A Phase IIa proof of concept, randomized, double-blind, placebo-controlled study of the effects of L-serine on early stage Alzheimer's disease patients

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Study Product: L-serine

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List of Abbreviations

AD: Alzheimer's Disease

AE: Adverse Event

BMAA: β-N-methylamino-L-alanine

BMP: Basic Metabolic Panel

CBC: Complete Blood Count

eAD: Early Stage Alzheimer's Disease

GCP: Good Clinical Practice

GMP: Good Manufacturing Practice

GRAS: Generally recognized as safe

IRB: Institutional Review Board

LFT: Liver Function Tests

MoCA: Montreal Cognitive Assessment

NFT: neurofibrillary tangles

SAE: Serious Adverse Event

CDR: Clinical Dementia Rating Scale

Hemoglobin A1C: Hgb A1C

Study Summary

Title	A Phase IIa proof of concept, randomized, double-blind, placebo-controlled study of the effects of L-serine on early stage Alzheimer's disease patients
Short Title	Phase IIa L-serine trial for eAD
Protocol Number	LSPI-2, D16180
Phase	Clinical study Phase IIa
Methodology	Randomized, double-blind, placebo controlled
Study Duration	Approximately2.5 years
Study Center(s)	Single-center
Objectives	Determine tolerability of L-serine for early stage Alzheimer's disease patients and assess preliminary indications of efficacy.
Number of Subjects	40
Diagnosis and Main Inclusion Criteria	Patients with a clinical diagnosis of early stage Alzheimer's disease.
Study Product, Dose, Route, Regimen	L-Serine 30g / day oral dose of 15 x 1g gummy chews morning and night with an ascending dose for two weeks of 2 x 1g gummies BID, two weeks of 8 x 1g gummies BID, and 246 days of 15 x 1g gummies BID.
Duration of administration	36 weeks
Reference therapy	Placebo, 15 gummies without L-serine morning and night with an ascending dose for two weeks of 2 gummies BID, two weeks of 8 gummies BID, and 246 days of 15 gummies BID.
Statistical Methodology	Non-parametric statistical analysis based on Mann-Whitney U test, with the critical value set at $p<0.05$.

1 Introduction

This document is a protocol for a human research study. 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.

1.1 Background

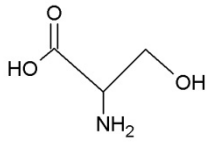
Early stage Alzheimer's disease (eAD) represents a clinical syndrome which encompasses an underlying neuropathological process, beginning with a long asymptomatic period followed by indications of mild cognitive impairment. Clinically this is characterized by cognitive declines with lapses in episodic memory, decline in speed of processing information, difficulty performing tasks, misplacing valuable objects, and a decreased ability to plan or organize activities. At this point, only palliative treatment is available, and no drug has been discovered which can slow the progression of eAD.

The Institute for Ethnomedicine and the Miller School of Medicine Department of Neurology recently were able to produce a model tauopathy with sparse amyloid deposits in a non-human primate, vervets (*Chlorocebus sabaeus*)(Cox et al. 2016) which has some aspects similarto Braak 1 early stage Alzheimer's disease pathology (Braak 1995, Cox et al. 2016). These Alzheimer's-type features include neurofibrillary tangles (NFT) formed from misfolded tau as determined by AT8 immunohistological staining and β-amyloid deposits determined byβ-amyloid(1-42) immunohistological stain; both of these neuropathological hallmarks of Alzheimer's disease were confirmed with Thioflavine-S IHC staining. Dense NFT were found in brain regions consistent with eAD although they occurred primarily in cortical layer I, rather than cortical layers I-II. The Institute for Ethnomedicine and the Miller School of Medicine Department of Neurology were unable to assess cognitive decline in these animals and so this model at this point in time is based solely on neuropathology. However, it is believed that an increasing density of NFT and β-amyloid plaques in human beings is correlated with cognitive declines in human Alzheimer's patients (Nelson et al. 2012).

The Institute for Ethnomedicine and the Miller School of Medicine Department of Neurologyused 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). L-serine is a naturally-occuring amino acid that is a normal part of the human diet, regarded as GRAS by the FDA with the average American receiving 3.5 grams/day from all sources. Since the Institute for Ethnomedicine and the Miller School of Medicine Department of Neurologyhave 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 tolerability of L-Serine and the potential of L-serine in slowing cognitive declines associated with early Alzheimer's disease.

1.2 Investigational Agent

L-serine (C3H7NO3; 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 a non-essential amino acid. L-serine is directly involved in the biosynthesis of purines, pyrimidines, and other amino acids. Serine residues are found in most proteins and within proteins functions as a site for phosphorylation.



L-serine is considered as 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. An FDA-approved Phase I human clinical trial of L-serine for ALS patients, sponsored by the Institute for Ethnomedicine and conducted by the Phoenix Neurological Associates, has now been published (Levine et al. 2016); patients were found to tolerate well doses up to 30 grams/day (ClinicalTrials.gov identifier NCT01835782). Similarly, 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 which for an average

American of 75.5kg is about 30 grams, the dose which we propose to use in this study (ClinicalTrials.gov identifier NCT01733407).

1.3 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 (see insert: Fig 4 from Dunlop et al. 2013). 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 melanogastor*, reveals that the presence of L-serine decreases the amount of BMAA incorporated into protein by half (Fig. 1, below). In addition, 40% of flies fed BMAA die within three days, however, co-administration with L-serine saves every fly within this time frame (Fig. 2, below).

