of questions and tasks pertaining to cognition and behavior. The ALS-CBS™ aims to detect frontal lobe-mediated cognitive and behavioral changes and was developed as an initial tool in identifying cognitive and behavioral dysfunction in a clinical setting (39). The ALS-CBS ™ measures attention, concentration and mental control. In one study where verbal fluency was used to screen cognition using a cut-off of eight words or less in 1 minute, the sensitivity was 88% but many normal patients scored poorly resulting in a specificity of only 70%. By comparison, the cognitive section of the ALS-CBS ™ had a sensitivity of 85% to detect any cognitive impairment and 100% sensitivity to detect ALS-FTD (39). Offering a test battery that takes only 5 minutes, but still illustrates high specificity and sensitivity, will be a reliable outcome measure for determining FTD and the effects of Memantine on patients with cognitive and behavioral impairment (frontotemporal dysfunction).

The Neuropsychiatric Inventory Questionnaire (NPI-Q) a non-ALS specific, validated clinical instrument for evaluating psychopathology in dementia will also be utilized as an outcome measure in this proposal. The NPI-Q provides brief, reliable, informant-based assessments of neuropsychiatric symptoms and associated caregiver distress that may be suitable for use in ALS patients to determine the effectiveness of assessing behavioral changes and how the use of Memantine can be beneficial.

The advantages of using these psychological measurements are to determine the frequency of frontotemporal dysfunction and FTD in our studied patients with ALS. They are sensitive and

reliable tools and can demonstrate the effects of Memantine on slowing the rate of progression of these neuropsychiatric changes.

The ALS-CBS™ and the NPI-Q will be administered at Screening, Week 4, Week 12, Week 24, and Week 36 by each site's study evaluator. These study evaluators who are certified to administer the ALSFRS-R will also administer the neuropsychological questionnaires. Only certified evaluators will be able to administer the questionnaires to limit inter-rater error.

The NPI-Q is given as a two-page questionnaire. The questionnaire consists of 12 symptom domains which the subjects' caregiver are asked to circle "yes" or "no" in response to each screening question, and to either proceed to the next question if the answer is "no" or to rate the symptoms present in the last 4 weeks if the answer is "yes." Neuropsychiatric symptoms are assessed in terms of severity on a three-point scale as (1-mild, 2-moderate, 3-severe), and distress which is based on a 0-5 scale. The total NPI-Q severity score represents the sum of individual symptom scores and ranges from 0 to 36. The total NPI-Q distress score represents the sum of individual symptom scores and ranges from 0 to 60 and tests can be administered in 5 minutes or less.

The ALS-CBS™ questionnaire measures both cognition and behavior. The cognitive section measures attention, concentration, working memory, fluency and tracking, and comprises four subscales with a total score of 20 (lower scores reflect greater impairment). Scoring is based on a combination of scores for each item and the number of errors made. The behavioral section measures empathy, personality, judgment, language and insight. This section includes 15 caregiver rated items that assess changes since disease onset. Items are scored from 0-3 (0 being largest and 3 being no change), total scores range from 0-45.

All of the sites who are participating in this study have previously participated in Neuropsychological ALS studies, therefore the study evaluators are well versed and experienced in administering the NPI-Q. We have good relationships with the physicians and psychologists at California Pacific Medical who have developed the ALS-CBS and therefore we do not expect any difficulties with the administration or the test itself. We anticipate that half of our study population will have characteristics of frontotemporal dysfunction, as previously reported and hope to see an improvement in the rate of progression of subjects with these characteristics over the course of Memantine treatment.

Although our previous open label trial with Memantine did not look at frontotemporal dysfunction of FTD, we do believe that Memantine may be a treatment that would benefit ALS patients with characteristics of FTD since it has been a first line FDA approved treatment for dementia. We will correlate the scores from the ALS-CBS and NPI-Q to clinical measures of ALS disease progression as determined by ALSFRS-R as well as FTD progression and compare results to placebo.

