Alzheimer's Disease Cooperative Study
UC San Diego

DISCOVER

Protocol Title:

A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics (PK) and Pharmacodynamic (PD) Effects of Posiphen® in Subjects with Early Alzheimer's Disease (AD)

Protocol Short Title: A Safety and PK/PD Study of Posiphen in Subjects with Early Alzheimer's Disease

Protocol Number: ADC-043-DISC (QR15001)

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LIST OF ABBREVIATIONS

5'UTR	5' untranslated region
αSYN	Alpha-synuclein
Aβ	Beta-amyloid
Aβ38	Beta Amyloid 38
Aβ40	Beta Amyloid 40
Aβ42	Beta Amyloid 42
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
ADAS-Cog12	Alzheimer's Disease Assessment Scale – Cognitive
ADCS	Alzheimer's Disease Cooperative Study
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
APOE/APOE4	Apolipoprotein E /Apolipoprotein epsilon 4
APP	Amyloid Precursor Protein
ANOVA	Analysis of variance
AUC	Area under the curve
BACE	β-secretase
BChE	Butyrylcholinesterase
BDNF	Brain-derived neurotrophic factor
BID	Twice a Day
BP	Blood pressure
CDR	Clinical Dementia Rating
CFR	Code of Federal Regulations
CTCAE	Common Terminology Criteria for Adverse Events
CLBP	Chronic low back pain
Cmax	Maximum plasma and CSF concentration
ChE	Cholinesterase
CJD	Creutzfeldt-Jakob Disease
C-SSRS	Columbia Suicide Severity Rating Scale
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CV	Coefficients of variation
CVD	Cerebrovascular Dementia
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FCR	Fractional clearance rate
FDA	Food and Drug Administration
FSR	Fractional Synthesis Rate
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
GRAS	Generally recognized as safe
GWAS	Genome Wide Association Studies
HEK 293	Human embryonic kidney cells

HEENT	Head/ears/eyes/nose/throat
hERG	Human ether-a-go-go related gene
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
IRE	Iron-responsible element
IRP1	Iron Regulatory Protein 1
LBD	Lewy Bodies Dementia
LP	Lumbar Puncture
MAD	Multiple Ascending Dose
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NIA	National Institute on Aging, under the NIH
NIA-AA	NIA and Alzheimer's Association
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NPH	Normal Pressure Hydrocephalus
NPI	Neuropsychiatric Inventory
OHRP	Office for Human Research Protections
p-tau	Phosphorylated tau
PD	Pharmacodynamic
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetic
PSP	Progressive Supranuclear Palsy
QD	Once a Day
QID	4 Times a Day
QT	Interval seen in electrocardiogram (ECG) test
QTc	Interval seen in QT (ECG) test
sAPP	Soluble Amyloid Precursor Protein
sAPPα	Soluble Amyloid Precursor Protein alpha
sAPPβ	Soluble Amyloid Precursor Protein beta
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SH-SY-5Y	Human Derived Cell Line
SILK™	Stable Isotope Labeling and Kinetics
SD	Standard Deviation
$t_{1/2}$	Half-life
tau	Tau protein
TID	Three Times a Day

SIGNATURE PAGE

(SIGNATURES ON FILE AT THE COORDINATING CENTER)

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1 PROTOCOL SYNOPSIS

PROTOCOL TITLE	A Multicenter, Randomized, Double-Blind, Placebo-Controlled Ascending Dose Study to Evaluate the Safety, Tolerability, PK and PD Effects of Posiphen® in Subjects with Early AD
PROJECT DIRECTORS	Martin Farlow, M.D. and Douglas Galasko, M.D.
COORDINATING CENTER	Alzheimer's Disease Cooperative Study (ADCS)
STUDY DESIGN	A Phase 1b multicenter, randomized, double-blind, placebo-controlled study evaluating safety, tolerability, PK and PD of 23-25 days exposure to multiple ascending doses of Posiphen in up to 24 participants with a clinical profile consistent with early AD including Mild Cognitive Impairment due to AD (MCI-AD) or Mild AD with the following planned doses: • 60 mg Once a Day (QD) (1x60mg) • 60 mg Twice a Day (BID) (2x60mg) • 60 mg Three Times a Day (TID) (3x60mg) Each dose group will include 8 subjects, 5 randomly assigned to Posiphen and 3 randomly assigned to placebo. Each dose group will be completed and evaluated for safety and tolerability by the standing ADCS Data Safety Monitoring Board (DSMB). The ADCS DSMB will determine whether to approve dose escalation. Subjects who do not tolerate study drug during the initial 3-week at-home treatment period will be discontinued and replaced by new subjects. If two subjects at a given dose are discontinued due to adverse events, no additional subjects will be added at this dose level until a safety review has been conducted. The safety committee will determine the appropriate dose level for additional subjects. The safety committee has the option to change the allocation of future patients to active drug versus placebo based on statistical advice to maintain an appropriate balance between placebo subjects and subjects on active drug.
DURATION OF STUDY PARTICIPATION	Each subject will have up to a 42-day screening period, followed by approximately 21-23 days of treatment at home, and an in-clinic visit that includes treatment and a 36-hour lumbar puncture (LP) Stable Isotope Labeling and Kinetics (SILK™) procedure.
SUMMARY OF INVESTIGATIONAL PRODUCT	Posiphen 60 mg QD, 60 mg BID or 60 mg TID, or placebo capsules taken orally for up to 25 days.

