CLINICAL RESEARCH PROTOCOL

Tolerability and Efficacy of L-Serine in Patients with Amyotrophic Lateral Sclerosis: A Phase IIa Study

Regulatory Sponsor: Elijah W. Stommel, MD, PhD
Dartmouth-Hitchcock Medical Center
Department of Neurology
One Medical Center Drive
Lebanon, NH 03576
603-650-5104

Funding Sponsor: Brain Chemistry Labs, Institute for Ethnomedicine
PO Box 3464
Jackson, WY 83001
A not-for-profit 501(c)(3) charity
801-375-6214

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List of Abbreviations

ALS: Amyotrophic Lateral Sclerosis

ALSA-Q40: Amyotrophic Lateral Sclerosis Assessment Questionnaire (long form)

ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised

BMAA: β-N-methylamino-L-alanine

FVC: Forced Vital Capacity

GRAS: generally recognized as safe

LAR: legally authorized representative

NFT: neurofibrillary tangles

PI: principal investigator

PHI: protected health information

PRO-ACT: Pooled Resource Open-access ALS Clinical Trials

C-SSRS: Columbia –Suicide Severity Rating Scale

Study Summary

Title	A Phase IIa study of the effects of L-serine in Patients with Amyotrophic Lateral Sclerosis: A Phase II Study
Short Title	Phase IIa L-serine trial for ALS patients
Phase	Clinical study phase IIa
Methodology	All patients receive the treatment at the same dose
Study Duration	Expected 4 years
Study Center(s)	Single-center
Objectives	Determine tolerability of L-serine oral doses for ALS patients and assess preliminary indications of efficacy.
Number of Subjects	50
Diagnosis and Main Inclusion Criteria	Patients with a clinical diagnosis of ALS.
Study Product, Dose, Route, Regimen	L-serine 15g oral dose twice daily morning and night.
Duration of administration	6 months
Reference therapy	Selected ALS patients in placebo groups in prior treatment trials with the same inclusion/exclusion criteria from the PRO-ACT database.
Statistical Methodology	Linear mixed effects model.

1 Introduction

This document is a protocol for a human research study. This study is to be conductedaccording to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background: Prevalence and Clinical Features of ALS

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease or Motor Neuron Disease, is a devastating progressive neurodegenerative disease characterized by death of primary motor neurons in the cerebral cortex, brain stem, and spinal cord (Brooks et al. 2000). Resultant denervation of the muscles leads to muscle wasting and paralysis. Generally, initial indications of ALS are development of a localized weakness, often in a peripheral part of the body such as a hand or foot, although sometimes problems with breathing, spasticity, and speech/swallowing signal the onset of the disease (Miller et al. 2005). Fasciculations are a common sign. Diagnosis of ALS requires evidence of both lower and upper motor neuron degeneration with spread of symptoms to other regions and evidence of progression (Brooks et al. 2000).

In Europe, the annual incidence rate of ALS in the general population is 2.16 per 100,000, with men having a slightly higher annual incidence rate (Logroscino et al. 2010). In the United States, estimates of annual incidence rates range between 1.6 per 100,000 (Hirtz et al. 2007) to the most recent estimate of the prevalence of 4.3 per 100,000 (Mehta et al. 2016). At any one time, roughly 20,000-30,000 Americans are living with diagnosis of ALS (Miller et al. 2005) with a prevalence rate of 4.3 per 100,000 in the U.S. population (Mehta et al. 2016). Thus, ALS fits within the FDA orphan disease category.

The burden that ALS places on patients and their families is enormous, with most patients dying within three years of diagnosis. Paralysis and loss of motor function is progressive, ultimately leading to the inability to walk, to write, to swallow, to speak, and to breathe. Although traditionally motor neuron deficits have been emphasized in clinical studies of ALS, it is now known that cognitive issues also occur in ALS patients with 51% of patients diagnosed with sporadic ALS showing some level of cognitive impairment (with 19% showing moderate to severe impairment). The pattern of cognitive problems is similar to that seen in familial ALS (fALS) and frontotemporal dementia (FTD) (Ringholz et al. 2005, Wheaton et al. 2007). In a study of 160 patients in Ireland, 13.8% fulfilled the criteria for FTD (Phukan et al. 2011).

Approximately 8-10% of ALS cases are familial, with 90% of cases being sporadic (sALS). Mutations in SOD-1, TDP-43, FUS/TLS, VCP, and TARDBP have all been shown as potential causes of ALS with some mutations such as TARDBP also being found in individuals with sporadic ALS (Kabashi et al. 2008). Environmental exposures including exposures to heavy metals such as lead, mercury, organic solvents, cyanobacteria, and pesticides are also being investigated as possible risk factors for ALS (Andrew et al. 2017, Caller et al. 2015).

The current standard of care in the United States is treatment with Riluzole—a glutamate antagonist—which was approved in 1995, although in May 2017 the FDA approved the use of Edaravone administered as an intravenous infusion for the treatment of ALS. Edaravone use in ALS patients in the early stages of their disease resulted in a reduced rate of functional decline of ALS patients by about 33% (Abe et al., 2014), and is estimated to cost about $145,000 per year for each patient.

1.2 Environmental Exposures as Risk Factors for ALS

Based on ethnomedical studies of a related paralytic disease, Guamanian Amyotrophic Lateral Sclerosis / Parkinsonism Dementia Complex (Guamanian ALS/PDC), there has been increased interest in chronic exposure to a cyanobacterial toxin, β-*N*-methylamino-L-alanine (BMAA) as an additional risk factor for ALS. Produced by endophytic cyanobacteria in specialized roots of cycad trees (*Cycas micronesica*) in Guam, BMAA contaminates traditional food items in the Chamorro diet (Cox et al. 2003, Murch et al. 2004, Banack et al. 2006). The hypothesis that chronic exposure to BMAA triggered an ALS-like disease among the Chamorro people of Guam was supported by recent replicated experiments with non-human primates in which chronic dietary exposure to BMAA triggered a dense tauopathy with sparse amyloid deposits in brain tissues of animals fed BMAA, but not in control animals (Cox et al. 2016). The resultant neuropathology had some aspects similar to Braak 1 early stage Alzheimer's disease pathology (Braak 1995, Cox et al. 2016) including neurofibrillary tangles (NFT) formed from misfolded tau as determined by AT8 immunohistological (IHC) staining and β-amyloid deposits determined by β-amyloid(1-42) IHC stain; both of these neuropathological features were confirmed with Thioflavine-S IHC staining. In addition, microglial activation similar to that observed in early stages of ALS was found in animals fed BMAA (Davis et al. 2016).

We used this non-human primate model to test the naturally-occurring dietary amino acid L-serine and found that dietary supplementation in non-human primates reduced the density of NFT by 35-50% in eight regions of the brain including the amygdala and anterior cingulate gyrus (Cox et al. 2016).

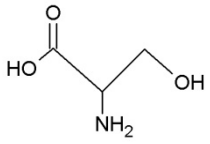
1.3 L-serine as a Possible Therapy for ALS

ALS, like other tangle diseases, is characterized by the presence of misfolded and tangled proteins. L-serine was initially investigated as a possible therapy for ALS because L-serine blocked misincorporation of BMAA into human neuroproteins, reducing protein misfolding, aggregation, and subsequent apoptosis (Dunlop et al. 2013). Subsequently, the ability of L-serine to alert the unfolded protein response (UPR) has been elucidated using *in vitro* data from human neuronal cell culture and *in vivo* data from non-human primates dosed with L-serine over 140 days compared to controls. Using a UPR real-time PCR array with genes from the ER stress and UPR pathways, as well as western blotting, it was found that L-serine functions as a small proteostasis regulator, alerting cells to quickly respond to oxidative insult (Dunlop, Powell, Guillemin, Cox 2017). This effect of L-serine occurs, in part, through upregulation of the ER stress chaperon protein disulphide isomerase (PDI), which is involved in refolding misfolded proteins (Dunlop, Powell, Metcalf, Guillemin, Cox 2017).

L-serine is a normal part of the human diet, with the average American receiving 3.5 grams/day from all sources. L-serine is synthesized by neurons and glial cells, but deficiencies in endogenous L-serine biosynthetic pathways are associated with a number of human neurological conditions (Canu et al, 2014; Metcalf et al. 2017). L-serine has shown effectiveness in treating polyneuropathy (Catsman-Berrevoets et al. 1997) and is currently being studied as a possible treatment for the hereditary neuropathy HSN-1 (Garofalo et al. 2011).

Since we have found in replicated studies in non-human primates that supplementing the diet with L-serine results in a decreased density of NFT, we now seek to evaluate the potential of L-serine in slowing disease progression and functional decline among ALS patients. Based on an earlier successful FDA-approved Phase I clinical trial of L-serine in 20 ALS patients (Levine et al. 2016), we now seek to extend studies of tolerability and potential efficacy of L-serine therapy in a Phase IIa trial in a larger number of ALS patients.

1.4 Investigational Agent



L-serine ($C_3H_7NO_3$; 105.09 g/mol; synonym (S)-2-amino-3-hydroxypropanoic acid) is a naturally-occurring dietary amino acid. It is abundant in soy products, some edible seaweeds, sweet potatoes, eggs, and meat. Since some L-serine is produced by astrocytes in the brain, it is considered by some authorities to be a non-essential amino acid, although as noted below, certain genetic diseases indicate that L-serine is a constituent essential amino acid. L-serine is directly involved in the biosynthesis of purines, pyrimidines, and other amino acids. L-serine residues in proteins serve, along with those of threonine, tyrosine, and histidine, as key sites for phosphorylation necessary for proper protein folding and functionality. L-serine is currently prescribed, sometimes in combination with glycine, for the treatment of two different genetic neurological diseases that result in L-serine deficiency, e.g., 3-phosphoglycerate dehydrogenase deficiency and 3-phosphoserine phosphatase deficiency (De Koning 2006). L-serine has been shown to reduce the production of neurotoxic deoxysphingolipids that are responsible for hereditary sensory autonomic neuropathy type 1, and has shown encouraging results in a pilot clinical trial (Garofalo et al. 2011).