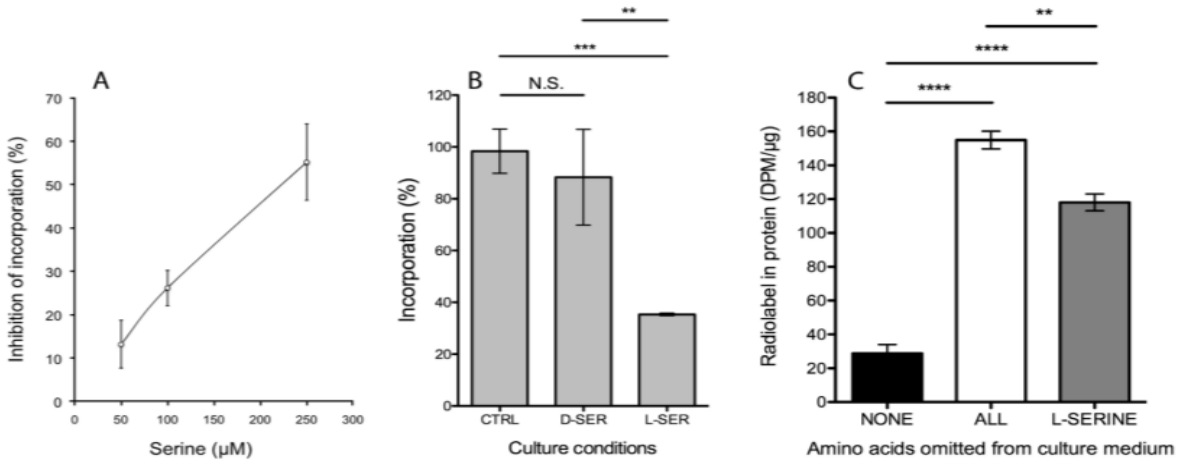


Figure 4. Inhibition of incorporation or radiolabel into cell proteins by L-serine. Panel A, Incorporation of radiolabeled BMAA was inhibited by L-serine in a concentration dependent manner. Panel B, D-serine (D-SER) had no significant impact on the incorporation of BMAA (NS, $P = 0.4419$). L-serine significantly inhibited the incorporation of BMAA compared to 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% ($^{***}P = 0.0009$). Student's two-tailed T-test, values are mean +/− SD for three independent experiments (n = 3).
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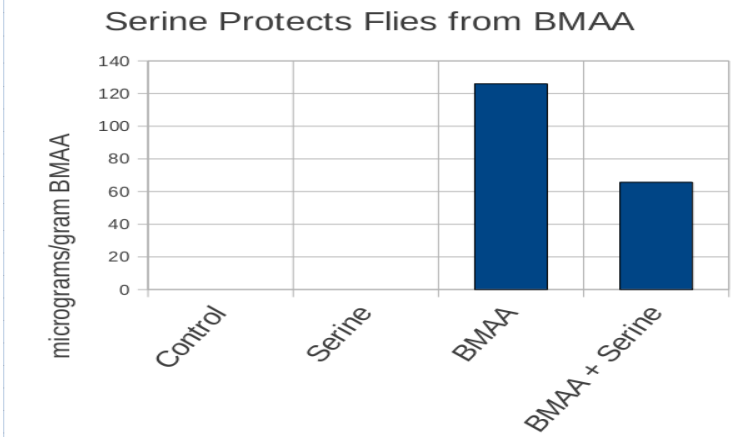
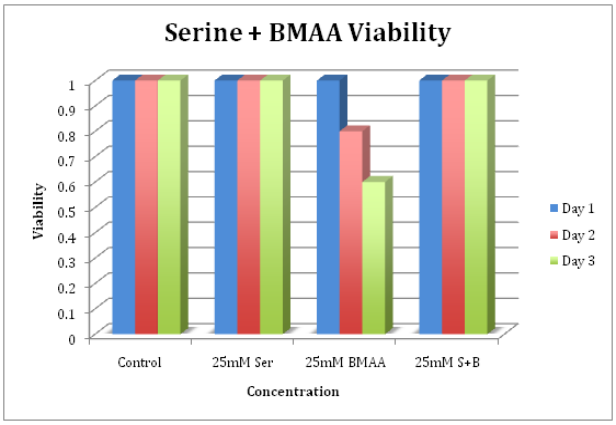
Figure 1: Protein incorporation in fruit flies fed BMAA and co-administered L-serine.

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

(Fig. 2)



(Fig. 1)



Based on these in vitro and in vivo results suggesting a neuroprotective function of L-serine, the Institute for Ethnomedicine and the Miller School of Medicine Department of Neurologysupplemented the diet of vervets (*Chlorocebus sabaeus*) 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 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 singulate cortex (Fig. 3).