SUMMARY OF KEY ELIGIBILITY CRITERIA	• Clinical profile consistent with early AD including MCI-AD or mild AD according to the core clinical criteria outlined in the National Institute Aging (NIA) and Alzheimer's Association (NIA-AA) Guidelines • Cerebrospinal fluid (CSF) AD biomarkers consistent with AD (Beta Amyloid 42(Aβ42)/Beta Amyloid 40(Aβ40) ratio less than 0.131 as measured by mass spectrometry) • Age 55-89 (inclusive), in good health with no frailty • Mini-Mental State Examination (MMSE) score between 17 – 30 (inclusive) - • Clinical Dementia Rating (CDR) global score of 0.5 with a memory score of 0.5 or greater, or global score of 1.0
PRIMARY OUTCOME MEASURE	Aim 1. Safety and Tolerability Aim 2. PK of Posiphen/metabolites in Plasma and CSF Aim 3. PD on fractional synthesis rate (FSR) of Beta Amyloid 40 (Aβ40) in CSF
OTHER KEY OUTCOME MEASURES / STUDY PROCEDURES	Aim 4. Feasibility of SILK™ in a multicenter trial Aim 5. PD on other biomarkers in CSF including Beta Amyloid 38 (Aβ38), Aβ40, Aβ42, Soluble Amyloid Precursor Protein alpha (sAPPα), Soluble Amyloid Precursor Protein alpha (sAPPβ), and T-Tau Aim 6. Cognitive and/or neuropsychiatric effects of Posiphen

2 INTRODUCTION

The medications that are currently approved for AD target symptoms such as memory loss, but the magnitude of the effect is small, and the clinical benefits are limited. Aducanumab (commercially known as Aduhelm™), an Aβ-directed monoclonal antibody, received accelerated approval by the FDA for the treatment of early AD in June 2021.

Posiphen® is a selective inhibitor of amyloid precursor protein (APP) production and has potential utility as a disease modifying treatment for AD (Cullen 2006; Utsuki 2006; Lahiri 2007). Posiphen was discovered at the National Institute on Aging and was selected from a series of structurally related compounds designed for APP specificity with no minimal or minimal acetylcholinesterase inhibitory activity. Posiphen was shown to reduce APP and consequently beta-amyloid (Aβ) production in relevant preclinical in vitro and in vivo studies.

Posiphen, developed by Annovis Bio, Inc., is a small molecule that is thought to act by lowering soluble APP protein levels through a post-transcriptional mechanism. Iron increases the translation of APP by binding to an iron-responsive element (IRE) that forms a stem loop structure in the 5' untranslated region (5'UTR) of APP Messenger Ribonucleic Acid (mRNA). Iron regulatory proteins bind to these stem loop structures and suppress the iron activation of mRNA translation. Iron Regulatory Protein 1 (IRP1) selectively binds the APP IRE with an affinity of 35 pM. When IRP1 is bound to the stem loop, it prevents the complex from binding to the ribosome and prevents translation of the mRNA. Addition of Posiphen to human derived cell line (SH-SY-5Y) neuroblastoma cells increases the affinity of IRP1 to the stem loop structure to 15 pM and lowers the rate of translation of the mRNA by the ribosome (Venti 2004; Cahill 2009; Cho 2010; Rogers 2011). A study conducted to analyze the effect of orally administered Posiphen on Aβ levels in the mouse brain, revealed that Posiphen lowered Aβ levels at plasma concentrations that are attainable with oral dosing in humans (IB 2015). Three Phase 1 studies have been completed with Posiphen. The first study was a single ascending dose (SAD) study in healthy volunteers; the second study was a multiple ascending dose (MAD) study in healthy volunteers, and the third study was one with a PK/PD study of biomarkers in CSF obtained from mild cognitively impaired participants.

The present study will confirm the pharmacokinetics of Posiphen and its metabolites in plasma and CSF. More importantly, it will expand on the biomarker reduction seen in the mechanism of action study (Maccecchini 2012) by measuring the effects of a 23-25 day treatment period with Posiphen on the CSF and plasma levels of a number of biomarkers, inflammatory factors and control proteins. It will also expand the safety data in humans by extending the treatment period from 10 days to a treatment period from 23-25 days. In addition, this study will measure concentrations of various soluble biomarkers in CSF and use the SILK™ assay methods to directly measure the effect of Posiphen on the fractional synthesis rate of Aβ40 in CSF, which will help guide the further development of Posiphen and determine the feasibility of SILK™ in a multicenter trial.

2.1 Primary Aims

Aim 1: To determine the safety and tolerability of multiple ascending doses of Posiphen on subjects with early AD with up to 23-25 days of daily usage.

Aim 2: To determine the PK of Posiphen and its metabolites in plasma and CSF of subjects with early AD treated with QD, BID and TID dosing regimens.

Aim 3: To assess the PD effects on the FSR of Aβ 40 using the SILK™ technique with multiple doses of Posiphen.