L-serine is considered GRAS (generally recognized as safe) by the FDA and has been approved as a normal food additive under CFR172.320. It is widely sold as a dietary supplement. No adverse incidents have been recorded by the FDA from its use. In an FDA-approved Phase I human clinical trial of L-serine for ALS patients, sponsored by the Brain Chemistry Labs, it was found that patients tolerated doses up to 30 grams/day (ClinicalTrials.gov identifier NCT01835782, Levine et al. 2016). Harvard University and Massachusetts General Hospital are conducting a two-year study on L-serine supplementation to correct biochemistry associated with hereditary sensory neuropathy type 1 at doses of 400mg/kg/day (ClinicalTrials.gov identifier NCT01733407).

1.5 Preclinical Data

In human neuronal cell cultures, L-serine blocks misincorporation of β-N-methylamino-L-alanine (BMAA) into neuroproteins, which otherwise leads to protein misfolding, aggregation, and cell apoptosis (Fig. 1). This protective effect of L-serine was replicated in a non-cell synthesis system, where protein incorporation of BMAA increased when L-serine was removed from the system and when human DNA templates were available in comparison with bacterial DNA templates (Glover et al. 2014). Data on fruit-flies, *Drosophila melanogaster*, reveals that co-feeding with L-serine decreased the amount of BMAA incorporated into protein by half (Fig. 2). In addition, 40% of flies fed BMAA died within three days, while co-administration with L-serine saved every fly within this time frame (Fig. 2).

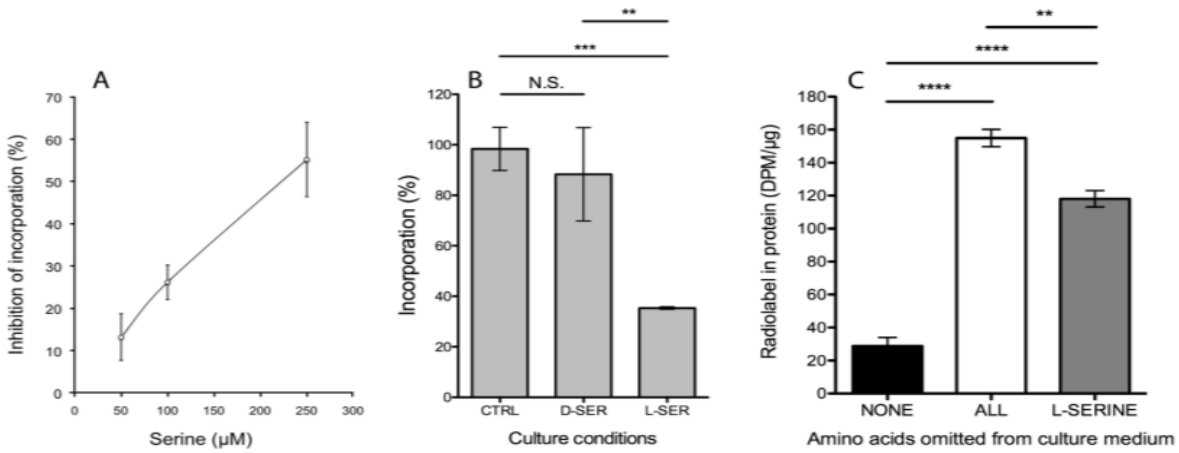


Figure 1: Inhibition of incorporation or radiolabel into cell proteins by L-serine. Panel A, Incorporation of radiolabeled BMAA was inhibited by L-serine in a concentration dependent manner. Panel B, D-serine (D-SER) did not significantly impact the incorporation of BMAA (NS, $P = 0.4419$). L-serine significantly inhibited the incorporation of BMAA compared to the control cells (CTRL, ***P=0.0002) and D-serine (D-SER, **P<0.01). Panel C, there was a significantly (****P<0.001) greater incorporation of BMAA when all protein amino acids were omitted from the culture medium (ALL) compared to when none were (NONE). When only L-serine was omitted (L-SERINE) incorporation was restored to approximately 80% (****P=0.0009). doi:10.1371/journal.pone.0075376.g004

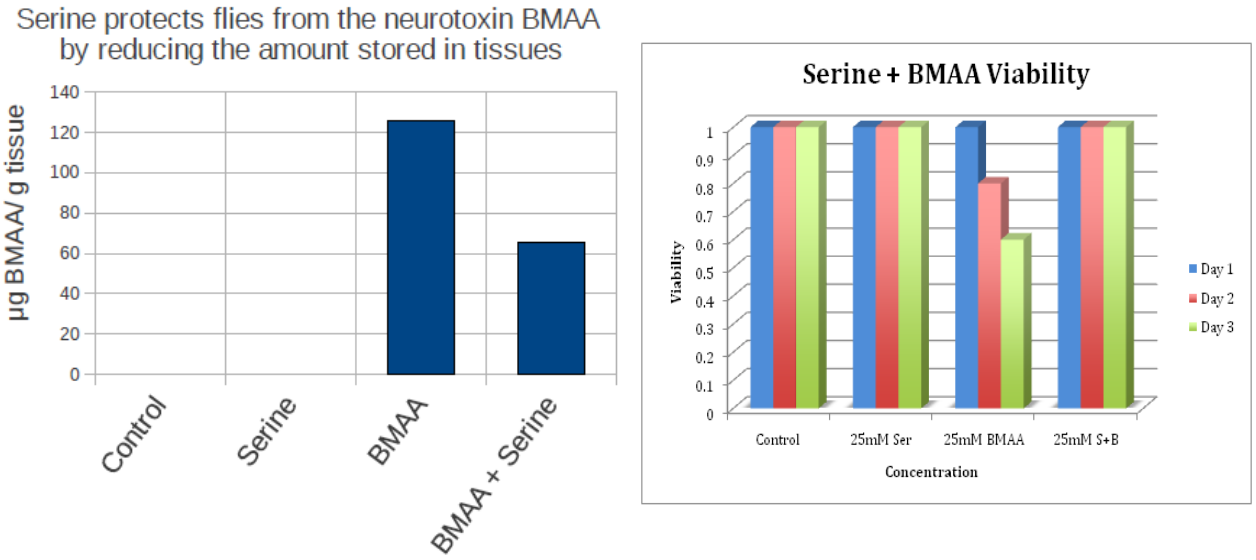


Figure 2: Survival of fruit flies fed BMAA and co-administered L-serine

Based on these *in vitro* and *in vivo* results suggesting a neuroprotective function of L-serine, we supplemented the diet of vervets exposed to BMAA with L-serine for 140 days. In replicated experiments, we found that co-administration of the dietary amino acid L-serine significantly reduced neuropathology including the density of NFT, with greater than 50% reduction in median NFT densities within the temporal (dorsal and ventral), primary motor, and entorhinal (posterior) and insula cortices, and a greater than 35% reduction of NFT density in the perirhinal cortex, amygdala, and anterior cingulate cortex (Fig. 3).

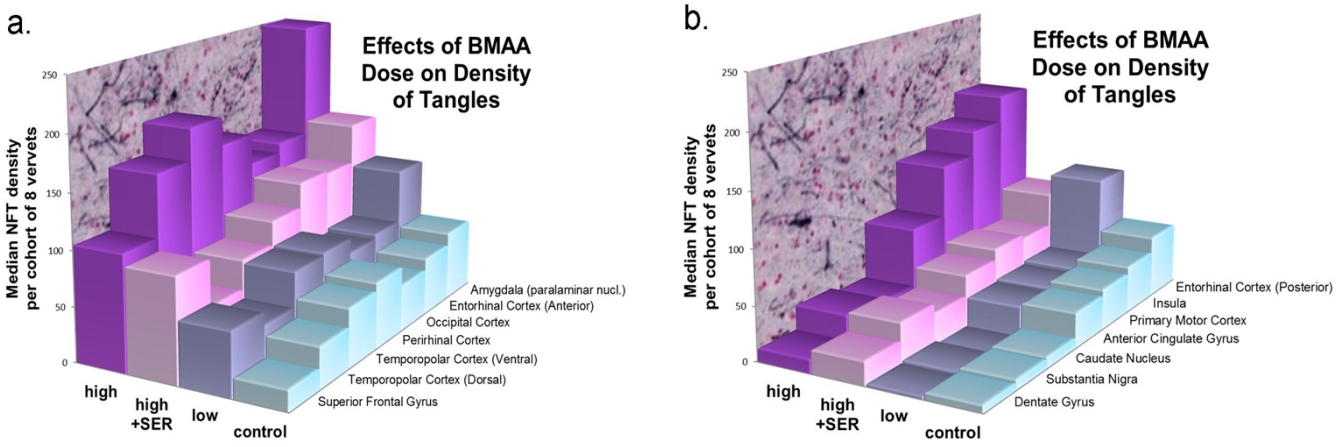


Figure 3: Reduction of NFT production in vervets fed both BMAA and L-serine (pink:high+SER) in comparison with equal amount of BMAA without L-serine (purple: high). Data from Cox et al. 2016.

1.6 Phase I Clinical Data

A phase I human clinical trial for safety of L-serine in ALS patients, funded by the Brain Chemistry Labs, was completed and published in 2016 (NCT01835782, Levine et al. 2016). Patients (n=20) with an ALSFRS-R score > 25 and a FVC score ≥ 60% predicted were randomly assigned to four different oral twice-daily dose regimens (0.5, 2.5, 7.5, or 15g) for six months. Two of the patients withdrew with gastrointestinal problems, but otherwise L-serine was well tolerated (Levine et al. 2016). Two ALS patients died during the trial plus a third patient who enrolled with a functional vital capacity (FVC) of 45% predicted, a protocol violation. The site investigator determined that for all three cases "death was due to progression of ALS and was not related to the study drug" (Levine et al. 2016, p. 3). No other adverse effects were noted among the remaining study participants, and no changes were seen throughout the trial in routine blood studies.

The ALS Functional Rating Scale-Revised (ALSFRS-R), which is strongly correlated with functional capacity and survival in ALS patients, was recorded over the course of the trial for each patient (Brooks et al. 2000, Cedarbaum et al. 1999, Kaufmann et al. 2005). The resultant ALSFRS-R scores for each patient were compared to 430 ALS patients with the same inclusion criteria from the placebo arms of five previous failed placebo-controlled clinical trials. As noted in the Phase I publication, "L-serine did not appear to accelerate functional decline of patients as measured by slope of their ALSFRS-R scores" (Levine et al. 2016, p. 1). Statistical analysis of the Phase I clinical data gave a preliminary indication that L-serine slowed functional decline of ALS patients in a dose-dependent fashion (Figure 4, Table 1).