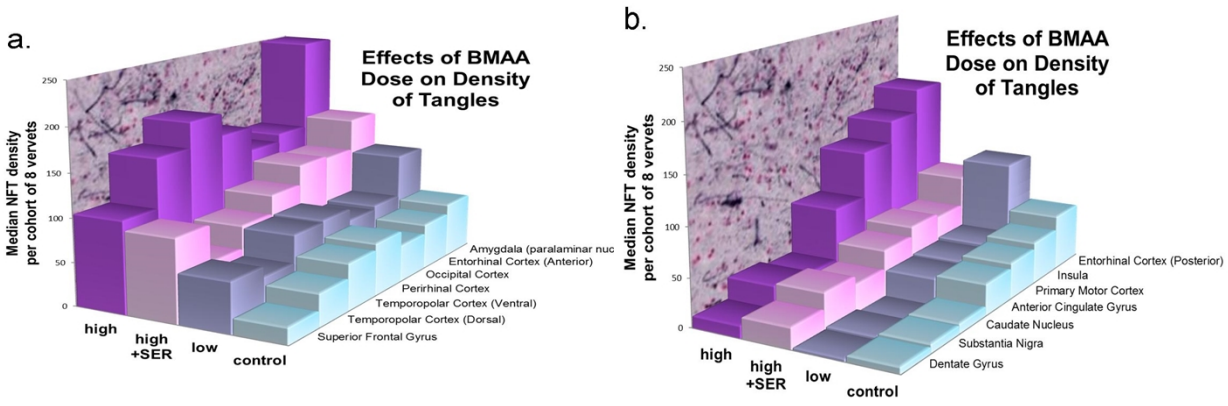


Figure 3: Reduction of NFT production in vervets fed both BMAA and L-serine (pink:high+SER) in comparison with equal amount of BMAA without L-serine (purple: high). Data from Cox et al. 2016.

1.4 Clinical Data to Date

A phase I human clinical trial for safety of L-serine for ALS patients (NCT01835782) has just been published (Levine et al. 2016). Patients (n=20) were given either 1, 5, 15, or 30 g/day for six months. Very few side-effects were reported (bloating, nausea, and loss of appetite; Levine et al., 2016). Analysis of the ALSFRS-R slopes indicates that ALS patients taking L-serine had a reduction in rate of functional loss compared to historical ALS control patients (5 studies that included patients with symptom duration <3 yrs and FVC>=60% to compare with the L-serine patients). There is no evidence of neurological harm to ALS patients as compared to controls. Sample sizes within a dose group are small, and so a Phase II trial of L-serine for 66 ALS patients is planned to see if this preliminary indication of efficacy can be replicated in a larger group. What we can currently conclude is that L-serine at doses up to 30g/ day is safe for ALS patients.

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 respectively) and no adverse effects were reported with either dose after 10 weeks of therapy (Garolfalo et al. 2011).

There are three 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) NCT02528994, 6, 12, 24, or 48 g/day, Short Term Dietary Serine Supplementation and Circulating Serine Levels; 3) NCT02599038, 20 or 200 mg/kg/day, Serine Supplementation for Obese Subjects With Fatty Liver Disease. None of these trials currently have reported data, but it is important to note that trials with L-serine at doses of 30 and 48 g/day are underway.

1.5 Dose Rationale and Risk/Benefits

Since there is no literature as to how much L-serine is tolerated or beneficial in eAD patients, we considered doses generally tolerated by humans in the above reported clinical trials. The dose selected for this trial will be 30g/day as this dose was well-tolerated by both ALS patients and HSAN1 patients. L-serine will be administered orally through gummies being produced in a GMP compliant facility (Knechtel, Chicago, IL). Each gummy contains 1 g L-serine (treatment) and will be packaged in a foil packet containing 15 pieces to be taken both morning and evening (with or without food) for nine months. The placebo will be a gummy with no L-serine, packaged and taken in the same manner. Although chewing this quantity of gummies, and the dose of 30g/day of L-serine has been well tolerated by other neurological patients, we will monitor side-effects in these eAD patients during a dose ramp-up period. We will also monitor amino acid balances in blood serum samples. In order to assess tolerability in patients, we have designed a 4 week dose ramp-up. Patients will take 2g (BID) for the first two weeks followed by 8g (BID) for the next two weeks. If patients tolerate these doses without negative effects, then they will begin taking 15g (BID) for the remainder of the study. If a patient cannot tolerate either 2 or 8g (BID) they will be dropped from the study, however, if a patient can tolerate 8g (BID) but not 15g (BID), they will remain in the study through an additional 246 day period taking a total of 15g per day (1 package of gummies split into two time periods within the day). The same ramp-up schedule and procedures will be observed for both placebo and L-serine patients.

2 Study Objective

Determine tolerability of L-serine for early stage Alzheimer's disease patients and assess preliminary indications of efficacy. Efficacy will be assessed by cognitive testing and biomarkers in plasma.

3 Study Design

3.1 General Design

This is a Phase IIa, randomized, double-blind, placebo controlled trial. Potential subjects for participation in this study will be identified by the Investigatorbased on their clinical and radiographic diagnosis of Alzheimer's disease, which will be completed as part of standard care.Up to 40 subjects will be enrolled. Subjects participating in the study will be randomized to receive either gummies containing L-Serine or placebo gummies, with the Investigator and study staff blinded to the group assignments. An individual subject's participation in the study will last approximately 40 weeks from time of enrollment. Study office visits will take place at Day 0, week 12, week 24 and week 36. Phone contact will be made at week 2, week 4 and week 8 to assess compliance, adverse events and concomitant medication. A follow-up phone call will also be made at week 40 to inquire about adverse events, concomitant medication, subject impression of study, subjective L-serine tolerability, and if they are continuing L-serine supplementation. If, due to study-related events, the PI judges that a participant should be seen in office during study participation, a visit will be scheduled (Unscheduled Visit)

The Day 0 visit includes subject consent, administration and scoring of the Clinical Dementia Rating Scale, review of medical history including any family history of Alzheimer's disease, a brief neurological and physical exam, capture and grading of baseline conditions, and a review of concomitant medications. Hemoglobin A1C, safety labs (CBC,BMP) and research only blood draw will be completed. Eligibility will be confirmed by the PI prior to randomization. If a participant fails to qualify based on their CDR score or Hgb A1 level, the portion of their blood drawn for research only will be discarded.