2.2 Secondary Aims

Aim 4: To implement a multicenter lumbar CSF catheter study employing standardized methodology and SILK™ technology. The following criteria will be evaluated as outcomes to determine feasibility:

- Enrollment comparisons of research subjects at each of the 3 sites
- Adequacy of sample collections to permit analyses of PK and PD effects of Posiphen
- Subject satisfaction questionnaires
- Comparison of protocol deviations and variances in data between the sites

Aim 5: To assess the PD effects of treatment with Posiphen versus placebo on the levels of the following biomarkers in CSF of subjects with early AD:

- Aβ 38 and 40
- sAPPα
- sAPPβ
- T-tau protein (T)

Aim 6: To assess whether there are short-term cognitive and/or neuropsychiatric effects of Posiphen in subjects with early AD, either positive or negative.

- ADAS-Cog12
- MMSE
- Neuropsychiatric Inventory (NPI)

3 PRELIMINARY STUDIES

The following is a brief summary of the available information on Posiphen. Detailed information can be found in the Posiphen Clinical Investigational Brochure (2015).

3.1 Summary of Non-Clinical Findings

In wild type mice, Posiphen lowered brain APP and Aβ levels by up to 60% (Lahiri 2007, Cullen 2006). In APP transgenic AD mice, Posiphen led to a decrease in APP levels, improved neuronal stem cell survival and increased levels of brain-derived neurotrophic factor (BDNF) (Marutle 2007; Kadir 2008, Lilja 2013). Chronic administration of Posiphen to APP transgenic mice totally prevented decline in memory and learning at plasma concentrations that are easily attainable with oral dosing in humans (Maccecchini, Sambamurti & Arancio in preparation). In

alpha-synuclein (αSYN) transgenic PD mice, Posiphen restored gut motility to normal (Maccecchini, Kuo & Nussbaum in preparation).

Posiphen was not mutagenic or clastogenic as assessed by in vitro assays.

The cardiac electrophysiological properties of Posiphen tartrate were negative in vitro using human ether-a-go-go related gene (hERG) transfected human embryonic kidney cells (HEK 293). Posiphen did not adversely affect the interval seen in an electrocardiogram (ECG) test (QT (or QTc)) interval.

Toxicity studies in dogs showed brain toxicity (ataxia and tremors/twitching) and gastrointestinal (GI) toxicity at 30 mg/kg/day, which was dose-dependent and reversible. The no observed adverse effect level (NOAEL) was 20 mg/kg/day in dogs. Posiphen readily crosses the blood-brain barrier. The signs/symptoms noted at high doses of Posiphen may be related to cholinergic manifestations. In in vitro assays, Posiphen showed minimal inhibition of AChE or BChE (Butyrylcholinesterase) activity, however, a metabolic product of Posiphen, N1-norposiphen demonstrated acetyl cholinesterase inhibitory activity (Yu 2013). There were no effects on the reproductive organs associated with 4-week exposure to Posiphen in male or female rats or dogs.

3.2 Summary of Clinical Findings

Three Phase 1 studies have been conducted with Posiphen (Annovis Bio, Inc. 2019). The first was an SAD study in healthy volunteers; the second was an MAD study in healthy volunteers, and the third one was a PK/PD study of CSF obtained from mild cognitively impaired participants.

In the multiple ascending dose trial, Posiphen was administered orally in doses of 20, 40, and 60 mg 4 times a day (QID). The first two treatments were administered for 7 days, and the third, for 10 days. Single doses were given on the first and last day to determine the pharmacokinetics of the drug. In general, the drug was well tolerated, resulting in no serious or severe adverse events and only one premature discontinuation, a subject in the 60 mg group discharged because of nausea, vomiting, dizziness and “feeling warm.” The incidence of adverse events, all of either mild or moderate severity also occurred with similar frequency in the placebo group. The most common AEs were dizziness, headache, and nausea/vomiting.

Posiphen was absorbed rapidly after oral administration, achieving maximum plasma and CSF concentration (C_{max}) within 1.2 - 1.5 hours. For the 40 and 60 mg doses, with fully defined plasma profiles, the mean terminal $t_{½}$’s were 3.80 ± 0.88 and 5.23 ± 1.24 hours, respectively after a single dose and 3.53 ± 1.03 and 4.104 ± 0.91 hours, respectively, after repeat dosing. The half-life ($t_{½}$) of the plasma concentrations at the lower doses could not be calculated accurately. The C_{max} increased disproportionally with dose (24, 144, and 2310 ng.h/mL after a single dose of 20, 40, and 60 mg, respectively and 110, 134, and 2101 ng.h/mL after multiple doses of 20, 40, and 60 mg, respectively).

In the proof of mechanism of action study, the PK of Posiphen was measured after 10 days of administration (4x60 mg) over 12 hours in CSF and plasma of the AD participants. The PD of a number of biomarkers was compared for 12 hours before the first dose at day 0 and after the last dose at day 11 of Posiphen administration. We found that the plasma concentrations of Posiphen overlapped with the plasma concentrations found in the MAD study. In this study, the N1 metabolite reached about 10 to 15%, while the level of the N8 metabolite reached about 20 to 25% of the Posiphen levels measured in plasma.