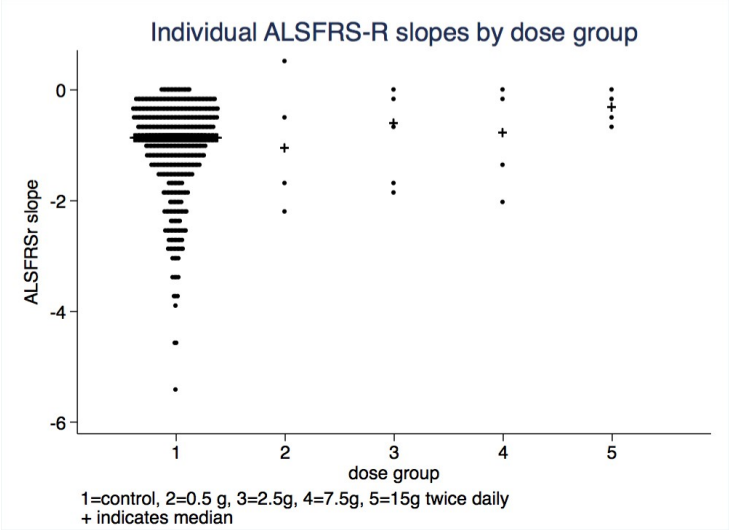


Figure 4: Phase I clinical data comparing increasing L-serine doses (groups 2-5) to 430 historical control patients from placebo arms of previous failed drug trials (group 1).

Table 1: ALSFRS-R Slope estimates for different statistical models

Method/Group	Slope (per mo decline)	95% Conf Int	p-value for L-serine effect	ALSFRS-R slope reduction
Separate Fits				
Placebo	1.09	(1.01,1.17)		
L-serine	0.76	(0.32,1.20)	0.14	30%
Linear mixed effects models				
Uncorrected				
Placebo	1.06	(0.98,1.14)		
L-serine	0.73	(0.33,1.13)	0.12	31%
Corrected for initial FVC and symptom duration				
Placebo	1.16	(1.07,1.25)	0.044	34%
L-serine	0.76	(0.38,1.41)		
Corrected and with dose[1]				
linear dose eff[2]	0.06		0.011	
separate dose eff[3]				
0.5 g	0.16	(-0.60,0.92)	0.68	14%
2.5 g	0.25	(-0.46,0.96)	0.49	22%
7.5 g	0.25	(-0.53,1.03)	0.53	22%
15.0 g	0.99	(0.20,1.78)	0.014	85%

[1] Dose in grams given twice daily
[2] Estimate is per gram reduction in slope of ALSFRS-R
[3] Estimates are reductions in slope at the specified dose

The p value of 0.014 shown above refers to the 85% reduction of functional decline for patients on the high dose 30g daily using a linear effects model. This model is comparable to a similar nonparametric model which demonstrates an increasing effect of L-serine with dose. A Jonckheere–Terpstra trend test based on medians of uncorrected ALSFRS-R slope estimates ordered by dose (ignoring the varying precision of each slope estimate) resulted in the null hypothesis H_o being rejected at the $p<0{\cdot}05$ level; thus, there was a significant relationship between increasing L-serine dose and slowing of disease progression as measured by the ALSFRS-R slope.

1.7 Relationship to Other Clinical Trials

In the Harvard study of 14 subjects with hereditary sensory autonomic neuropathy (HSAN1) L-serine was given at either 200 mg/kg or 400 mg/kg (roughly 15 and 30 g/day for a 75 kg patient, respectively) and no adverse effects were reported with either dose after 10 weeks of therapy (Garolfalo et al. 2011).

There are two additional trials currently reported by clinicaltrials.gov involving L-serine: 1) NCT01733407, 400mg/kg/d L-serine, L-Serine Supplementation in Hereditary Sensory Neuropathy Type 1; 2) NCT02599038, 20 or 200 mg/kg/day, Serine Supplementation for Obese Subjects With Fatty Liver Disease. None of these trials currently has reported data, and the fatty liver disease trial was withdrawn prior to

enrollment due to logistical reasons. It is important to note that trials with L-serine at doses of 30 g/day are underway.

On March 1, 2017, the FDA approved a clinical trial of L-serine for ALS patients funded in part by the Brain Chemistry Labs of the Institute for Ethnomedicine, a 501(c)(3) charitable research organization, with Drs. Robert Miller, Todd Levine, and Walter Bradley serving as co-PIs. This trial (IND 133995) is for a multi-site, nine-month Phase II trial for 66 ALS patients using inclusion criteria that select for ALS patients earlier in their disease course than in the completed Phase I trial. In this planned larger trial, ALSFRS-R scores of the patients in the trial will be compared to 225 subjects selected from the PRO-ACT database.

The complex nature of this planned Phase II trial and the multiplicity of institutions involved, caused the estimated costs of this trial to escalate since submission of the initial IND application. Additionally, difficulties in obtaining external funding have resulted in concomitant delays in the projected date of trial initiation. While remaining committed to funding this larger, more complex multi-site trial, Dartmouth-Hitchcock Medical Center and Brain Chemistry Labs proposes to initiate a single site Phase IIa trial prior to the initiation of the planned larger Phase II trial (IND 13399). Hence, with funding from Brain Chemistry Labs, Dartmouth-Hitchcock Medical Center proposes a single site Phase IIa trial of the tolerability of L-serine for ALS patients, with the added potential of providing additional indications of efficacy, as well as exploratory biomarker studies based on measures of the neuroprotective effects of L-serine.

1.8 Study Objectives

1.8.1 Primary Objective

To assess the tolerability of taking 30g/day of L-serine in ALS patients.

1.8.2 Secondary Objective

To assess the efficacy of L-serine in subjects with probable or definite ALS.

1.8.3 Tertiary Objectives

To explore the possibility of new ALS progression biomarkers by using neurally-derived exosomes from blood samples. Assess effects within subgroups defined by serine synthetic gene polymorphisms.

2 Study Design

2.1 General Design

This is a Phase IIa study of the effects of L-serine on ALS patients. Potential candidates for participation in this study will be identified by the Investigator as having probable or definite ALS by the modified El Escorial criteria (Brooks et al., 2000), an ALSFRS-R score >25 and FVC ≥60%. These measures are completed as part of standard practice. Patients currently taking an L-Serine supplement at any dose will be required to have a 30 day washout period prior to the start of study drug. Up to 50 subjects will be enrolled

over the course of approximately four years. Subjects participating in the study will receive a 15 gram twice daily dose. Study duration will last approximately 48 weeks from time of enrollment for each enrolled subject. Study treatment administration will last approximately 6 months (24 weeks). After an initial screening/baseline visit, patients will be instructed to take one 15 g dose of L-serine per day for a two-week period. If they tolerate the drug well, they will be instructed at the end of the second week to increase their dose to one 15 g dose of L-serine in the morning and one 15 g dose of L-serine in the evening, for a total of 30 g per day for the remainder of the six-month trial. At any stage in the trial, if a patient experiences unacceptable side effects, such as nausea or vomiting, the affected patient's daily dose will be reduced to 15 g/day for the duration of the trial. Patients will return at month 3 for assessments and drug dispensing and at month 6 for assessments. All remaining study drug will be collected at month 6 and returned to the study sponsor.

2.2 Use of Historical Placebo Patient Control Data

In this Phase IIa trial, we will use historical control data from 430 placebo patients in the PRO-ACT database for comparison purposes to test the secondary objective. We will assess the efficacy of L-serine in subjects with probable or definite ALS, in the same way that occurred in the earlier FDA-approved Phase I clinical trial of L-serine for ALS patients (Levine et al, 2016). The use of historical control data in human clinical trials is recognized as having "the potential to further streamline the development of drugs" (Viele et al, 2014). In this single-arm open label clinical trial the use of historical control data presents significant advantages over a more traditional clinical model such as a double-blinded, randomized Phase IIa controlled trial (Williams, 2001) of the type we are currently conducting at Dartmouth-Hitchcock Medical Center for early stage Alzheimer's patients (ClinicalTrials.gov Identifier NCT03062449).

First, unlike early stage Alzheimer's disease patients, a rapidly growing segment of the ALS patient population take L-serine as a dietary supplement. The primary objective of this trial is to assess tolerability of ALS patients taking 30 g/day of L-serine, with a secondary objective of assessing the efficacy of L-serine for ALS patients. A commonly marketed dietary supplement, L-serine is considered as GRAS and has FDA approval as a normal food additive under CFR172.320. It is inexpensive and can be easily purchased from the internet. Thus, it is becoming increasingly difficult to recruit ALS patients for clinical trials who have not taken, either with the advice of their physician or as a self-dose regimen, L-serine. At this point, there is a "paucity of willing participants for randomization into control and experimental groups" (Desai et al., 2013).

Second, the study outcome of the Phase I clinical trial (Levine et al., 2016) yielded a preliminary, but statistically significant, indication that L-serine may slow functional decline in ALS patients as measured by the slopes of their ALSFRS-R scores. Thus, there are ethical concerns about randomly assigning patients to a placebo group for an extended period (in this case six months) if there is a distinct possibility that they could benefit from the study drug. Such ethical concerns have been raised in randomly assigning patients to other placebo or suboptimal treatments in clinical studies (Desai et al., 2013; Polman et al., 2008; Spławiński and Kuźniar, 2004).

Third, this Phase IIa trial is considered to be preliminary to a larger Phase II trial (IND 133995) of L-serine for ALS patients (see Section 1.7: Relationship to Other Clinical Trials). If the primary objectives of the current trial are not met, and if tolerability of L-serine at 30 g/day is not demonstrated, or if the secondary objective of assessing efficacy of L-serine in subjects with probable or definite ALS does not yield a positive result, this could result in redesign or reconsideration of the larger Phase II trial. For these purposes, it is important that the proposed Phase IIa trial, if approved by the FDA, be quickly initiated and that patient enrollment occur at a rapid pace.