All subsequent study visits include a brief physical/neurological exam, adverse event assessment, study medication compliance assessment, concomitant medications review, safety blood draws (CBC, BMP) and research-specific blood draws.

Study medication will be dispensed at Day 0, week 12, and week 24, and collected at week 12, week 24 and week 36. All unused study drug will be collected and returned to the study sponsor.

The MoCA will be performed at Visits 0, week 24, week 36, and at early withdrawal or unscheduled visits. Three different MoCA versions will be administered in a random order during these assessments.

At week 24 and week 36, we propose to utilize a portion of the blood sample to examine neurally derived blood exosomes as potential biomarkers. Exosomes are a class of endosome-derived membrane vesicles which are shed by neural cells and transported in blood plasma. Thus, they provide an accessible measure of proteins excreted by neuronal cells. Since two proteins, phosphorylated tau and beta-amyloid-42, are implicated in the formation of neurofibrillary tangles and amyloid plaques, they can potentially be used as biomarkers of disease status. Fiandaca et al. (2015) found that the concentrations of phosphorylated tau and beta-amyloid present within exosomes were significantly higher for AD patients than case controls and that they increased progressively with time and disease progression. We propose to examine, in this proof-of-concept trial, if these exosome biomarkers change as a result of L-serine treatment. Furthermore, we will assess whether the concentrations or presence of these biomarkers show any correlation with cognitive decline.

3.2 Primary Endpoint

This proof-of-concept trial will assess the MoCA score at the week 36 study visit in patients with L-serine treatment compared to placebo. Slopes reflecting the change in MoCA score from Day 0 to the week 36 visit will be compared between treatment and placebo groups.

3.3 Secondary Endpoints

The Institute of Ethnomedicine will also assess plasma neurally derived exosome protein levels of beta-amyloid, as a biomarker which may be related to cognitive decline in eAD (Fiandaca et al. 2015). Slopes reflecting the change in biomarker protein level will be compared between treatment and placebo groups. In addition, we will assess the relationship between the MoCA scores and the concentration of plasma neuronal derived exosome biomarkers (Fiandaca et al. 2015) to demonstrate that this biomarker is related to cognitive decline, and that the MOCA assessment and neural exosome biomarkers are useful for future studies.

3.4. Primary Safety Endpoint

Patients will be asked to report on the tolerability of the gummies. Safety labs will be drawn at baseline and at subsequent study visits to monitor for any adverse effects. These labs include CBC, BMP, and LFT's which will allow the Investigator to screen for any baseline blood values that could be considered clinically significant prior to study entry, and then continue to monitor throughout the trial. Additional blood from the draw will be sent to the Institute of Ethnomedicine to be tested for the amount of L-serine present.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Diagnosis of early stage Alzheimer's disease as scored by the Clinical Dementia Rating Scale score of 0.5 -1.0 upon enrollment.
2. Participants able to provide informed consent.
3. Participants taking NMDA receptor antagonist medications or acetylcholinesterase inhibitor medications must be on a stable dose of these medications for at least 30 days prior to enrolling in this clinical trial.
4. Participants able to consume study gummy chews throughout the course of the clinical trial.

4.2 Exclusion Criteria

1. Diagnosis or previous history of ischemic stroke, astrocytoma, meningioma or oligodendroma.

2. Diagnosis or previous history of any other comorbid diagnosis of neurodegenerative disease including amyotrophic lateral sclerosis, Parkinson's disease, Lewy Body Disease, Pick's Disease, Huntington's Disease, or Progressive Supra Nuclear Palsy.
3. Undergoing any chemotherapy or radiation therapy for any tumor or carcinoma.
4. Diagnosis or previous history of type I or type II diabetes. Subjects with no history of diabetes will have a hemoglobin A1C test as part of their initial labs at V1. Subjects with a Hgb A1C value of less than 6.5 will be considered eligible. Subjects will be referred to their PCP if their Hgb A1C indicates they may be at increased risk to develop diabetes, though will be allowed to participate if they meet all other inclusion/exclusion criteria, and if deemed appropriate by the Investigator.
5. Diagnosis or previous history of psychiatric illness that in the investigator's opinion would affect the subject's ability to successfully participate in the study.
6. In the Investigator's opinion, subject would be unable to successfully participate in the study for any reason.

4.3 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. Subjects must be able to consent for themselves to be able to participate in this study and have 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 the Dartmouth-Hitchcock Medical Center Department of Neurology in Dr. Stark's practice or referred by outside neurologists to the department. All study enrollment, visits and procedures will take place at Dartmouth Hitchcock Medical Center, Lebanon, NH.