Because a substantial proportion of the adverse events observed in AD participants treated with cholinesterase inhibitors appear to reflect the cholinomimetic properties of molecules in this

class, Posiphen's highest tolerated dose is determined by the levels of the N1-norposiphen in blood and brain.

While the $t_{1/2}$ of Posiphen in plasma was 5 hours as seen in the SAD and MAD studies, the $t_{1/2}$ in CSF/brain was longer than 12 hours. Concentrations of Posiphen in the brain, extrapolated from blood and CSF, were 8 times higher than in plasma. 10 days of treatment with Posiphen normalized CSF levels of Soluble Amyloid Precursor Protein (sAPP) and tau, reduced αSYN and a series of inflammatory markers. The concentration and persistence of Posiphen in the brain suggest that much lower doses of drug administered once daily could achieve the desired pharmacological effect.

4 BACKGROUND AND SIGNIFICANCE

A major pathological hallmark of AD is the appearance in the brain of senile plaques composed of aggregated forms of Aβ, which is a proteolytic product of APP. Both accumulation of Aβ and other pathological changes, such as development of neurofibrillary tangles, synaptic loss and brain atrophy, are thought to occur one or two decades prior to overt dementia. Soluble Aβ oligomers have been demonstrated to show toxicity in vitro and in vivo and represent a target for drug development in AD. Recent research has shown that soluble forms of phosphorylated tau (p-tau) and αSYN may also contribute to neuronal loss and that AD as well as Parkinson's Disease carry a high load of inflammation and microglia activating factors that contribute to neurodegeneration.

For small molecules that target Aβ protein metabolism or other key biochemical pathways in AD, studies using CSF and plasma sampling in humans can provide data to demonstrate that a drug crosses the blood-brain barrier and engages the relevant target, and to describe the relationship between blood levels and central nervous system (CNS) effects. Repeated sampling through a lumbar CSF catheter for up to 36 hours, and the additional technique of SILK™ to measure parameters such as fractional synthesis rate of Aβ, can help guide the development of drugs targeting Aβ.

Posiphen is the (+) enantiomer of phenserine, an AChE inhibitor which had been tested in several AD studies without significant evidence of efficacy. Preliminary data shows that Posiphen is affected by food; however, when the drug is taken in a series there is no effect on the PK analyses results. However, the failure of those studies with phenserine may have been related to the formulation, which is significantly affected by food. While phenserine inhibits AChE, Posiphen has no AChE activity itself and develops some activity in vivo with the metabolism to N1-bisnorposiphen. In vivo phenserine is about 200 times more potent in regards to AChE activity than Posiphen, when metabolized to N1-bisnorposiphen.

Posiphen has been found to significantly reduce soluble APP and Aβ as well as tau, p-tau and αSYN in the rodent brain and in human CSF. In preliminary studies in animals and humans, inhibition of APP, Aβ, tau, p-tau and αSYN occurs at levels 6 to 10 times lower than the levels causing cholinomimetic effects.

Posiphen is thought to act at the 5'UTR of the APP mRNA and lowers APP and Aβ protein levels in animal models, and decreased sAPP levels in human CSF. Preliminary data suggests that these effects are achieved via the same mechanism of binding to the 5'UTR of mRNAs and inhibiting their translation.

As Posiphen is thought to inhibit the synthesis of APP, as well as other neurotoxic aggregating proteins, it might have a broader spectrum of activity and protect neurons and have a disease-modifying effect in AD as well as other neurodegenerative disorders.

CSF Sampling and SILK™

Sampling CSF repeatedly for as long as 36 hours through a lumbar catheter had been initially used in a few studies related to AD treatment during the 1990s (Cutler 1998). The technique has gained greater utility in relation to measuring pharmacological effects on Aβ with the addition of SILK™, a technique developed by Dr. R. Bateman and colleagues at Washington University (Bateman 2006). This technique involves placement of a lumbar CSF catheter, followed by intravenous infusion of $^{13}C_6$-labeled "heavy" leucine for 9 hours. Leucine is an essential amino acid that undergoes active transport into the brain. $^{13}C_6$ leucine is non-radioactive, harmless to humans and the environment, and is physically identical to unlabeled ($^{12}C_6$) leucine except for an additional proton difference in the mass of the amino acids. The leucine is incorporated into a fraction of newly synthesized proteins throughout the body, including the brain, which are secreted into the CSF. Samples of CSF are collected and Aβ is immune-precipitated from the CSF, and the ratio of Aβ containing $^{13}C_6$-leucine to that containing unlabeled leucine is measured using mass spectrometry. During hours 4-12 after start of the leucine infusion, the fraction of labeled Aβ in the lumbar CSF increases, reaching a maximum of about 10%. The slope (change in % labeled Aβ/time) is called the fractional synthesis rate (FSR) and is a measure of the rate of Aβ synthesis in the brain. During late stages of the catheter procedure (hours 24-36), the fractional % of Aβ that is labeled decreases linearly, and the slope is measured to calculate the fractional clearance rate (FCR). Using this method, Bateman and colleagues estimated that Aβ has a rapid FSR and FCR in CSF.