Fourth, the historical control placebo patient data from the PRO-ACT database are voluminous. There are 430 control patients in that database who match our inclusion criteria for this proposed single-arm trial. This large control patient data base sample has the advantage of constrained variability of the range of ALSFRS-R slopes; it is unlikely that further increase in control patient numbers will significantly impact either the sample mean or sample variance for placebo patients as an estimate of disease progression within the ALS patient population. In other words, the large size of the comparison group serves to reduce the possibility of a type I error in which the null hypothesis would be incorrectly rejected and we would falsely detect an effect that is not present.

For these four reasons, we have designed a single-arm open label trial in which all patients will receive L-serine at 30 g/day and we will use historical control patient data to determine the secondary objective of the study, e.g. possible efficacy.

2.3 Study assessments

See section 5.1 for list of assessments performed at each visit If unable to obtain any of the assessment due to restrictions on patient visit by Dartmouth-Hitchcock or the participant choice regarding

- Vital signs (blood pressure, heart rate, temperature, respiratory rate, height, weight pulse oximetry)
- Neurological exam
- Brief physical exam
- ALSA-Q40 (Amyotrophic Lateral Sclerosis Assessment Questionnaire (long form))
- ALSFRS-R (Amyotrophic Lateral Sclerosis Functional Rating Scale - Revised)
- FVC % predicted (Forced Vital Capacity % predicted)
- C-SSRS (Columbia-Suicide Severity Rating Scale)

2.4 Blood and Urine Samples

If unable to obtain urine or study blood work due to restrictions on patient visit by Dartmouth-Hitchcock or the participant choice regarding illness prevention the urine and or bloodwork will be obtained at first in person visit after the study visit scheduled date. Study participants that require urine pregnancy testing will be sent home at

baseline visit or shipped urine pregnancy tests to be done at home. The study coordinator will contact the participant for the reminder and the results of test.

Urine samples for pregnancy testing will be collected at baseline, 3 months, and 6 months (Visit 1, Visit 2, Visit 3). Blood samples will also be collected at baseline, 3 months, and 6 months (Visit 1, Visit 2, Visit 3). In addition to safety labs, serum pregnancy, and L-serine levels, blood will be used to extract neurally-derived exosomes (Goetzl et al., 2015; Winston et al., 2016; Thompson et al, 2016). The RNA content of these exosomes will be analyzed for potential biomarkers for L-serine efficacy based on analysis of transcripts associated with endoplasmic reticulum stress and the unfolded protein response using extraction of mRNA and proteins Exosomes are a class of endosome-derived membrane vesicles shed by neural cells (among other cells) and transported in blood plasma. We isolate the neurally-derived exosomes using LiCAM antibodies, which react with a neural cell adhesion molecule attached to exosome membranes. These exosomes provide an accessible source of proteins, RNA, and DNA excreted by neuronal cells and have been used to distinguish early stage Alzheimer's patients from controls (Fiandaca et al. 2015). We will examine neurally-derived exosomes for biomarkers that can temporally associate with ALS progression using baseline, mid, and endpoint blood samples. This proof-of-concept study will determine the potential utility of exosome biomarkers in future trials of L-serine, or other treatments.

We also study chiral ratios of L- and D-serine in blood samples from patients, comparing baseline ratios to endpoint ratios. In combinations with genetic testing, we will determine if there is a relationship between human serine synthetic gene deficiency and chiral ratios. We will also determine if L-serine therapy alters the ratio of these enantiomers (Metcalf et al., 2017). The chiral ratio alterations could be used as a potential biomarker of L-serine supplementation biological effect.

2.5 Saliva Samples

In order to look for polymorphisms in key human serine synthetic genes, a saliva sample will be taken from each patient enrolled in the clinical trial on a consented voluntary basis. Samples will be collected in saliva tubes specific for study of DNA, stabilizing the samples for up to 12 months at room temperature. Exon DNA (protein coding DNA) will be sequenced for analysis of serine racemase, 3-phosphoglycerate dehydrogenase, phosphoserine phosphatase and phosphoserine aminotransferase to determine if positive patient responses to administration of L-serine may be due to mutations in their serine biosynthetic genes. Responses of individual patients (slope of ALSFRS-R and FVC) to L-serine treatment will be compared between patients with normal gene sequences for serine racemase, 3-phosphoglycerate dehydrogenase, phosphoserine phosphatase and phosphoserine aminotransferase and those who have different sequences for those genes.

2.6 Primary Study Endpoints

The primary endpoint of this study is tolerability. We will assess the proportion of enrolled patients who tolerate and are compliant with the 30g/day dose, and the proportion who scale back to 15g/day. We will also assess reasons for any withdrawals

from the study. Rates of adverse events will be compared between the treatment and historical control groups.

2.7 Secondary Study Endpoints

A secondary endpoint to be analyzed is the possibility that L-serine slows functional decline. The slopes of the ALSFRS-R and FVC through time will be used to test for treatment effect, comparing L-serine patients with the 430 historical placebo controls from the PRO-ACT database.

3 Subject Selection and Withdrawal

3.1 Inclusion Criteria

1. Valid informed consent by subject.
2. Diagnosis of probable or definite ALS based on the El Escorial criteria (Brooks et al., 2000) within the last three years prior to study enrollment.
3. ALSFRS-R score > 25 and a FVC score ≥ 60% predicted.
4. Patients currently taking Riluzole and Edaravone/Radicava will be allowed into the trial as long as they have been taking these FDA-approved drugs for three months prior to baseline/screening at a stable dose. If the patient has been instructed by his or her physician to stop or pause Riluzole or Edaravone/Radicava due to an adverse event the requirement for inclusion into the study will be off Riluzole or Edaravone/Radicava for seven (7) days, This also applies if the dosing has not been stable for three (3) months prior to enrollment and treatment. Initiating or restarting these medications after enrollment and treatment has begun is allowed
5. Age ≥ 18 years old.

3.2 Exclusion Criteria

1. Diagnosis of probable or definite ALS more than three years prior to study enrollment.
2. Diagnosis or previous history of ischemic stroke, brain tumor, uncontrolled diabetes, renal insufficiency (an estimated GFR <60mg/dl)or severe hypertension. Severe hypertension (asymptomatic or hypertensive urgency) is defined as severely elevated blood pressure (180 mm Hg or more systolic, or 110 mmHg or more diastolic) without acute target organ injury.
3. Diagnosis or previous history of progressive neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, Lewy Body Disease, Pick's Disease, Huntington's Disease, Progressive Supranuclear Palsy. However, ALS patients diagnosed with frontotemporal dementia will not be excluded from this study.
4. Diagnosis or previous history of symptomatic peripheral neuropathy. Patients with findings of peripheral neuropathy on electrodiagnostic tests only but no clinical symptoms at the time of enrollment are eligible.
5. Undergoing any chemotherapy or radiation therapy for any cancer.
6. Any medical condition likely to interfere with the conduct of the trial or survival of the patient during this study period.
7. Pregnant women or women who are breast-feeding.

8. If subject has taken an L-Serine supplement less than 30 days prior to start of study drug. a 7 day waiting period is required.
9. In the Investigator's opinion, subject would be unable to successfully participate in the study for any reason.

3.3 Subject Recruitment and Screening

Participation in this research requires informed consent according to Institutional Review Board (IRB) guidelines. A signed IRB approved Consent Form is the means of documenting this understanding. No one is allowed to participate without having signed this statement personally and received an exact copy (at point of signature acquisition). Potential recruits are instructed that their participation is completely voluntary and that their medical care will not be altered in any way should they elect not to participate. Subjects are recruited from patients presenting to or referred to the Department of Neurology with a diagnosis of ALS using El Escorial criteria (Brooks et al., 2000). Potential subjects that do not present to or are not referred to the Department of Neurology may also be prescreened by the principle investigator, sub-investigator, study coordinator, or research nurse listed with the IRB for this study to determine potential eligibility for participation in this study. PHI may be retrieved from medical records, and/or from the subject. Subjects will be prescreened based on their medical history for study inclusion/exclusion criteria. Subjects deemed to be potentially eligible will be initially contacted by the investigator and then by the study coordinator or research nurse to gather eligibility criteria. Subjects may be given a copy of the consent form and subject study information to review prior to enrollment.

3.4 Early Withdrawal of Subjects

3.4.1 When and How to Withdraw Subjects

Participants who cannot tolerate the study drug, have adverse reactions to the L-serine, or would like to withdraw for any other reason can withdraw from the study at any time. If a participant experiences an adverse event which is serious or life threatening in nature, and deemed to be drug related, the study medication will be discontinued immediately. The Investigator may reserve the right to discontinue a subject from study treatment if the PI feels it is not in the best interest of the patient to continue.

If a participant discontinues study medication, they will be encouraged to remain in the study and complete study related procedures and assessments while not receiving study medication.

If a participant discontinues study medication and does not wish to return for the remainder of study visits, this decision will be documented. They will be asked to return to the site for a final study visit. This visit will be conducted with the same study procedures and assessments as "Visit 3" listed in Section 6.5.

3.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a patient is terminated early from the study because of an adverse event, every effort will be made to follow the patient until the adverse event resolves and to have the

ALSFRS-R score recorded, by telephone if necessary, at the 6-month end of study time period.

4 Study Drug

4.1 Description

L-serine is a non-essential amino acid already present in the human diet. This drug will be presented as a powder in packets containing 15g of L-serine each. The L-serine will be packaged into a foil or Mylar package. Patients will be instructed to begin the trial with a two-week dose period where they will take one 15g dose of L-serine in the morning. If the 15g daily dose is well-tolerated, the patients will be instructed to then take one package in the morning and one in the evening, for a total of 30g of L-serine/day for the remainder of the trial. Patients will be encouraged to dissolve the L-serine powder in water or another beverage or to mix it with their food.

4.2 Manufacturer and Manufacturing Details of Bulk Drug Substance

Parchem Fine & Specialty Chemicals (415 Huguenot Street, New Rochelle, New York 10801) is the supplier of L-serine for the trial, and the L-serine was manufactured by Wuhan Grand Hoyo Co., Ltd. (No. 1 Industrial Park, Gedian Economy Development Zone, E'hou City, Hubei, China). L-serine is produced via fermentation.