Dosing Regimen

Patients will start at 10 mg a day of Memantine and titrate up to 20 mg bid over seven weeks. Titration schedule as follows:

Week 1-2: (1) 10mg capsule in the morning
Week 3-4: (1) 10 mg capsule in the morning; (1) 10 mg capsule in the evening
Week 5-6: (1) 10 mg capsule in the morning; (2) 10 mg capsules in the evening
Week 7: (2) 10 mg capsules in the morning; (2) 10 mg capsules in the evening

Patients will continue on their same treatment throughout the remainder of the study unless they experience treatment related adverse events (see dosing reduction/discontinuation). Memantine will be supplied at 10 mg tablets.

Dosing Reduction/Discontinuation

Participants who cannot tolerate or have adverse reactions to Memantine based on the PIs discretion, can chose to terminate the study, reduce their daily intake by 10 mg per day, or can remain on the same study dose for another week. If the AE has not abated, then the PI or patient can again elect to terminate the study, decrease the dose by another 10 mg per day, or remain on the same dose. If the AE is still present, intolerable or medically unsafe after two dose titrations, then the patient must be terminated from the study. If the patient is terminated early, and the patient has not recovered from their adverse events, it must be reported and every effort should be made to follow the patient until the adverse event is resolved. 10mg is the lowest dose the patient can remain on in the study.

When a patient experiences intolerable adverse reactions to a higher dose of medication, we will challenge the higher dose twice before determining the most efficacious dose for the patient. We will wait one week (7 days) before challenging the patient again. For example, if a patient is currently taking 20mg daily (10mg BID) and starts experiencing adverse reactions when taking 30mg daily, we will ask the patient to reduce the dose back down to 20mg daily for one week before attempting to take 30mg again. If the patient experiences adverse reactions again, we will repeat the same procedure once more and have the patient remain at the lower dose for another week (7 days) before challenging the patient again at 30mg. At this point, the PI will make the determination to leave the patient on the lower dose or proceed with the higher dose.

If a patient is unable to tolerate the lowest dose of medication (10mg QD), sites should ask the patient if they are willing to complete the study as scheduled so that we may obtain important study data. If the patient is unwilling, an early termination visit should be performed that will match the Week 36 visit.

Blinding

The Randomization ID number is the kit number. All study participants, Investigators, Coordinators and all other study personnel will be blinded to treatment groups throughout the course of the study.

Emergency UnBlinding

An emergency unblinding procedure will allow the site investigator the option of learning the treatment assignment for a participant in case of a medical emergency. Each site will be provided an unblinding envelope for emergency use only. These unblinding envelopes will be kept with non-study personnel. For unblinding, the PI must contact the Coordinating Center and Safety Monitor immediately.

D. Subject Criteria

1. Study Population

Men and women from 18-85 years of age inclusive, diagnosed with possible, probable-lab supported, probable or definite ALS. Patients must have an FVC greater than 60% of the predicted value and have an ALSFRS-R score greater than 25. They must also have had the disease for less than 3 years from symptom onset. They will also have to meet all of the inclusion and exclusion criteria detailed in the eligibility section of the study design. Once patients are screened and meet criteria, data entered and screening data monitored, they will then be randomized.

2. Eligibility

Inclusion Criteria

1. Age 18-85
2. Male or Female
3. Clinically definite, probable, probable lab-supported, or possible ALS by El Escorial criteria
4. ALSFRS-R > 25
5. Must be willing to undergo longitudinal blood draws for biomarker analysis
6. Availability and willingness to complete the study
7. Capable of providing informed consent and complying with trial procedures
8. If patients are taking Riluzole and/or Radicava, they must be a on a stable dose for at least thirty days prior to the baseline.