As part of their CSF sampling studies, Bateman and colleagues measured CSF Aβ hourly for 36 hours in healthy adults and participants with AD. They found that CSF Aβ in normal subjects fluctuate from hour to hour, in a circadian pattern, and to a much greater extent than in participants with AD (Huang 2012). This variability complicates the design of studies in which CSF is sampled in a serial manner through a single lumbar puncture before and after treatment from the same person.

In this study $^{13}C_6$-leucine will be infused during the CSF catheterization procedure. CSF samples will be collected for mass spectrometry analysis of Aβ40 and Aβ42 to see if treatment with Posiphen decreases CSF levels of Aβ40 and of sAPPβ measured initially by mass spectrometry.

During the leucine infusion, participants must comply with a low leucine diet – approximately 1500 mg or less of leucine per day (refer to Appendix 4, Sample Low Leucine Menu). The kitchen on site will be responsible for preparing low leucine meals and snacks while maintaining a food journal for each subject. Subjects who break the low leucine diet will not be excluded from the study, since this will not be evident until the leucine analyses are completed.

At the end of the study, all unused leucine should be returned to C2N.

Study population: The study will be conducted in participants with a clinical profile consistent with early AD including MCI due to AD and mild Alzheimer's disease, consistent with the core clinical criteria outlined in the NIA-AA guidelines (2011), MMSE inclusion score between 17 – 30 (inclusive), Clinical Dementia Rating (CDR) global score of 0.5 with a memory score of 0.5 or

greater, or global score of 1.0, and by the presence of a CSF Aβ42/ Aβ40 ratio less than 0.131 consistent with AD (as measured by C2N at screening). All subjects will have a screening lumbar puncture to measure the CSF Aβ42/40 ratio. These biomarkers can support a diagnosis of AD. CSF will be stored to allow analysis of baseline (pre-treatment) levels of larger biomarkers.

Dose selection: Posiphen will be tested at doses of 60 mg QID, 60 mg BID (120 mg total daily), and at 60 mg TID (180 mg total daily). Whereas the plasma t½ of Posiphen has been determined to be 5 hours, the CNS t½ is estimated to be over 12 hours, allowing that once daily dosing may be feasible. However, previous studies indicated that likely cholinergic adverse events are mostly driven by Cmax. In particular, in the SAD study significant nausea, vomiting and dizziness occurred at a single dose of 160mg. In the MAD study 40mg QD (=160 mg/day) was well tolerated. Thus, the same total daily dose is better tolerated if divided into several doses. The use of multiple doses compared to once a day in this study will help evaluate the PK/PD relationship in the context of tolerability of different administration schemes.

Study Design: This study is designed as a Phase 1b multicenter, randomized, double-blind, placebo-controlled study evaluating safety, tolerability, PK and PD of 23-25 days exposure to multiple ascending doses of Posiphen in up to 24 participants with a clinical profile consistent with early AD including MCI-AD or Mild AD with the following planned doses:

- 60 mg QD (1x60mg)
- 60 mg BID (2x60mg)
- 60 mg TID (3x60mg)

Each dose group will include 8 subjects, 5 randomly assigned to Posiphen and 3 randomly assigned to placebo.

There is some risk of excess cholinergic adverse events given that some subjects will be included who are on cholinesterase inhibitor treatment for Alzheimer's disease which may become additive with the first metabolite of Posiphen (N1-norPosiphen) that has anticholinesterase properties.

Therefore, each dose group will be completed and evaluated for safety and tolerability by the standing ADCS DSMB before the next dose group is initiated.

Subjects who do not tolerate study drug during the initial 3-week at home treatment period will be discontinued and replaced by new subjects. If two subjects at a given dose are discontinued due to adverse events, no additional subjects will be added at this dose level until a safety review has been conducted. The safety committee will determine the appropriate dose level for additional subjects. The safety committee has the option to change the allocation of future patients to active drug versus placebo based on statistical advice to maintain an appropriate balance between placebo subjects and subjects on active drug.

Outcome measures to assess pharmacodynamics: Transcriptional inhibition of the APP gene will result in decreased production of APP, which may be reflected by decreased secretion of the processed parts fragments of APP, namely sAPP and Aβ, in CSF. We have selected Aβ40 as a primary outcome measure because it has a relatively high concentration in CSF and is unaffected by the deposition of Aβ as plaques in the brain, which will be present in the study population. We also will measure secreted forms of APP (sAPPα and sAPPβ) and levels of tau in CSF as secondary outcome measures. sAPP reflects processing of APP by α-secretase to

produce sAPPα and β-secretase (BACE) to produce sAPPβ. Tau levels are being measured since tau transcription may be under the regulation of a similar IRE that may also be affected by Posiphen treatment.

Safety outcome measures: Reports of adverse events (AEs) and serious adverse events (SAEs) during exposure to Posiphen will be collected to evaluate if there are any significant clinical safety issues for the study population. All SAEs occurring up to 30 days after last study drug administration that are considered drug-related must also be reported. Extensive clinical and laboratory safety data already exist for Posiphen, therefore, this approach will be sufficient in the proposed study.

Clinical and cognitive assessment measures: Although clinical benefit is unlikely due to the short duration of this study, standard cognitive measures (MMSE, Logical Memory subtest of the Wechsler memory Scale (Story 1 only), and ADAS-cog) will be collected to describe the study cohort, which allows comparison to subjects in other clinical trials. It is predicted that cognition will remain stable over the course of drug exposure; however, this will be assessed formally in clinic.