The specifications for the drug substance (including the tests performed, analytical procedures, and acceptance criteria) are provided by the manufacturer in the Investigator Brochure. Brain Chemistry Labs will independently screen L-serine powder from lot number 170603 during packaging and throughout the trial using triple quadrupole liquid chromatography mass spectrometry in Jackson Hole. Our experience in screening L-serine provided by Parchem is that, to date, the lots we have analyzed have met the necessary criteria as determined by triple quadrupole mass spectrometry for use in human clinical trials and is spectroscopically consistent with an authenticated standard provided by Sigma-Aldrich (St. Louis, MO).

4.3 Stability Data for the Drug Substance

L-serine, as a small (105.09 MW) proteinogenic amino acid, is extraordinarily stable. We include in the Investigator Brochure long-term stability data from the manufacturer for L-serine over 24 months when held at room temperature (<30°) with relative humidity <75%. As can be seen, no significant changes were observed in optical rotation or purity over this period. We have also performed our own studies on L-serine stability in general based on samples from two different manufacturers over a 3.5-year period using triple quadrupole mass spectrometry. We were able to independently confirm the stability of L-serine powder when maintained at room temperature.

4.4 Packaging

- L-serine dose preparation will be conducted in a cGMP FDA-compliant facility by Knechtel (7341 Hamlin Avenue, Skokie, IL 60076) and the dose will be placed in sealed foil packages. Each package will contain 15 g L-serine powder. The L-serine screening and milling procedures by Knechtel are shown in the Investigator

Brochure. The specification materials used for pouches to package L-serine powder are manufactured by ESP Packaging LLC (3001 Red Hill Avenue 1-105, Costa Mesa, CA 92626).

- Each individual foil pouch containing a single patient dose will be labeled with the lot number containing the Julian code date. Each box that is prepared to be delivered to the patients will contain multiple pouches designated for use in a specified period of time, will include the following statement: "Caution: New Drug—Limited by Federal (or United States) law to investigational use."
- Foil packages sufficient for three months will be given to the patients at their first visit and also at their three-month visit. Doses will be sent to Dartmouth-Hitchcock Medical Center in periodic shipments over the length of the project.

4.5 Blinding of Study Drug

All subjects are receiving L-Serine, and no blinding is necessary.

4.6 Treatment Regimen

All subjects will receive the same dose (15 g twice daily) after the initial two-week dose period of 15 g per day, unless they develop unacceptable side effects (such as nausea or vomiting). In the event of such intolerable side effects, patients will be instructed to reduce the daily dose to 15 g per day, and may increase to 30g once the intolerable side effect has been resolved, according to PI . The study has prepared an optional drug diary for the subjects to utilize to assist with accountability. These diaries are optional.

4.7 Method for Assigning Subjects to Treatment Groups

4.8 Preparation and Administration of Study Drug

Study drug will be dispensed to the subject at study visits or shipped bv the Investigational Pharmacy to the participant to arrive at the scheduled visit date To be taken orally by the subject at home. Subjects will be instructed to mix the study drug powder with a liquid or food.

4.9 Subject Compliance Monitoring

Subjects will have the opportunity to use study-provideddrug diaries in effort to improve compliance and provide assistance to study staff in compliance monitoring. This is an optional task for the subject, and some may choose not to participate. Those who do will be asked to bring their drug diaries, and used and unused foil packages with them to each study visit for the study coordinator to review and log reported compliance into the CRF. For subjects who do not choose to utilize a drug diary, accountability and compliance will occur by counting the amount of empty packets returned and the comparing it to the number of doses that should have been taken.

4.10 Prior and Concomitant Therapy

Information regarding a subject's current medication regimen will be collected to assist with safety monitoring and for data collection purposes. Patients will be encouraged to follow their physician instructions in taking Riluzole and Earavone/Radicava at the same dose if they have already been taking these medications. If the patient has been instructed by his or her physician to stop or pause Riluzole or Edaravone/Radicava due

to an adverse event the requirement for inclusion into the study will be off Riluzole or Edaravone/Radicava for seven (7) days, This also applies if the dosing has not been stable for three (3) months prior to enrollment and treatment. Initiating or restarting these medications after enrollment and treatment has begun is allowed. Patients will record medication, doses, and any changes to the dosing in their provided diaries. This information will be confirmed with the patient by the study coordinator during Visit 1, 2, and/or 3 as well as phone visits and then will be entered into the CRF for data collection and analysis.

4.11 Receiving, Storage, Dispensing, and Return

4.11.1 Receipt of Drug Supplies

The L-serine will be shipped directly from Knechtel to Dartmouth-Hitchcock Medical Center in batch shipments. Upon receipt of the study treatment supplies by the receiving pharmacy, the designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator will notify the Brain Chemistry Labs of any damaged or unusable study treatments that were supplied to the site.

4.11.2 Storage

L-serine foil packages should be kept dry location at 10-20 degrees C during storage. Patients should be advised to keep their monthly packets in a cool, dry location.

4.11.3 Dispensing of Study Drug

Each subject will be given foil packages that contain the correct dose (15 g) to be taken once a day during the initial two-week period, and twice a day thereafter. Subjects will be given at the baseline visit the appropriate number of packages to last three months, plus one week (186 packages). At the 3-month visit, any unused drug will be collected, as well as the used foil packets to check study drug compliance. Subjects will be given the appropriate number of packages to last three months (186 packages). At the 6-month visit any unused study drug and used foil packets will be collected by study staff. If the subject is unable to attend an in person study visit due to Covid19 or any other widespread illness visit restrictions, the study drug will be shipped to the subject to be received at the scheduled study visit date. The subject will receive notification that the study drug will be shipped, and may includea new diary and detailed instructions, depending on if the subject is using the study-provided diary The return of the used packets and unused medication will be brought in by the subject at the next in person visit or mailed to the study team by the subject.

Patients should return any unused packets and report compliance during study visits. A log of study drug issued and study drug returned with date of issue and return will be kept by study staff

4.11.4 Return or Destruction of Study Drug

At the completion of the trial, a final reconciliation of all study drug shipped, consumed and remaining will be performed. All remaining drug will be returned to the Brain Chemistry Labs, PO Box 3464, Jackson, WY 83001.

5 Study Procedures

5.1 Visits and telephone calls

The study timeline includes three in-clinic visits as well as five follow-up phone calls, with one occurring 6 months after treatment completion (month 12). The study visits and timeline are as follows with a -/+ 2 week window Visits that cannot be attended by the subject due to restrictions will be done by telephone by the investigator and the study coordinator to obtain information from the subject. Assessments that cannot be done remotely, will be obtained at the next in person visit.

Visit 1: Baseline: Screening and Enrollment

- Informed consent obtained
- Inclusion /Exclusion Criteria
- Demographics
- Vital signs (blood pressure, heart rate, temperature, respiratory rate, height, weight, pulse oximetry)
- Brief medical history and neurological and brief physical exam
- Concomitant medications
- ALSFRS-R
- ALSA-Q40
- Pulmonary Function - FVC % predicted
- Blood draw
- Urine pregnancy test
- Dispense study drug
- Dispense study drug diary (optional)
- Dispense instructions for dosing and storage of L-Serine
- Saliva sample (optional, and may be obtained at any visit)
- C-SSRS

Telephone Call 1: Week 3 (+ or – 2 weeks)

- Adverse events
- Concomitant medications
- L-serine tolerability and dosing

Telephone Call 2: Week 6 (+ or – 2s weeks)

- Adverse events
- Concomitant medications
- L-serine tolerability and dosing

Visit 2: 3 month visit (+ or – 2 weeks)

- Vital signs
- Neurological and brief physical exam
- ALSFRS-R
- ALSA-Q40
- FVC % predicted

- Concomitant medications
- Adverse events
- Blood draw
- Urine pregnancy test
- Collection of unused study drug and diary (if applicable)
- Dispense study drug
- Dispense study drug diary (optional)
- Saliva sample (optional, and may be obtained at any visit)
- C-SSRS

Telephone Call 3: Week 18 (+ or – 2 weeks)
- Adverse events
- Concomitant medications
- L-serine tolerability and dosing

Visit 3: 6 month visit (+ or – 2weeks)
- Vital signs
- Neurological and brief physical exam
- ALSFRS-R
- ALSA-Q40
- FVC % predicted
- Concomitant medications
- Adverse events
- Blood draw
- Urine pregnancy test
- Collection of unused study drug and diary (if applicable)
- Saliva sample (optional, and may be obtained at any visit)
- C-SSRS

Telephone Call 4: Week 28 (+ or – 2 weeks)
- Adverse events
- Concomitant medications
- Continued supplementation status

Telephone Call 5: Week 48 (+ or – 2 week)
- Adverse events
- Concomitant medications
- Continued supplementation status
- ALSFRS-R
- Overall study impression

5.1.1 **Study Timeline and Events** (+ / - 2 week for each visit/phone call)

Visit / Telephone	Visit 1	Telephone 1	Telephone 2	Visit 2 3 Months	Telephone 3	Visit 3 6 Months Treatment ends	Telephone 4	Telephone 5
Week	Week 0	Week 3	Week 6	Week12	Week 18	Week 24	Week 28	Week 48
Informed Consent	X							
Inclusion/ Exclusion Criteria	X							
Demographics	X							
Brief Medical History	X							
Concomitant Medications	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
Brief Physical Exam	X			X		X		
Neurological Exam	X			X		X		
Vital Signs- weight, height, temperature, blood pressure, pulse, respiratory rate, pulse oximetry	X			X		X		
Pulmonary Function (FVC % predicted)	X			X		X		
ALSFRS-R	X			X		X		X
ALSA-Q40	X			X		X		
C-SSRS	X			X		X		
Optional Saliva Sample	X			X		X		
Blood samples drawn	X			X		X		
Safety labs (CBC, CMP)	X			X		X		
Serum pregnancy test	X							
Urine pregnancy test	X			X		X		
Saliva Sample (optional)	X			X		X		
Dispense study drug	X			X				
Dispense study drug diary (optional) and information	X			X				
Collection of diary and unused study drug (optional)				X		X		
Study drug compliance				X		X		
L-serine tolerability		X	X	X	X	X		
Continued Supplementation Status							X	X
Study Impression								X

5.2 Blood Draws and Specimen Processing, Storage, and Shipment

Safety labs will be drawn at three time points: Visit 1 (baseline: screening/enrollment), Visit 2, and Visit 3. Safety labs, sent to DHMC Pathology laboratory will include:

- Complete Blood Count
- Comprehensive Metabolic Panel

Safety labs may be repeated at the discretion of the investigator
Serum pregnancy test to be done at Visit 1 (screening)
Urine pregnancy testing will be done at Visit 1 (screening), Visit 2 and Visit 3. Blood draw and urine pregnancy tests will be done at next in person visit if the subject is unable to attend the visit due to restrictions. Urine pregnancy testing, if required, will be performed at home by the subject with study dispensed urine pregnancy tests. The coordinator will call the subject as a reminder to do the test at the visit date, and will also document in source the result as reported by the subject.