Exclusion Criteria

1. Patients with FVC ≤ 60%*
2. History of liver disease
3. Severe renal failure

4. History of intolerance to Memantine
5. Onset of weakness for greater than 3 years
6. Any other co-morbid condition which would make completion of the trial unlikely
7. If female, pregnant or breast-feeding; or, if of childbearing age, an unwillingness to use birth control.
8. Taking any investigational medications. If the patient was previously on investigational medications, a 30-day washout period is required before the baseline visit. Non-trial medications are not cause for exclusion.
9. Unwillingness to provide consent

3. **Remote Enrollment**

Subjects may be enrolled remotely if they meet the following criteria:

1. Age 18-85
2. Male or Female
3. Clinically definite, probable, probable lab-supported, or possible ALS by El Escorial criteria
4. ALSFRS-R > 25
5. Must be willing to undergo longitudinal blood draws for biomarker analysis. This may be foregone during the screening visit.
6. Availability and willingness to complete the study
7. Capable of providing informed consent and complying with trial procedures
8. If patients are taking Riluzole and/or Radicava, they must be a on a stable dose for at least thirty days prior to the baseline.
9. Documentation of not clinically significant liver enzymes within the previous 6 months.

Subjects may be enrolled remotely if they do not meet the following criteria:

1. Patients with FVC ≤ 60%*
2. History of liver disease
3. Severe renal failure
4. History of intolerance to Memantine
5. Onset of weakness for greater than 3 years
6. Any other co-morbid condition which would make completion of the trial unlikely
7. If female, pregnant or breast-feeding; or, if of childbearing age, an unwillingness to use birth control.
8. Taking any investigational medications. If the patient was previously on investigational medications, a 30-day washout period is required before the baseline visit. Non-trial medications are not cause for exclusion.
9. Unwillingness to provide consent

When enrolling subjects remotely, the visit may take place over the phone or via a meeting with video capabilities. All assessments should be performed with the exception of the physical exam, forced vital capacity, and biomarker/safety lab draws. Informed consent should take place before performing any study procedure. Use the guidance below when consenting study subjects remotely.

1. The study team confirms the potential subject's interest in learning more about the study and verifies the mailing address or email address.
    a. If the consent form will be sent by postal mail, the blank consent form is mailed, along with a cover letter that introduces the study and explains when the phone conversation will occur. A stamped, self-addressed envelope is provided so the subject can return the signed consent document to the study team.
    b. Alternatively, if the subject agrees to email communication, the consent form is sent by secure email.
2. After the potential subject has received the document, a member of the study team calls the subject and walks through the entire document over the phone, answering questions and

making notes about the subject's questions. Time and date of the conversation should be recorded.

3. Once all questions are answered, the subject signs the consent form if they are willing to participate.
    a. S/he returns the consent form by mail in the provided self-addressed envelope.
    b. Alternatively, the consent form should be scanned by the subject and returned to the study team by email.
4. Once the signed version is returned, the study team member who conducted the consent conversation should sign the consent form and date with today's date. If postal mail is used, explain the discrepancy in dates. The study team member should write a note on the consent form stating that the subject's consent was obtained by phone on xx date (the date the subject signed.)
5. The subject should receive back a full-signed copy of the consent form for their records

* Since FVC cannot be captured during a remote screening visit, an acceptable FVC done within the previous 90 days will be accepted. If an FVC is not available within the previous 90 days, the subject may be enrolled if the local site PI believes the subject has no significant shortness of breath or respiratory issues. Because of the ongoing COVID-19 pandemic, it may not be possible to obtain an FVC due to potential spread of the coronavirus via respiratory equipment. Therefore, these criteria may be waved at enrollment if there is a prior FVC from the previous clinic visit >60% of if the investigator does not believe the patient has significant shortness of breath or respiratory issues.