5 POTENTIAL RISKS AND BENEFITS OF INVESTIGATIONAL PRODUCT AND STUDY PROCEDURES

5.1 Risks and Benefits Associated with Posiphen or Placebo

There are no benefits to the subjects other than receiving medical and selective cognitive evaluations.

The clinical investigator should advise all potential subjects of the possibility of unexpected side effects and carefully evaluate each person exposed to Investigational Product for possible AEs.

Side effects to placebo are not uncommon but are obviously not due to a pharmacological agent as an industry standard placebo (non-lactose compound) will be provided. The placebo used for the study consists of microcrystalline cellulose which is generally recognized as safe (GRAS), with no known side effects anticipated.

In clinical studies to date, Posiphen has been well tolerated with single doses of 80 mg or less and QID doses of 60 mg. A higher single dose of 160 mg was associated with an increased incidence of nausea and vomiting, potentially cholinergic based side effects, which resulted in the decision not to test higher single doses. Please refer to table below from the single, ascending dose study, which included healthy men and women:

Table 5.4-1: Summary of Adverse Events in More Than One Subject Per Treatment Group (Males and Females Combined) Study AX-PO-101

Body System Adverse Event[a] (Preferred Term)	Number (%) of Patients in Treatment Group (n = 72)						
	10 mg (n = 10)	20 mg (n = 20)	40 mg (n = 10)	80 mg (n = 10)	160 mg (n = 10)	All Posiphen (n = 60)	Placebo (n = 12)
All AEs, mild	2 (20.0)	4 (20.0)	1 (10.0)	3 (30.0)	3 (30.0)	13 (21.7)	2 (16.7)
All AEs, moderate	1 (10.0)	2 (10.0)	0 (0)	0 (0)	4 (40.0)	7 (11.7)	1 (8.3)
All AEs, severe	0 (0)	1 (5.0)	0 (0)	0 (0)	0 (0)	1 (1.7)	1 (8.3)
Gastrointestinal Disorders							
Nausea	0 (0)	2 (10.0)[b]	0 (0)	0 (0)	4 (40.0)	6 (10.0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	3 (30.0)	3 (5.0)	0 (0)
General Disorders and Administration Site Conditions							
Feeling hot	0 (0)	2 (10.0)	0 (0)	0 (0)	0 (0)	2 (3.3)	0 (0)
Investigations							
Heart rate increased	2 (20.0)	1 (5.0)	0 (0)	0 (0)	0 (0)	3 (5.0)	2 (16.7)[b]
Nervous System Disorders							
Dizziness	1 (10.0)	4 (20.0)[b]	1 (10.0)	3 (30.0)	4 (40.0)	13 (21.7)[b]	3 (25.0)[b]
Fainting[c]	1 (10.0)	1 (5.0)	0 (0)	0 (0)	0 (0)	2 (3.3)	1 (8.3)
Vascular Disorders							
Orthostatic hypotension	0 (0.0)	1 (5.0)[b]	0 (0)	0 (0)	0 (0)	1 (1.7)[b]	1 (8.3)[b]

[a] = A subject may have experienced more than one AE in more than one category at more than one severity, each counted as a separate event.
[b] = Includes at least one AE reported as severe.
[c] = *Dizziness* is the preferred MedDRA term, which encompasses descriptive terms *fainting*, *dizzy*, and *lightheadedness*.

Aside from nausea and vomiting, which are well-known responses to treatment with ChE inhibitors, the only consistent pattern of AEs entailed dizziness/fainting, headache, and reduction in total serum protein. These effects were seen to varying degrees at all doses of Posiphen and also in the placebo group. There was a tendency, but no definitive pattern of increased incidence of AEs with increasing dose of Posiphen. There have been no SAEs in prior clinical studies with Posiphen.

Definitive reproductive and developmental toxicity studies have not been conducted with Posiphen. As a result, women of childbearing potential will be excluded from participating in this study.

5.2 Risk/Benefit Associated with Blood Collections

Phlebotomy is associated with mild to moderate discomfort due to piercing of the skin. This can be minimized with the use of a well-trained phlebotomist/nurse. Sometimes the blood draw site may become discolored with a “bruised” appearance that is transient and not painful. Rarely, the blood draw site may become infected and require antibiotic treatment.

5.3 Risk/Benefit Associated with SILK™ Procedure and CSF Sampling

The most frequent adverse event associated with these studies was post-lumbar puncture headache. This was successfully managed by blood patching if a participant is unresponsive to orally administered fluids and/or caffeine.

Other potential risks include meningitis, pain or tingling sensation in the lower extremities, temporary eye weakness or double vision, infection or bleeding at the site of catheter insertion, pain at the site of insertion, backache, epidural or subdural bleeding, infection, paralysis and potential injury to the nerve root.

In studies conducted at C2N Diagnostics and at Washington University, the rate of post-lumbar puncture headache requiring a blood patch is about 10% in older individuals (Randall Bateman, MD, personal communication).