In addition, concurrent with the safety labs, blood samples will be drawn and sent to the Brain Chemistry Labs for analysis of L-serine concentrations and neurally-derived exosomes at three time points (Visit 1 (baseline/enrollment), Visit 2, and Visit 3).

Optional saliva samples can be obtained once during the study at any visit, and will be sent to the Brain Chemistry Labs where they will be stored for batch genetic sequencing as indicated in Section 2.5.

5.3 Optional Consent for Saliva Sample, Biobanking, and Future Analysis of Blood Samples

There will be an option in the informed consent document for a saliva sample to be obtained to look for polymorphisms in key human serine synthetic genes and for patients to have a portion of their blood sample drawn to be de-identified, saved, and used for future research by the Brain Chemistry Labs. The researchers at Brain Chemistry Labs wish to use the optional donations for future ALS research. This may help researchers learn about factors that can help prevent or promote disease in the future. No results will be reported back the patient. No extra blood is required for the blood donation. Opting in is not required for study participation.

5.4 Administration of the Columbia Suicide Severity Rating Scale (C-SSRS)

The Columbia Suicide Severity Rating Scale (C-SSRS) will be administered at Visits 1, 2, and 3. The investigator will review answers given by the patient before the end of the visit in order to identify risk as presented with this evidence based tool. Any score greater than 0 may indicate the need for mental health intervention as decided by the investigator. A score of 4 (active suicidal ideation with some intent to act) or 5 (active suicide ideation with specific plan and intent) will be used to trigger further evaluation or immediate contact with a prompt referral to a mental health professional and/or possibly the emergency department.

6 Statistical Plan

6.1 Sample Size Determination

Anticipating that patients may drop out of the study before the six month visit for various reasons, which we conservatively estimate at 20%, or 9 patients, enrollment of n=50 patients will be adequate to provide the needed statistical power for data analysis. With n=36 participants completing the full six month study visit in the L-serine group, we expect to have 80% power to detect a meaningful difference in functional decline, defined as a difference in mean ALSFRS-R slope of 0.43 between the L-serine treatment and historical placebo control groups (ratio 10 placebo patients: 1 L-serine patient, standard deviation (SD) = 0.88, alpha = 0.05). However, as noted below in 6.4, we reserve the right to terminate the trial at n = 30 participants completing the full six-month trial in the L-serine group if it is statistically determined that the trial is either futile or efficacious.

6.2 Statistical Methods

The primary outcome of the study is tolerability of the L-serine oral formulation. We will assess the proportion of patients in the treatment group who: report experiencing GI symptoms on the 15 g/day dose, report experiencing GI symptoms on the 30 g/day dose, or withdraw prior to the 6-month visit.

We will begin by assessing the comparability of the baseline functional status of the patients by comparing symptom duration, ALSFRS-R scores, and FVC measures for the historical placebo group vs. the L-serine treatment group at time zero using a t-test. If the data are not normally distributed, we will transform the data or use a non-parametric statistical analysis.

We will then longitudinally assess the declining slopes of the ALSFRS-R scores throughout the study period by constructing linear mixed-models to analyze repeated measures over the ALSFRS-R assessment time points. We will assess the between-patient effect of treatment. Our null hypothesis is that there is no difference between the mean ALSFRS-R slopes for the fixed-effect of the historical placebo vs. L-serine treatment. The models will include individual random-effects to account for time-invariant factors related to genetic susceptibility, and other unobservable individual confounders. Models will be adjusted for differences in baseline ALSFRS-R or FVC and symptom duration, as needed. In addition, we will assess trends in the within-patient effect of L-serine treatment on ALSFRS-R scores, FVC%, as well as the exosomal biomarkers compared to the baseline visit.

6.3 Subject Population(s) for Analysis

The primary study analysis and any applicable secondary analyses will include any subject enrolled into the study who has received at least one treatment dose.

6.4 Efficacy/Futility May Result in Early Termination of the Study

In 2010, the FDA issued a draft Guidance for Industry entitled, “Adaptive Design Clinical Trials for Drugs and Biologics.” While carefully considering the limitations of adaptive

designs, including potential to increase the chance of erroneous positive conclusions, and loss of study power in blinded interim analyses, the FDA noted in Section V.D. that "Methods have been developed that allow valid analyses of interim data and provide well-recognized alpha spending approaches to address the control of the Type I error rate . . . If the drug is more effective than expected, the accumulating data can offer strong statistical evidence of the therapy's success well in advance of the planned completion of the study."

One of the obvious benefits of an adaptive design is that not only can a trial be stopped on early indications of efficacy or futility, but less patients are needed to complete the trial as compared to a fixed sample design. Although there has been some consideration given to sequential monitoring of patients in a single-arm trial (Thall et al., 1995), with the benefit of data from the Phase I clinical trial of L-serine for ALS patients, we have been able to estimate the minimal sample size necessary to replicate preliminary indications of efficacy if we have in the Phase IIa trial $n \geq 27$ patients, based on the differences between slopes of the ALSFRS-R scores of the low-dose patients (1 g/day) and the high-dose patients (30 g/day) in the Phase I trial. Since we propose an open label single-arm Phase IIa trial using data from historical placebo control patients, we will make an initial analysis of the secondary objective after the 30th patient finishes the six-month trial. If the magnitude of efficacy observed in the Phase I trial is replicated, or if no efficacy whatsoever is detected, we may choose to terminate the Phase IIa trial without continuing to the planned 50 patients. However, if there are preliminary indications of efficacy that do not rise to the level of replication of the possible efficacy detected in the Phase I trial, or if the analysis of biomarkers which constitutes the tertiary objective appears productive, we may choose to continue the trial to the full n = 50 patient design.

7 Safety and Adverse Events

Information obtained from any standard of care visits as well as study visits and telephone calls will be reviewed for adverse events. Adverse events will be recorded in the participants study file and CRF, along with date of occurrence, date of resolution, severity and any actions taken.

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- U<u>nexpected in nature, severity, or frequency (</u>i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- R<u>elated or possibly related to participation in the research (</u>i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- S<u>uggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

Adverse Event
An **adverse event** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A **serious adverse event** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition.

Post-study Adverse Event
At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
The PI will make the determination of clinical significance of lab values that are out of normal range. Those of clinical significance will be reported as adverse events.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

7.3.1 Investigator reporting: notifying the Dartmouth - HitchcockIRB

Adverse events and unanticipated problems posing risks to subjects or will be reported to the Dartmouth- Hitchcock IRB as required by the DHH- IRB, within the required timeframe and using the appropriate form or as a written report of the event.

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

7.4 Unblinding Procedures

All patients receive the same dose, therefore no blinding is necessary.

7.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events. In addition to PI oversight, a medical monitor external to the study will be identified. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

- Currently, the Medical Monitor is Dr. Victoria Lawson, MD, Neurologist at Dartmouth Hitchcock Medical Center. Dr. Lawson is not involved in the study in any way other than as a medical monitor.

This monitoring will take place once every year, and will include a thorough review of the annual report, which includes enrollment numbers, withdrawals, unexpected and expected adverse events, and deaths. Each annual report will also include a passage about the study, including length and procedures performed.

After completion of the monitoring review, the monitor will sign off on her review, and signal if a follow-up discussion is required. This form will be signed by both the medical monitor and by the study PI.

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Information obtained during prescreening also applies to the Health Insurance Portability and Accounatabily Act of 1996. No PHI used for prescreening will be released for presentation or publication.

Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for this study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked a written notation will be made. If an item is not applicable to the individual case, written notation will be made.

8.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there

are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

The Clinical Trials Office will be monitoring this study. The investigator will allocate adequate time for monitoring activities which includes but is not limited to reviewing source documents and case report forms; checking for study medication accountability and study drug dispensation documentation; reviewing regulatory documents and the protocol. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

9.2 Auditing and Inspecting

The study investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and Dartmouth compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The study investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Dartmouth compliance and quality assurance offices.

10 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

10.1 Funding Source

This study will be funded by the Brain Chemistry Labs at the Institute for Ethnomedicine, a not-for-profit 501(c)(3) charity in Jackson Hole, Wyoming.

10.2 Conflict of Interest

All Dartmouth investigators will follow the Dartmouth conflict of interest policy. The Brain Chemistry Labs has applied for patents for the use of L-serine to treat neurodegenerative illness (US 13/683,821).

10.3 Subject Stipends or Payments

Patients will not be paid, but will be given a travel stipend and/or allowance, and as needed, overnight accommodations near Dartmouth-Hitchcock to participate in clinical visits.

10.4 Publication Plan

Publication of results will be the responsibility of the Brain Chemistry Labs in conjunction with Dartmouth-Hitchcock Medical Center.