4.4 Early Withdrawal: When and How to Withdraw Subjects

Participants taking NMDA receptor antagonist or Acetylcholinesterase inhibitor medications must be on a stable dose for 30 days prior to beginning the study. Participants may come off these medications during the course of the study if it is in their best interest, though should consult with the PI regarding this decision. Participants will be asked to refrain from starting NMDA receptor antagonist or Acetylcholinesterase inhibitor medications throughout the duration of this trial. If they wish to begin these therapies, they may opt to do so and be withdrawn from this clinical trial

Participants will be placed on an ascending dose for two weeks of 2 x 1g gummies BID, two weeks of 8 x 1g gummies BID, and then provided the target dose of 15 x 1g gummies BID for 36 weeks if they are able to tolerate these doses without negative effects. If a patient cannot tolerate 2 or 8g (BID) they will cease treatment. If a patient can tolerate 8g (BID) but not 15g (BID), they will remain in the study through the remainder of the study timeline taking a total of 15g per day (1 package of gummies split into two time periods within the day). The same escalating dose schedule and procedures will be observed for both placebo and L-serine patients. If a participant experiences an adverse event which is serious 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 treatment if the PI feels it is not in the best interest of the participant to continue in the study.

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 4" listed in Section 6.5.

4.4.1 Dosing delays and dose modifications

Dose modification for the following adverse events: nausea, vomiting, diarrhea will proceed as follows:

Nausea, vomiting, diarrhea	**Management/Next Dose for *L-serine***
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions should go off protocol therapy.	
Recommended management: antiemetics, or antidiarrheal therapy.	

Adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, June 14, 2010.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a patient is terminated early from the study as a result of an adverse event, every effort will be made to follow the patient until the adverse event resolves.

5 Study Drug

5.1 Description

L-serine is a naturally-occurring, non-essential amino acid present in the human diet. L-serine will be presented in gummies containing 1g serine each. Gummies will be packaged into a foil package containing 2, 8, or 15 gummies with instructions to take one package in the morning and one in the evening, for a total of 4, 16, and 30g of L-serine/day, respectively. Placebo gummies containing no L-serine will be packaged in the same manner and given to patients to take two times a day.

5.2 Treatment Regimen

Participants will take the study drug or placebo in the form of gummies as described above. Subjects randomized into the L-serine arm will take 15 grams of L-Serine (15 gummies containing 1g of L-serine) orally twice daily for 246 days after the initial ascending dose period to confirm tolerability of the dose. Participants will be given three boxes of gummies to take home at each study visit. If the participant is unable to attend the study visit within the date range for the

scheduled visit the three boxes of gummies may be shipped to the participant. Email or phone contact from the participant confirming receipt of the study medication is required. Each box will weigh approximately five pounds and contain thirty days (60 packets) of gummies. During the initial period of accelerating doses, the gummies will be grouped and labeled into packages containing weeks one and two and weeks three and four respectively.

5.3 Method for Assigning Subjects to Treatment Groups

Participants will be randomized using an urn randomization procedure to ensure equal numbers of patients in both the placebo and L-serine groups receive either Placebo gummies or L-Serine gummies.

Foil packets will be pre-numbered with a lot number and then boxed by the manufacturer prior to shipment and delivery to DHMC. Boxes will contain either 60 packets of L-serine gummies or 60 packets of placebo gummies exclusively. The manufacturer will randomly assign kit numbers 1 through 40 to be used for either the L-serine arm or the placebo arm, with 20 kit numbers associated with each arm. Boxes will be labeled with a kit number corresponding to the correct arm, L-serine or placebo. Each kit number will be assigned to 9 boxes, the total number to be given to the subject. Randomization of the kit numbers and labeling of the corresponding boxes will take place at the manufacturer prior to being shipped to the study site. Patients will be assigned randomized kits in order of numbers 1-40 as they are consented into the study by study coordinators at DHMC.

The Investigational Pharmacy at DHMC, which does not have patient contact in the study, will have access to a sealed envelope which will contain the numbered box randomization key if necessary in an emergency unblinding scenario. The participants and DHMC study team will be unaware of the L-serine or placebo assignment.

5.4 Preparation and Administration of Study Drug

L-serine and placebo preparation will be produced in a GMP compliant facility by Knechtel Laboratories, Inc. (7341 Hamlin Ave, Skokie, IL 60076). The dose will be placed in sealed foil packages. Foil packages sufficient for 3 months will be given to the patients at each visit. Doses will be sent to Dartmouth Hitchcock Medical Center at the beginning of the study.

5.5 Subject Compliance Monitoring

Participants will be given study drug diaries and reminder sheets to attach to their wall/refrigerator in order to improve compliance and provide assistance to study staff in compliance monitoring. Participants will be asked to bring their drug diaries with them at each in person study visit for the study coordinator to review and log reported compliance into the CRF. Written instructions on how to take the medication and what to save and return will also be issued with each medication dispensation.

5.6 Prior and Concomitant Therapy

There are no prohibited concomitant medications. Participants are discouraged from taking any additional L-Serine supplementation as there is not any research to show the effects of higher dosing of L-serine. As part of the patient's history intake, concomitant medications will be recorded and then reviewed at each study visit. Patients must be on a stable dose of NMDA receptor antagonist medications or acetylcholinesterase inhibitor medication for at least 30 days prior to enrolling in the study and taking their first dose of study drug.

5.7 Packaging

L-serine and placebo preparation will be conducted in a GMP facility by Knechtel Laboratories, Inc. Foil packets will be pre-numbered with a lot number and then boxed by the manufacturer prior to shipment and delivery to DHMC. The dose will be placed in sealed foil packages. Stability testing will be completed on all lots by the Brain Chemistry Laboratory on a quarterly basis throughout the study. Packages will not be labeled with an expiration date. Packages are not expected to expire during the course of the study. Each package will contain 2, 8, or 15 gummy pieces either 1 g of L-serine or serine-free pieces.