5.4 Risk/Benefit Associated with Investigational Drugs Intended to Reduce β-amyloid in the Brain

In 2010, Food and Drug Administration (FDA) sent a notice to all sponsors of investigational compounds that are intended to treat Alzheimer's disease by reducing Aβ in the brain of the occurrence of imaging abnormalities believed to represent cerebral vasogenic edema. These imaging abnormalities were noted in a study of a monoclonal antibody (Salloway, 2013) and were, in the majority of instances, asymptomatic, and their presence was detected by routine brain magnetic resonance imaging (MRI). Symptoms, when present in association with such imaging abnormalities, were reported to include headache, worsening cognitive function, alteration of consciousness, seizures, unsteadiness, and vomiting. In most instances, the occurrence of such imaging abnormalities, even when symptomatic, did not require treatment beyond discontinuation of the investigational compound, with imaging abnormalities then resolving. Infrequently, high-dose steroid therapy was administered in the presence of prominent symptoms.

More recently (2014), FDA has determined that, following a comprehensive review of existing data, development programs investigating use of a monoclonal antibody that targets β-amyloid (regardless of mechanism) must incorporate serial clinical and MRI monitoring. However, development programs for small molecules that target Aβ are only required to inform investigators of the potential risk of cerebral vasogenic edema including its imaging manifestations, accompanying clinical signs and symptoms, and appropriate management should symptomatic cases occur. Informed consent documents for these programs should also include a description of the manifestations of cerebral vasogenic edema, including its possible symptoms (i.e., headache, worsening cognitive function, alteration of consciousness, seizures, unsteadiness, and vomiting).

6 SAMPLE SIZE AND STATISTICAL PLAN

A sample size of up to 24 subjects (5 randomized to Posiphen and 3 randomized to placebo per treatment group) is planned across 3-6 clinical research centers.

6.1 Randomization

Subjects who have signed an informed consent and meet screening eligibility requirements will be randomly assigned to receive 60 mg Posiphen daily or placebo (with an allocation of 5 on Posiphen, 3 on placebo) by a stratified, random permuted blocked treatment assignment method, stratified by site. Following dosing of the initial 8 subjects and review of safety and tolerability, the next dose group will be 60 mg BID Posiphen or placebo (5 on Posiphen, 3 on placebo). Following the dosing of this group of 8 subjects and review of safety and tolerability, the next dose group will be 60 mg TID Posiphen or placebo (5 on Posiphen, 3 on placebo).

6.2 Power and Sample Size Determination

SILK™ Analyses

Power estimates for SILK™ studies involving measurements of FSR of Aβ40 were determined by analysis of data provided by C2N, Inc., and modeled to evaluate a primary analytic goal of demonstrating a monotonic dose response relationship to Posiphen treatment.

Based on a two-sided t-test for the difference between two independent means at a significance level (alpha) of 0.05 and a statistical power of 80% (1-beta), a sample size of 5 subjects in each treatment group (n.active = 15) and 9 controls will provide at least 80% statistical power to detect a 27% change in the mean Aβ40 FSR. The following table provides estimated power analysis to detect different effect size reductions of FSR Aβ40 of 27% with 2-sided alpha and significance set at 0.05, assuming no dropouts during the SILK™ study:

Table 2. Estimated Power Analysis

	n.active= 12	n.active= 15	n.active= 18
n.cont= 8	0.783	0.822	0.848
n.cont= 9	0.812	0.851	0.876
n.cont= 10	0.836	0.874	0.898

This serves as justification for our sample size estimate of 24. We expect that if dropouts are to occur, they will occur before the SILK™ studies at day 23-25 and can be replaced to allow the full sample size of 24 for SILK™ being achieved.

6.3 Safety and Tolerability Analysis

Safety and tolerability will be assessed with physical examinations, vital signs, clinical laboratory values, use of concomitant medications, and AE reports. The frequencies of adverse events, serious adverse events and laboratory abnormalities between the participants across the three groups will be compared. The severity of cholinergic adverse events will be carefully evaluated taking the criteria in Appendix 5 into consideration (modified from Common Terminology Criteria for Adverse Events (CTCAE)" version 4.03) as there is potential for an interaction between Posiphen and Donepezil through the inhibition of AChE, thereby resulting in increased cholinergic activity.

Each dose group will be completed and evaluated for safety and tolerability by the standing ADCS DSMB before the next dose group is initiated. Drug administration at the next higher dose level may not proceed until written confirmation is provided from the ADCS DSMB indicating that the results of the previous dose level were evaluated and that it is permissible to proceed to a higher dose level.

Subjects who do not tolerate study drug during the initial 3-week at-home treatment period will be discontinued and replaced by new subjects. If two subjects at a given dose are discontinued due to adverse events, no additional subjects will be added at this dose level until a safety review has been conducted. The ADCS DSMB will determine the appropriate dose level for additional subjects. The ADCS DSMB has the option to change the allocation of future patients to active drug versus placebo based on statistical advice to maintain an appropriate balance between placebo subjects and subjects on active drug.

6.4 PK Analysis

Plasma concentration-time data will be analyzed by non-compartmental methods using WinNonlin version 6.2.1 or greater. Calculations will be based on the actual sampling times recorded during the study. Only the subjects receiving Posiphen will be included in the PK analysis. From the plasma concentration-time data, the following PK parameters will be determined, as data permit: Area under the curve (AUC)τ, $AUC_{0-\infty}$, C_{max}, t_{max}, t½, eff, Vss, Cτ, AR, and CL.