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12 Appendices

Appendix 1. ALS Functional Rating Scale - Revised (ALSFRS-R)

Appendix 2. ALSA – Q40

Appendix 3. C-SSRS

L-serine IIa in ALS	Patient Number: _____	Visit: _____ Date: _____

ALS FUNCTIONAL RATING SCALE (ALSFRS-R)		
1. Speech	4	Normal Speech processes
	3	Detectable speech with disturbances
	2	Intelligible with repeating
	1	Speech combined with nonvocal communication
	0	Loss of useful speech
2. Salivation	4	Normal
	3	Slight but definite excess of saliva in mouth; may have nighttime drooling
	2	Moderately excessive saliva; may have minimal drooling
	1	Marked excess of saliva with some drooling
	0	Marked drooling; requires constant tissue or handkerchief
3. Swallowing	4	Normal eating habits
	3	Early eating problems – occasional choking
	2	Dietary consistency changes
	1	Needs supplemental tube feeding
	0	NPO (exclusively parenteral or enteral feeding)
4. Handwriting	4	Normal
	3	Slow or sloppy; all words are legible
	2	Not all words are legible
	1	Able to grip pen but unable to write
	0	Unable to grip pen
5a. Cutting Food and Handling Utensils (patients without gastrostomy)	4	Normal
	3	Somewhat slow and clumsy, but no help needed
	2	Can cut most foods, although clumsy and slow; some help needed
	1	Food must be cut by someone, but can still feed slowly
	0	Needs to be fed
5b. Cutting Food and Handling Utensils (patients with gastrostomy)	4	Normal
	3	Clumsy but able to perform all manipulations independently
	2	Some help needed with closures and fasteners
	1	Provides minimal assistance to caregivers
	0	Unable to perform any aspect of task
6. Dressing and Hygiene	4	Normal function
	3	Independent and complete self-care with effort or decreased efficiency
	2	Intermittent assistance or substitute methods
	1	Needs attendant for self-care
	0	Total dependence

L-serine IIa in ALS	Patient Number: _____	Visit: _____ Date: _____

7. Turning in bed and adjusting bed clothes	4	Normal
	3	Someone slow and clumsy, but no help needed
	2	Can turn alone or adjust sheets, but with great difficulty
	1	Can initiate, but not turn or adjust sheets alone
	0	Helpless
8. Walking	4	Normal
	3	Early ambulation difficulties
	2	Walks with assistance
	1	Nonambulatory functional movement only
	0	No purposeful leg movement
9. Climbing Stairs	4	Normal
	3	Slow
	2	Mild unsteadiness or fatigue
	1	Needs assistance
	0	Cannot do
10. Dyspnea	4	None
	3	Occurs when walking
	2	Occurs with one or more of the following: eating, bathing, dressing
	1	Occurs at rest, difficulty breathing when either sitting or lying
	0	Significant difficulty, considering using mechanical respiratory support
11. Orthopnea	4	None
	3	Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
	2	Needs extra pillow in order to sleep (more than two)
	1	Can only sleep sitting up
	0	Unable to sleep
12.Respiratory Insufficiency	4	None
	3	Intermittent use of NIPPV
	2	Continuous use of NIPPV during the night
	1	Continuous use of NIPPV during the night and day
	0	Invasive mechanical ventilation by intubation or tracheostomy

Total Score for ASLFRS-R: _____ / **48**

L-serine IIa in ALS	Patient Number: _____	Visit: _____ Date: _____

ALSA-Q40

Please complete this questionnaire as soon as possible. If you have any difficulties filling in this questionnaire by yourself, please have someone to help you. However it is **your** responses that we are interested in.

The questionnaire consists of a number of statements about difficulties that you may have experienced **during the last 2 weeks**. There are no right or wrong answers: your first response is likely to be the most accurate for you. **Please check the box that best describes your own experience or feelings**.

Please answer every question even though some may seem very similar to others, or may not seem relevant to you.

All the information you provide is **confidential**.

The following statements all refer to difficulties that you may have had **during the last 2 weeks**.

Please indicate, by checking the appropriate box, how often the following statements have been true for you.

The following statements all refer to certain difficulties that you may have had during the last 2 weeks. Please indicate, by checking the appropriate box, how often the following statements have been true for you.

If you cannot walk at all *please check* **Always/cannot walk at all.**

How often* during the last 2 weeks *have the following been true?

Please check **one box** for each question

	Never	Rarely	Sometimes	Often	Always/ cannot walk at all
1. Have found it difficult to walk short distances, e.g. around the house	☐	☐	☐	☐	☐
2. I have fallen over while walking.	☐	☐	☐	☐	☐
3. I have stumbled or tripped while walking.	☐	☐	☐	☐	☐
4. I have lost my balance while walking.	☐	☐	☐	☐	☐
5. I have had to concentrate while walking.	☐	☐	☐	☐	☐
6. Walking has worn me out.	☐	☐	☐	☐	☐
7. I have had pains in my legs while walking.	☐	☐	☐	☐	☐
8. I have found it difficult to go up and down the stairs.	☐	☐	☐	☐	☐

	Never	Rarely	Sometimes	Often	Always/ cannot walk at all
9. I have found it difficult to stand up.	☐	☐	☐	☐	☐

10. I have found it difficult to move from sitting in a chair to standing upright.	☐	☐	☐	☐	☐
11. I have had difficulty using my arms and hands.	☐	☐	☐	☐	☐
12. I have found turning and moving in bed difficult.	☐	☐	☐	☐	☐
13. I have had difficulty picking things up.	☐	☐	☐	☐	☐
14. I have had difficulty holding books or newspapers, or turning pages.	☐	☐	☐	☐	☐
15. I have had difficulty writing clearly.	☐	☐	☐	☐	☐
16. I have found it difficult to do jobs around the house.	☐	☐	☐	☐	☐
17. I have found it difficult to feed myself.	☐	☐	☐	☐	☐
18. I have had difficulty combing my hair or brushing and/or flossing my teeth.	☐	☐	☐	☐	☐

	Never	**Rarely**	**Sometimes**	**Often**	**Always/ cannot walk at all**
19. I have had difficulty getting dressed.	☐	☐	☐	☐	☐
20. I have had difficulty washing at the bathroom sink.	☐	☐	☐	☐	☐

21. I have had difficulty swallowing.	☐	☐	☐	☐	☐
22. I have had difficulty eating solid food.	☐	☐	☐	☐	☐
23. I have had difficulty drinking liquids.	☐	☐	☐	☐	☐
24. I have had difficulty participating in conversations.	☐	☐	☐	☐	☐
25. I have felt that my speech has not been easy to understand.	☐	☐	☐	☐	☐
26. I have stuttered or slurred my speech.	☐	☐	☐	☐	☐
27. I have had to talk very slowly.	☐	☐	☐	☐	☐
28. I have talked less than I used to do.	☐	☐	☐	☐	☐
29. I have been frustrated with my speech.	☐	☐	☐	☐	☐
30. I have felt self-conscious about my speech.	☐	☐	☐	☐	☐
31. I have felt lonely.	☐	☐	☐	☐	☐

	Never	**Rarely**	**Sometimes**	**Often**	**Always/ cannot walk at all**
32. I have been bored.	☐	☐	☐	☐	☐
33. I have felt embarrassed in social situations.	☐	☐	☐	☐	☐
34. I have felt hopeless about the future.	☐	☐	☐	☐	☐

35. I have worried that I am a burden to other people.	☐	☐	☐	☐	☐
36. I have wondered why I keep going.	☐	☐	☐	☐	☐
37. I have felt angry because of the disease.	☐	☐	☐	☐	☐
38. I have felt depressed.	☐	☐	☐	☐	☐
39. I have worried about how the disease will affect me in the future.	☐	☐	☐	☐	☐
40. I have felt as if I have lost my independence.	☐	☐	☐	☐	☐

Please make sure that you have checked **one box for each question**

Thank you for completing the questionnaire

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Baseline/Screening Version

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form,*** *developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION		
Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.	**Lifetime: Time He/She Felt Most Suicidal**	**Past __ Months**
1. Wish to be Dead Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up. ***Have you wished you were dead or wished you could go to sleep and not wake up?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
2. Non-Specific Active Suicidal Thoughts General non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period. ***Have you actually had any thoughts of killing yourself?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g. thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it...and I would never go through with it."* ***Have you been thinking about how you might do this?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."* ***Have you had these thoughts and had some intention of acting on them?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
5. Active Suicidal Ideation with Specific Plan and Intent Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out. ***Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?*** If yes, describe:	**Yes** ☐ **No** ☐	**Yes** ☐ **No** ☐
INTENSITY OF IDEATION		
The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe). Ask about time he/she was feeling the most suicidal. Lifetime - ***Most Severe Ideation:*** _____ (*Type # (1-5)*) _____ (*Description of Ideation*) Past X Months - ***Most Severe Ideation:*** _____ (*Type # (1-5)*) _____ (*Description of Ideation*)	Most Severe	Most Severe
Frequency ***How many times have you had these thoughts?*** (1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day	____	____
Duration ***When you have the thoughts how long do they last?*** (1) Fleeting - few seconds or minutes (2) Less than 1 hour/some of the time (3) 1-4 hours/a lot of time (4) 4-8 hours/most of day (5) More than 8 hours/persistent or continuous	____	____
Controllability ***Could/can you stop thinking about killing yourself or wanting to die if you want to?*** (1) Easily able to control thoughts (2) Can control thoughts with little difficulty (3) Can control thoughts with some difficulty (4) Can control thoughts with a lot of difficulty (5) Unable to control thoughts (0) Does not attempt to control thoughts	____	____
Deterrents ***Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?*** (1) Deterrents definitely stopped you from attempting suicide (2) Deterrents probably stopped you (3) Uncertain that deterrents stopped you (4) Deterrents most likely did not stop you (5) Deterrents definitely did not stop you (0) Does not apply	____	____
Reasons for Ideation ***What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?*** (1) Completely to get attention, revenge or a reaction from others (2) Mostly to get attention, revenge or a reaction from others (3) Equally to get attention, revenge or a reaction from others and to end/stop the pain (4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling) (0) Does not apply	____	____

 C-SSRS—Baseline/Screening (Version 1/14/09) Page 1 of 2

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Lifetime**	**Past __ Years**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act.* Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or Did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe:	Yes ☐ No ☐ Total # of Attempts _____	Yes ☐ No ☐ Total # of Attempts _____
Has subject engaged in Non-Suicidal Self-Injurious Behavior?	Yes ☐ No ☐	Yes ☐ No ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred).* Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of interrupted _____	Yes ☐ No ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	Yes ☐ No ☐ Total # of aborted _____	Yes ☐ No ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	Yes ☐ No ☐	Yes ☐ No ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	Yes ☐ No ☐	Yes ☐ No ☐

Answer for Actual Attempts Only	Most Recent Attempt Date:	Most Lethal Attempt Date:	Initial/First Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____
Potential Lethality: Only Answer if Actual Lethality=0 Likely lethality of actual attempt if no medical damage (the following examples, while having no actual medical damage, had potential for very serious lethality: put gun in mouth and pulled the trigger but gun fails to fire so no medical damage; laying on train tracks with oncoming train but pulled away before run over). 0 = Behavior not likely to result in injury 1 = Behavior likely to result in injury but not likely to cause death 2 = Behavior likely to result in death despite available medical care	*Enter Code* _____	*Enter Code* _____	*Enter Code* _____

COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS)

Since Last Visit

Version 1/14/09

Posner, K.; Brent, D.; Lucas, C.; Gould, M.; Stanley, B.; Brown, G.; Fisher, P.; Zelazny, J.; Burke, A.; Oquendo, M.; Mann, J.