5.8 Blinding of Study Drug

Packages will be labeled with a lot number. Kits will be assigned a number 1-40 to indicate subject number, and will include 9 boxes, each labeled with the subject number, box number and lot number. Randomization will take place at the manufacturer. Forty sealed envelopes will be sent by the Institute of Ethnomedicine to study site, DHMC, for emergency unblinding. Unblinding information will be kept in the Investigational Pharmacy and accessible 24/7 in case of emergency unblinding.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

The L-serine will be shipped directly from Knechtel Laboratories to Dartmouth-Hitchcock Medical Center Department of Neurology, 4th Floor Research Offices, One Medical Center Drive, Lebanon, NH 03756. Upon receipt of the of the study treatment supplies, an inventory will be performed and a drug receipt log will be filled out and signed by the person accepting the shipment. 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 (active drug or comparator) will be documented in the study files. The investigator will notify the Institute for Ethnomedicine of any damaged or unusable study treatments that were supplied to the site.

5.9.2 Storage

L-serine foil packages should be kept in a cool, dry location during storage and patients will be advised to keep their monthly packets in a cool, dry location.

5.9.3 Dispensing of Study Drug

The boxes of packets will be dispensed at study visits or shipped to the participant.Patients should return any unused packets or gummies and report compliance during study visits. The Dartmouth Hitchcock Investigational Pharmacy will keep record of all study drug issued and study drug returned by participants. Notation will also be made in the participants' electronic medical records regarding the dispensation and collection of the study medication.

5.9.4 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 Institute for Ethnomedicine, 240 East Deloney Avenue, PO Box 3464, Jackson, WY 83001.

6 Study Procedures

The study timeline includes four clinic visits and three telephone calls as well as one follow-up phone call that will occur at 0 weeks (30 days after the last clinic visit). The study visits and timeline are as follows:

6.1 Visit 1: Screening and Enrollment (Day 0)

- Obtain Informed Consent
- Clinical Dementia Rating Scale
- Brief review of medical history and demographic collection
- Neurological and physical exam
- Capture and grade baseline conditions
- Concomitant medication assessment
- Montreal Cognitive Assessment (MoCA)
- Blood Draw:
    - Safety Labs completed locally (BMP, CBC)
    - Hemoglobin A1C level
    - Samples sent to the Brain Chemistry Laboratories for L-serine levels and neurally-derived exosomes with optional excess blood set aside for future protein expression and RNA blueprints
- Review of Inclusion/Exclusion Criteria
- Randomization
- Dispense study medication, dosing instructions and calendar

6.2 Follow-Up Phone Call #1: Week 2

- Medication Compliance
- Adverse Events
- Concomitant Medications

6.3 Follow-Up Phone Call #2: Week 4

- Medication Compliance
- Adverse Events
- Concomitant Medications

6.4 Follow-Up Phone Call #3: Week 8

- Medication Compliance
- Adverse Events
- Concomitant Medications

6.5 Visit 2: Week 12 (+/- 2 weeks)

- Brief neurological and physical exam
- Review of adverse events
- Review current medications
- Blood Draw: Safety Labs completed locally and samples sent to the Brain Chemistry Laboratories for L-serine levels optional excess blood set aside for future protein expression and RNA blueprints at scheduled visit date or next in person visit if there are restrictions in place for in person visits
- Collect unused study medication and calendar at next in person visit
- Dispense study medication and calendar (may be shipped to the participant to be received at scheduled visit date)

6.6 Visit 3: Week 24 (+/- 2 weeks)

- Brief neurological and physical exam
- Montreal Cognitive Assessment, (MoCA)
- Review of adverse events
- Review current medications
- Blood draw: Safety Labs completed locally and samples sent to the Brain Chemistry Laboratories for levels and neurally-derived exosomes with optional excess blood set aside for future protein expression and RNA blueprints at scheduled visit date or next in person visit if there are restrictions in place for in person visits
- Collect unused study medication and calendar at next in person visitDispense study medication and calendar (may be shipped to the participant to be received at scheduled visit date)

6.7 Visit 4: Week 36 (+/- 2 weeks)

- Brief neurological and physical exam
- MoCA exam
- Review of adverse events
- Review current medications
- Blood draw: Safety Labs completed locally and samples sent to the Brain Chemistry Laboratories for L-Serine levels and neurally-derived

exosomes with optional excess blood set aside for future protein expression and RNA blueprints

- Collect unused study medication and calendar. If participant unable to do this in person due to restrictions in place, provisions will be made to assist participant to mail the study medication and calendar to the research team at no expense to the participant.

6.8 Follow-Up Study Coordinator Phone Call: 4 weeks (+/- 2 weeks) after Visit 4:

- Review of adverse events
- Continued supplementation status
- L-Serine Tolerability
- Overall study impression

At study conclusion, the data will be collected and analyzed. Unblinding of study participants will occur after final analysis of all data.

6.9 Blood Draws and Specimen Processing, Storage and Shipment

Safety labs will be drawn at four time points: Visit 1 (Baseline/enrollment), Visit 2 Visit 3 and Visit 4. Blood draws may be delayed and obtained at a later date (next in person visit due to restrictions placed on participant in person visits by Dartmouth-Hitchcock or the participant).