4. Premature discontinuation, protocol violation or loss to follow-up

All attempts will be made to enhance patient compliance. Early withdrawal may occur for any of the following reasons: a) the patient requests to; b) the investigator decides that it is in the patient's interest; c) a serious adverse event that is related to study medication; d) there is any other reason at the investigator's discretion. If the patient withdraws early, the patient will be encouraged to continue with scheduled visits. If they choose to not return, termination evaluations will be completed. If a patient is unable to return to the center, attempts will be made to follow the patient via telephone calls to complete the ALSFRS-R, adverse events and con meds until study completion.

E. Specific Methods

1. Study Schema (see table below in Appendix 1)

Visit Windows

Study visits are based on a 28-day month, so if a patient comes in on a Monday, their next visit is 4 weeks (28 days) from that visit (on a Monday). Study visits are calculated from the baseline visit, which is the day the patient starts taking the medication. Maximum length of time between screening visit and baseline visit is 28 days but can be shorter as applicable.

As stated, visits from baseline are calculated on a 28-day month $\pm$ 7 days.

Visit 1-Screening

All patients must sign and date the most current Institutional Review Board (IRB)/Ethics Committee's approved written informed consent before any study specific assessments or procedures are performed. A screening examination (medical history and physical and neurological examination including vital signs, height and weight) will be performed. Concomitant medications will be recorded

Laboratory blood tests will be assessed. A CBC with differential and comprehensive metabolic panel (CMP), will be attained using each site's local laboratory. CRP biomarkers will be drawn, processed and stored for batch shipping. A urine pregnancy test will be performed for females of childbearing potential. ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS will be administered. The ALSFRS-R and Forced Vital Capacity will be used to also determine eligibility.

Randomization

After the patient fulfills screening criteria, data entered into the database, and their screening visit is monitored remotely, the patient will be randomized using a predetermined randomization formula. KUMC will provide the site with proper kit numbers for each randomized patient.

Visit 2 –Baseline

1. Dispense medication

Patients who are randomized will start at 10 mg per day for 2 week, and then titrate up to 20 mg twice daily over the next 7 weeks. One month (28 days) of Memantine or placebo will be supplied to the patient.

Visit 3 – Week 4

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. Administer the ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS.
4. CBC with differential and CMP labs, urine pregnancy test
5. Measure vital signs
6. FVC
7. Plasma for biomarker analysis
8. Two months of study medication will be supplied and prior medication collected. Drug accountability will be performed.

Visit 4 – Week 8

The study coordinator will contact patients by phone to assess the ALSFRS-R. Assess and document all adverse events and concomitant medications.

Visit 5– At Week 12

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. Administer the ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS.
4. CBC with differential and CMP labs, urine pregnancy test
5. Measure vital signs
6. FVC
7. Plasma for biomarker analysis

8. Three months of study medication will be supplied and prior medication collected. Drug accountability will be performed.

Visit 6 & 7 Telephone Contact – At Week 16 and Week 20

The study coordinator will contact patients by phone to assess the ALSFRS-R. Assess and document all adverse events and concomitant medications.

Visit 8 Week 24

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. Administer the ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS.
4. CBC with differential and CMP labs, urine pregnancy test
5. Measure vital signs
6. FVC
7. Plasma for biomarker analysis
8. Three months of study medication will be supplied and prior medication collected. Drug accountability will be performed.

Visits 9 & 10 Telephone Contact – At Week 28 and Week 32

The study coordinator will contact patients by phone to assess the ALSFRS-R. Assess and document all adverse events and concomitant medications.

Visit 11 Week 36

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. Administer the ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS.
4. CBC with differential and CMP labs, urine pregnancy test
5. Measure vital signs
6. FVC

7. Plasma for biomarker analysis
8. All medication collected

Visit 12 Week 40

1. Phone call to reconcile adverse events and concomitant medications.

Unscheduled Visit (If needed)

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. CBC with differential and CMP labs, urine pregnancy test
4. Measure vital signs

Early Termination (ET) Visit (If needed)