Disclaimer:

This scale is intended to be used by individuals who have received training in its administration. The questions contained in the Columbia-Suicide Severity Rating Scale are suggested probes. Ultimately, the determination of the presence of suicidal ideation or behavior depends on the judgment of the individual administering the scale.

Definitions of behavioral suicidal events in this scale are based on those used in ***The Columbia Suicide History Form****, developed by John Mann, MD and Maria Oquendo, MD, Conte Center for the Neuroscience of Mental Disorders (CCNMD), New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY, 10032. (Oquendo M. A., Halberstam B. & Mann J. J., Risk factors for suicidal behavior: utility and limitations of research instruments. In M.B. First [Ed.]* Standardized Evaluation in Clinical Practice, *pp. 103 -130, 2003.)*

For reprints of the C-SSRS contact Kelly Posner, Ph.D., New York State Psychiatric Institute, 1051 Riverside Drive, New York, New York, 10032; inquiries and training requirements contact posnerk@nyspi.columbia.edu

SUICIDAL IDEATION

Ask questions 1 and 2. If both are negative, proceed to "Suicidal Behavior" section. If the answer to question 2 is "yes", ask questions 3, 4 and 5. If the answer to question 1 and/or 2 is "yes", complete "Intensity of Ideation" section below.

Since Last Visit

1. Wish to be Dead
Subject endorses thoughts about a wish to be dead or not alive anymore, or wish to fall asleep and not wake up.
Have you wished you were dead or wished you could go to sleep and not wake up?
If yes, describe:
Yes ☐ **No** ☐

2. Non-Specific Active Suicidal Thoughts
General, non-specific thoughts of wanting to end one's life/commit suicide (e.g., *"I've thought about killing myself"*) without thoughts of ways to kill oneself/associated methods, intent, or plan during the assessment period.
Have you actually had any thoughts of killing yourself?
If yes, describe:
Yes ☐ **No** ☐

3. Active Suicidal Ideation with Any Methods (Not Plan) without Intent to Act
Subject endorses thoughts of suicide and has thought of at least one method during the assessment period. This is different than a specific plan with time, place or method details worked out (e.g., thought of method to kill self but not a specific plan). Includes person who would say, *"I thought about taking an overdose but I never made a specific plan as to when, where or how I would actually do it...and I would never go through with it."*
Have you been thinking about how you might do this?
If yes, describe:
Yes ☐ **No** ☐

4. Active Suicidal Ideation with Some Intent to Act, without Specific Plan
Active suicidal thoughts of killing oneself and subject reports having some intent to act on such thoughts, as opposed to *"I have the thoughts but I definitely will not do anything about them."*
Have you had these thoughts and had some intention of acting on them?
If yes, describe:
Yes ☐ **No** ☐

5. Active Suicidal Ideation with Specific Plan and Intent
Thoughts of killing oneself with details of plan fully or partially worked out and subject has some intent to carry it out.
Have you started to work out or worked out the details of how to kill yourself? Do you intend to carry out this plan?
If yes, describe:
Yes ☐ **No** ☐

INTENSITY OF IDEATION

The following features should be rated with respect to the most severe type of ideation (i.e., 1-5 from above, with 1 being the least severe and 5 being the most severe).

Most Severe Ideation: ____ _____

Type # (1-5) ***Description of Ideation***

Most Severe

Frequency
How many times have you had these thoughts?
(1) Less than once a week (2) Once a week (3) 2-5 times in week (4) Daily or almost daily (5) Many times each day
____

Duration
When you have the thoughts, how long do they last?
(1) Fleeting - few seconds or minutes
(2) Less than 1 hour/some of the time
(3) 1-4 hours/a lot of time
(4) 4-8 hours/most of day
(5) More than 8 hours/persistent or continuous
____

Controllability
Could/can you stop thinking about killing yourself or wanting to die if you want to?
(1) Easily able to control thoughts
(2) Can control thoughts with little difficulty
(3) Can control thoughts with some difficulty
(4) Can control thoughts with a lot of difficulty
(5) Unable to control thoughts
(0) Does not attempt to control thoughts
____

Deterrents
Are there things - anyone or anything (e.g., family, religion, pain of death) - that stopped you from wanting to die or acting on thoughts of committing suicide?
(1) Deterrents definitely stopped you from attempting suicide
(2) Deterrents probably stopped you
(3) Uncertain that deterrents stopped you
(4) Deterrents most likely did not stop you
(5) Deterrents definitely did not stop you
(0) Does not apply
____

Reasons for Ideation
What sort of reasons did you have for thinking about wanting to die or killing yourself? Was it to end the pain or stop the way you were feeling (in other words you couldn't go on living with this pain or how you were feeling) or was it to get attention, revenge or a reaction from others? Or both?
(1) Completely to get attention, revenge or a reaction from others
(2) Mostly to get attention, revenge or a reaction from others
(3) Equally to get attention, revenge or a reaction from others and to end/stop the pain
(4) Mostly to end or stop the pain (you couldn't go on living with the pain or how you were feeling)
(5) Completely to end or stop the pain (you couldn't go on living with the pain or how you were feeling)
(0) Does not apply
____

SUICIDAL BEHAVIOR *(Check all that apply, so long as these are separate events; must ask about all types)*	**Since Last Visit**
Actual Attempt: A potentially self-injurious act committed with at least some wish to die, *as a result of act*. Behavior was in part thought of as method to kill oneself. Intent does not have to be 100%. If there is ***any*** intent/desire to die associated with the act, then it can be considered an actual suicide attempt. ***There does not have to be any injury or harm***, just the potential for injury or harm. If person pulls trigger while gun is in mouth but gun is broken so no injury results, this is considered an attempt. Inferring Intent: Even if an individual denies intent/wish to die, it may be inferred clinically from the behavior or circumstances. For example, a highly lethal act that is clearly not an accident so no other intent but suicide can be inferred (e.g., gunshot to head, jumping from window of a high floor/story). Also, if someone denies intent to die, but they thought that what they did could be lethal, intent may be inferred. ***Have you made a suicide attempt?*** ***Have you done anything to harm yourself?*** ***Have you done anything dangerous where you could have died?*** ***What did you do?*** ***Did you_____ as a way to end your life?*** ***Did you want to die (even a little) when you_____?*** ***Were you trying to end your life when you _____?*** ***Or did you think it was possible you could have died from_____?*** ***Or did you do it purely for other reasons / without ANY intention of killing yourself (like to relieve stress, feel better, get sympathy, or get something else to happen)?*** (Self-Injurious Behavior without suicidal intent) If yes, describe: **Has subject engaged in Non-Suicidal Self-Injurious Behavior?**	**Yes** ☐ **No** ☐ Total # of Attempts _____ **Yes** ☐ **No** ☐
Interrupted Attempt: When the person is interrupted (by an outside circumstance) from starting the potentially self-injurious act *(if not for that, actual attempt would have occurred)*. Overdose: Person has pills in hand but is stopped from ingesting. Once they ingest any pills, this becomes an attempt rather than an interrupted attempt. Shooting: Person has gun pointed toward self, gun is taken away by someone else, or is somehow prevented from pulling trigger. Once they pull the trigger, even if the gun fails to fire, it is an attempt. Jumping: Person is poised to jump, is grabbed and taken down from ledge. Hanging: Person has noose around neck but has not yet started to hang - is stopped from doing so. ***Has there been a time when you started to do something to end your life but someone or something stopped you before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of interrupted _____
Aborted Attempt: When person begins to take steps toward making a suicide attempt, but stops themselves before they actually have engaged in any self-destructive behavior. Examples are similar to interrupted attempts, except that the individual stops him/herself, instead of being stopped by something else. ***Has there been a time when you started to do something to try to end your life but you stopped yourself before you actually did anything?*** If yes, describe:	**Yes** ☐ **No** ☐ Total # of aborted _____
Preparatory Acts or Behavior: Acts or preparation towards imminently making a suicide attempt. This can include anything beyond a verbalization or thought, such as assembling a specific method (e.g., buying pills, purchasing a gun) or preparing for one's death by suicide (e.g., giving things away, writing a suicide note). ***Have you taken any steps towards making a suicide attempt or preparing to kill yourself (such as collecting pills, getting a gun, giving valuables away or writing a suicide note)?*** If yes, describe:	**Yes** ☐ **No** ☐
Suicidal Behavior: Suicidal behavior was present during the assessment period?	**Yes** ☐ **No** ☐
Suicide:	**Yes** ☐ **No** ☐
Answer for Actual Attempts Only	Most Lethal Attempt Date:
Actual Lethality/Medical Damage: 0. No physical damage or very minor physical damage (e.g., surface scratches). 1. Minor physical damage (e.g., lethargic speech; first-degree burns; mild bleeding; sprains). 2. Moderate physical damage; medical attention needed (e.g., conscious but sleepy, somewhat responsive; second-degree burns; bleeding of major vessel). 3. Moderately severe physical damage; *medical* hospitalization and likely intensive care required (e.g., comatose with reflexes intact; third-degree burns less than 20% of body; extensive blood loss but can recover; major fractures). 4. Severe physical damage; *medical* hospitalization with intensive care required (e.g., comatose without reflexes; third-degree burns over 20% of body; extensive blood loss with unstable vital signs; major damage to a vital area). 5. Death	*Enter Code* _____
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