Safety labs include:

- Complete Blood Count
- Liver Function Test
- Basic Metabolic Panel

Blood samples will be drawn and sent to the Brain Chemistry Laboratories for analysis of L-Serine levels and neurally-derived exosomes at four time points: Visit 1 (Baseline/enrollment), Visit 2, Visit 3 and Visit 4 which may include delays in obtaining due to participant unable to attend the visit at the scheduled date

6.10 Optional Blood Donation

There will be an option in the consent for patients to have a portion of their blood drawn to be saved and used for future use by the Institute of Ethnomedicine. The researchers at the Brain Chemistry Laboratories wish to utilize the optional

donations to possibly evaluate for protein expression and corresponding RNA blueprints. These exploratory evaluations will not be reported back to patients. No extra blood is required for the blood donation. Opting in is not required for study participation.

7 Statistical Plan

7.1 Sample Size Determination

In a previous study (Fiandaca, 2014), the neurally derived blood exosomal beta-amyloid protein level of eAD patients had a standard deviation of 2.8 pg/ml. The minimum detectable difference in the means of the treatment and placebo subjects is 2.5 pg/ml, with power 0.8 (alpha = 0.05). An increase of approximately 7 pg/ml was observed between preclinical AD and AD patients (Fiandaca, 2014).

Based on Nasreddine et al. (2005), the standard deviation of the MoCA is approximately 4 points. Power calculations suggest that the minimum detectable difference in mean MoCA scores for two groups, each with 20 patients, is 3.65 points. A similar result for sample size comes from Mead's resource equation to predict adequate sample size, e.g. E= N-B-T, where N is the required sample size, E is the degrees of freedom of the error component (usually around 20), B is the blocking component for environmental effects, and T is the number of Treatments plus controls,

Placebo:20 patients
L-serine:20 patients
Total treatments:2
Total patients: 40

Subtracting one degree of freedom from each value before inserting it in the equation, we have E= 39- 1-1 = 37, Understanding that individual patients may drop out of the study before the 36 week dosing completion for various reasons, this sample size will be adequate to provide the needed statistical power.

7.2 Statistical Methods

Nonparametric statistics are proposed since it is not known in advance if MoCA scores or levels of neurally-derived exosomes will approximate, or can be transformed, to a normal distribution. A Mann-Whitney U-test will compare exosomal protein levels or MoCA scores between the L-serine treatment and placebo patients at a $p<0.05$ level. Parametric equivalents will be considered if appropriate to the distribution of the data.

First, we will check the effectiveness of the randomization by comparing the baseline characteristics of the L-serine and placebo groups according to demographic factors (e.g. age, gender). We will also assess whether the levels of biomarkers or cognitive impairment in the treatment group differ from the placebo group at time zero. This first test will check whether the randomization procedures were sufficient for the study. Next we will compare the exosomal protein levels or MoCA scores at the last study visit (month 9). (H_0 = there is no difference between the median exosomal protein level or MoCA score for treatment patients and control patients; H_1 = there is a difference between the median exosomal protein level MoCA score for treatment patients and control patients). A Mann-Whitney U-test will examine exosomal protein levels or MoCA scores of each patient, by comparing median values between groups. Second, we will assess the slope of the exosomal protein levels or MoCA scores over time to see if they differ among the treatment and control groups by Mann-Whitney U-test. We will use Spearman's Rank correlation, in which rho is the equivalent of the parametric correlation coefficient, to determine if there is a monotonic relationship between levels of proteins in the neurally-derived exosomes and the MoCA scores.

7.3 Subject Population(s) for Analysis

The subject populations whose data will be subjected to the study analysis – both for the primary analysis and any applicable secondary analyses include:
Any subject randomized into the study that received at least one dose of study drug.

8 Safety and Adverse Events

8.1 Definitions

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

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (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)
- Suggests that the research places subjects or others at greater risk of harm (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. Any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. 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 FDA 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 FDA 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

Study lab results will be reviewed by the PI when results are available, prior to dispensation of the following visit study medication. 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. No lab values are identified as exclusionary but the PI may withdraw a subject if they feel it is in the subject's best interest.

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 and 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.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning, review of medical records, 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.

8.3 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

8.3.1 Investigator reporting: notifying the Dartmouth IRB

This section describes the requirements for safety reporting by investigators who are Dartmouth faculty, affiliated with a Dartmouth research site, or otherwise responsible for safety reporting to the Dartmouth IRB. The Dartmouth College IRB (CPHS) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Dartmouth IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

AND

Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

1. Unanticipated problems that are serious adverse events should be reported to the IRB within 1 week of the investigator becoming aware of the event.
2. Any other unanticipated problem should be reported to the IRB within 2 weeks of the investigator becoming aware of the problem.

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Dartmouth IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

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

Reporting Deaths:

Concerning deaths that occur during the course of a research study:

- Report the event when the death is unforeseen (unexpected) and possibly related indicating participants or others may be at increased risk of harm. The AE/Unanticipated Problem Form is required.
- Report the event at the time of continuing review, for all other deaths, regardless of whether the death is related to study participation.