1. Assess and document all adverse events and concomitant medications.
2. Physical exam
3. Administer the ALSFRS-R, ALS-CBS, NPI-Q, C-SSRS.
4. CBC with differential and CMP labs, urine pregnancy test
5. Measure vital signs
6. FVC
7. Plasma for biomarker analysis
8. All medication collected

F. Subject Safety and Data and Safety Monitoring Plan

Safety Variables

A standard physical exam, including vital signs, will be performed at every in-person visit to ensure safety and to follow ALS progression. Laboratory tests will be performed at every in-person visit to monitor comprehensive metabolic values. Adverse Events (AEs) will be reviewed

and documented at every visit. Patients will be instructed to contact their PI or study coordinator with any adverse reactions they have regardless of causality to the medication. If patients have any adverse reactions believed to be caused from the medication, it will be the PIs discretion whether to reduce the patient's medication or to early terminate the patient from the study. At each visit, patients will be questioned about the development of any new symptoms. If an unscheduled visit is deemed necessary, the patient will be asked to return for a clinic visit within 48 hours. The site investigator will then evaluate any changes and determine the need for any intervention.

The C-SSRS is frequently asked for or recommended by various international agencies such as the FDA, WHO, JCAHO Best Practices Library, AMA Best Practices Adolescent Suicide, Health Canada, Korean Association for Suicide Prevention, Japanese National Institute of Mental Health and Neurology, and the Israeli Defense Force. The C-SSRS has been administered several million times and has exhibited excellent feasibility – no mental health training is required to administer it. The C-SSRS, initially designed for an NIMH-funded suicide study, addresses this goal by showing successful suicide attempt prediction not only in suicidal adolescents, but in non-suicidal adults as well. In the past, typical screening has only identified suicide attempts, omitting some of the most important behaviors that are critical for risk assessment and suicide prevention (e.g. collecting pills, buying a gun). The C-SSRS is the only screening tool that assesses the full range of evidence-based ideation and behavior items, with criteria for next steps (e.g. referral to mental health professionals); thus, the C-SSRS can be exceptionally useful in initial screenings. Training is required before administering the C-SSRS and must be completed and documented. The web-based training can be accessed at the following web site and takes approximately 30 minutes to complete.
http://c-ssrs.trainingcampus.net/uas/modules/trees/windex.aspx

Monitoring

This study will utilize remote monitoring. After the patient is screened, all screening source documents and signed consent forms in their entirety will be sent to the Project Manager (Andrew Heim or alternate, Maureen Walsh) at the University of Kansas Medical Center over a secured website. The University of Kansas Medical Center will retain the signed consent forms

and stored on a secure site. All other documentation will be destroyed. Sites will be asked to send source documentation periodically throughout the study.

Other items that the Project Manager will request and retain will be medical licenses or nursing licenses (if applicable), human subjects or GCP training, signed CV's of all key personnel, original 1572, laboratory director credentials, CV's, laboratory normative values and other documentation as needed. Each site is expected to maintain a regulatory binder with such documents and approvals.

Laboratory Test Abnormalities

In the event of medically significant unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to the normal range and/or an adequate planation of the abnormality is found. For abnormalities that fall in the range of critical values that would affect safety then Dr. Farmakidis will instruct the site to hold further treatment dosing. Dr. Farmakidis will be notified within 24 hours of any unscheduled visits or SAEs. Once he is notified he will contact the DSMB to inform them if one of these events occurs.

Treatment and Follow-up of AEs

The site investigators will be responsible for reviewing laboratory results and serious adverse events to determine the relationship to the study medication. Dr. Barohn and the Data and Safety Monitoring Board will determine whether changes in the protocol or consent form is needed based on reported AEs.

Recording Adverse Events

Definition - adverse events are clinical abnormalities (illness, signs, or symptoms) that begin or worsen during the course of the study and are not thought to be directly related to the expected course of ALS itself, whether or not the abnormality is believed by the investigator to be related to the study medication.

Recording - the investigator will monitor each patient closely and record all adverse events on the adverse event page of the case report form.