Other Reportable events:

For clinical drug trials, the following events are also reportable to the Dartmouth IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.2 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section The contact information for submitting IND safety reports is noted below:
FDA Contact: Teresa Wheelous, Division of Neurology Phone: 1 (301) 796-1161

8.4 Unblinding Procedures

Randomization information may be made available to the Investigator only in the event of a medical emergency or an AE that necessitates identification of the study drug for the welfare of that patient. Except in a medical emergency, the Investigator (or designee) and study site clinical staff will remain blinded for the duration of the study.

8.5 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 as noted above. In addition to PI oversight, a medical monitor external to the study team will be identified. Medical monitoring will include an assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 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).

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.

9.2 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.

9.3 Case Report Forms

Both written study case report forms (CRF) and RedCap will be data collection instruments 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. All changes made to written CRF's will be initialed and dated. Changes made to data recorded in RedCap will be recorded by an audit trail.

9.4 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. In such an instance, it is the responsibility of the sponsor to inform the institution as to when these documents no longer need to be retained.

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.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in

writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

This study will be funded by the Institute for Ethnomedicine private donations to Dartmouth-Hitchcock Medical Center.

11.2 Conflict of Interest

All Dartmouth investigators will follow the Dartmouth conflict of interest policy.

11.3 Subject Stipends or Payments

Patients will be paid a $50 travel and time spent on study activities stipend per study visit

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14 Attachments

- L-Serine Study Visit Grid
- Lab Processing and Shipping Manual/Instructions

Blood Processing for Exosome recovery

A Phase IIa proof of concept, randomized, double-blind, placebo-controlled study of the effects of L-serine on early stage Alzheimer's disease patients

1. Fill one EDTA tube (lavender top at least 6 mls) completely and **record collecting time**. Time between blood collection and freezing should be less than one hour.

2. Mix immediately by gently inverting the tube(s) at least 8-10 times.

3. Centrifuge immediately at **2,200 x g (5000 rpm) for 15 minutes** to separate cells and platelets from the plasma.

4. Transfer plasma (top layer) into pre-labeled cryovials* in **1 ml aliquots (n = 2-3)**. Verify that cryovials have patient ID, visit #, date on them. Spreadsheet with draw and process time, process date, and ship date will be completed by coordinator and shipped with samples.

5. Transfer buffy coat (middle layer) and erythrocytes (bottom layer) in separate, labeled cryovials. Freeze at **-80°C** in same box as plasma. Study coordinator will document cryovial placement and number on spreadsheet included with subject information.

5. Immediately put the cryovials upright into a small box suitable for storing cryovials in a unique space in a **-80°C freezer** and store at -80°C until shipping on dry ice.

***Please Note:**

Plasma = **Yellow** tops
Buffycoat = **Clear** tops
Erythrocyte = **Red** tops

Shipping instructions

1. Prior to placing the specimens in the shipping containers, verify that all tubes have been properly identified.
2. Verify that the specimens match those listed on the Laboratory Requisition Form.
3. Be sure to inspect the shipping boxes for accurate labeling information.
4. Remove the box containing the vials from the freezer.

5. Place a layer of dry ice at the bottom of the frozen shipper.
6. Ensure that the absorbent sheet is placed in the transport bag with the box of cryovials. Place the lab requisition into the outer pouch of the bag. It is acceptable to ship the entire box of specimens from more than one patient together in the transport bag.
7. Seal the transport bag and place it into the bottom of the shipping box.
8. Fill the remainder of the box with dry ice (minimum of 8 lbs or ~4 kg). Place the Styrofoam cover on the inner Styrofoam container. **DO NOT TAPE THE STYROFOAM BOX CLOSED.**
9. Close box flaps and seal the exterior box with tape.
10. Complete the necessary sections on the pre-printed FedEx Air Waybill. Date all forms where necessary and retain the "Sender's Copy" for files. Place completed air waybill into the clear plastic air bill pouch face up. Make sure outside of box has been properly marked with amount of dry ice.

- **Packages will be shipped frozen on dry ice to Brain Chemistry Lab approximately every 4-6 weeks.**

- **Brain Chemistry Lab will be notified by email with fed ex tracking number when labs have been shipped.**

- **Other arrangements can be made prior to shipping with prior approval by both parties.**

L-Serine Study Timeline of Events-LSPI-2 D16180		+/- 2 week	+/- 2 week	+/- 2 week	+/- 2 week	+/- 2 week	+/- 2 week	+/- 2 week	
	Day 0 Visit 1	Week 2 Phone Call	Week 4 Phone Call	Week 8 Phone Call	Week 12 Visit 2	Week 24 Visit 3	Week 36 Visit 4	Week 40 Follow-Up Call	Early Withdrawal/ Unscheduled Visit
Procedures									
Consent	x								
Clinical Dementia Rating Scale	x								
Medical History	x								
Adverse event tracking		x	x	x	x	x	x	x	x
Drug Collect/accountability					x	x	x		x
Medication compliance inquiry		x	x	x	x	x	x		x
Concomitant Medication	x	x	x	x	x	x	x	x	x
Neuro/Physical Exam	x				x	x	x		x
MoCA (Montreal Cog Assessment)	x					x	x		x

Gummy dispense and instruction	x				x	x			
Medication calendar issue and instruction	x				x	x			
Continued Supplementation Status								x	
Subject Impression of Study								x	
L-Serine tolerability								x	
LFT, (liver function), CBC,BMP	x				x	x	x		x
Hemoglobin A1C	x								
Blood Sample for Brain Chemistry Labs	x				x	x	x		x