Expectedness - the investigator will determine whether the AE is expected or unexpected

Severity - adverse events should be graded for severity:

Mild: causing no limitation of usual activities

Moderate: causing some limitation of usual activities

Severe: causing inability to carry out usual activities

Adverse events should be followed up until they have stabilized or have returned to baseline status (in the event of a reaction to the medication). This is especially important for those events where the reported causal relationship to the study medication is anything other than "unrelated" (e.g. probable, possible). If patients have an adverse event that the PI assesses as "related" to the study medication then the PI will, or the patient can choose, to terminate the study or can decrease the dose of the medication by 10 mg per day. If the AE still does not abate then the PI or patient can again elect to terminate the study or decrease the dose by another 10 mg per day again. If the AE is still present after two dose titrations, then the patient must be terminated from the study. If the patient is terminated early, and the patient has not recovered from their adverse events, every effort should be made to follow the patient until the adverse event is resolved.

Reporting of Serious Adverse Events (Immediately Reportable)

Serious Adverse Events

Definition: Serious adverse events are life threatening, fatal, result in hospitalization or prolonged hospitalization, permanent disability, congenital anomaly, cancer, or overdose, or are any event that the investigator believes is very unusual or potentially serious.

Report: Any serious adverse event will be reported immediately (within 24 hours). All serious adverse events will be recorded on the standard adverse events page of the case report form, and a serious adverse event form will be completed. The coordinating center will be notified within 24 hours.

Follow-up of Adverse Events: The site investigator is responsible for appropriate medical management and laboratory tests for adverse events until the event is resolved. The management and resolution of each adverse event should be recorded on the adverse event page of the case report form.

Any clinical AE or abnormal test value that is serious (defined as fatal or life-threatening, one which results in inpatient hospitalization, one which results in persistent disability, congenital anomaly or is medically significant or requires intervention to prevent one of the other outcomes listed), and which occurs during the course of the study, regardless of the treatment group, must be reported within one working day of the investigator becoming aware of the event. In addition, for fatal and life threatening events, the Medical Monitor (Dr. Constantine Farmakidis at the University of Kansas Medical Center) should be contacted immediately. The chair of the Data and Safety Monitoring Board (Dr. Nicholas Silvestri, Neurologist at University of Buffalo), and the DSMB, will review SAEs in a similar manner and make a determination if the patient can continue in the trial. If the subject needs to stop study medication Dr. Farmakidis will contact the PI and study coordinator at that subjects' site.

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STUDY TABLE:

Procedures	**V1** **SC**	**V2** BL	**V3** Wk 4	**V4** Wk 8	**V5** Wk 12	**V6** Wk 16	**V7** Wk 20	**V8** Wk 24	**V9** Wk 28	**V10** Wk 32	**V11** Wk 36	**V12** Wk 40
Consent	**X**											
Eligibility (Inclusion/Exclusion)	**X**											
Vitals	**X**		**X**		**X**			**X**			**X**	
Height	**X**											
Labs/Urine pregnancy test	**X**		**X**		**X**			**X**			**X**	
Med History	**X**											
Physical Exam	**X**		**X**		**X**			**X**			**X**	
FVC	**X**		**X**		**X**			**X**			**X**	
ALSFRS-R	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
ALS-CBS	**X**		**X**		**X**			**X**			**X**	
NPI-Q	**X**		**X**		**X**			**X**			**X**	
C-SSRS	**X**		**X**		**X**			**X**			**X**	
Biomarkers	**X**		**X**		**X**			**X**			**X**	
Phone call				**X**		**X**	**X**		**X**	**X**		**X**
Drug dispensing		**X**	**X**		**X**			**X**				
Drug Acct			**X**		**X**			**X**			**X**	
Adverse Events			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Concomitant Medication	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Physician	**X**		**X**		**X**			**X**			**X**	
Study Coordinator	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Data Entry	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**