Dose-proportionality may be assessed on C_{max} and AUCτ as appropriate. Serial pre-dose trough concentrations Cτ will be assessed for the attainment of steady-state.

The focus of the PK analysis will be to characterize the preliminary PK of Posiphen. Plasma concentration-time data for Posiphen will be listed and summarized descriptively (number of subjects (N), mean, median, standard deviation (SD), minimum (min), and maximum (max)) and graphically presented. PK parameters will be listed and summarized descriptively (N, mean, median, SD, min, max, and 95% CI) by each dose regimen. Also, a plasma/CSF comparison of the Posiphen PK will be conducted.

In addition, geometric means and between-subject coefficients of variation (CV) will be calculated for AUCτ, $AUC_{0-\infty}$, C_{max}, t_{max}, t½, eff, Vss, Cτ, AR, and CL. All PK analyses will be performed by the ADCS Biomarker Core.

6.5 SILK™ Analysis

In SILK™ studies, the mass spectrometry data will be used to calculate the fraction of labeled Aβ for Aβ38, 40, and 42 during 36 hours after administration of study drug for each subject, as described. The FSRs will be calculated by fitting a line to this data. FSRs for treated and untreated subjects will be compared by analysis of variance (ANOVA). The percentage of newly synthesized and degraded Aβ will be estimated over 36 hours by multiplying the mass spectrometry concentration of Aβ by the % of Aβ that contains $^{13}C_6$-leucine at each time point. This will be used to estimate the AUC of the drug for decreasing the amount of newly produced Aβ (Bateman 2009).

6.6 Criteria for the termination of the trial

The trial may be terminated by the Project Directors and/or Annovis Bio, Inc., based on issues of safety, feasibility, and Data Safety Monitoring Board (DSMB) recommendations.

7 STUDY DRUG AND CONCOMITANT MEDICATIONS

7.1 Name and Description of IP and Comparator

Posiphen will be prepared in 60 mg gelatin capsules, without excipients or fillers. Matching placebo capsules (non-lactose compound) will be prepared with an inert inactive excipient generally recognized as safe for human pharmaceutical use. Annovis Bio, Inc.'s vendor, Frontage Laboratories, will provide both study drug and placebo.

7.2 Dosage

The study drug is to be taken orally; one capsule, up to three times per day depending on treatment group (one in the morning, one in the middle of the day, and one in evening/bed time), with or without food, for 21-23 days at home (depending on when the confinement visit is scheduled). An additional 2 days of drug will be administered during the confinement visit for a total 23-25 days of Posiphen exposure. The study drug will be size 5, hard gelatin capsules. The following table describes the number and type of capsules to be taken each day based on treatment group. The quantity of 60 mg Posiphen capsules and placebo capsules to be taken each day will depend on the assigned treatment group:

Table 3. Quantity of Capsules per Day

	Quantity of Capsules per day	
Treatment Group	**60 mg Posiphen**	**Matching Placebo**
Posiphen 60 mg Treatment Group	1	2
Posiphen 120 mg Treatment Group	2	1
Posiphen 180 mg Treatment Group	3	0
Placebo	0	3

Based on when the confinement visit is scheduled, participants will take 21-23 days of the drug at home. Participants must take 80% of all doses (across 21-23 days), and should take at least 80% of all doses in the last week before the confinement visit. Blister packs will be examined in clinic at the pre-confinement visit to assess compliance overall and in the last week.

7.3 Packaging/Dispensing/Labeling

Frontage Laboratories will prepare the study drug (Posiphen capsules and placebo capsules) to preserve the blind, i.e., identical color and shape opaque hard gelatin capsule shells. The study drug (Posiphen) and placebo capsules will be packaged in identical blister packs with labels including the requisite cautionary statement "CAUTION: Limited by Federal (United States) Law to Investigational Use Only".

In order to preserve the blind, Frontage Laboratories will provide the initial drug supply and subsequent resupply to the sites to dispense to participants.

The dosing schedule and storage requirements will be clearly explained to the participants and caregiver before dispensing the study drug, and it will be printed on the labels.

All clinical trial material dispensed under this protocol will bear the requisite cautionary statement “CAUTION: Limited by Federal (United States) Law to Investigational Use Only”.

7.4 Storage

Both Posiphen capsules and the matching placebo capsules (non-lactose compound) must be stored at room temperature (15-30 °C; 59-86 °F), protected from moisture, in a locked area with limited staff access.

7.5 Drug Accountability

The investigator is responsible for investigational product reconciliation and records maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. The responsible person(s) will document the amount of investigational product dispensed to and returned by subjects. All used and unused study drug containers must be returned to the site for accountability. Once authorized by a clinical monitor, unused study drug (including partially used blister packs) may be destroyed on site per appropriate site SOPS or should be shipped back to Annovis Bio, Inc. for destruction.

7.6 Compliance

Site personnel will assess compliance based on the amount of study drug dispensed to and returned by the participants, together with any related information, including the administration of study drug by study staff during the confinement visit. Participants must take 80% of all doses at home (21-23 days) depending on the confinement visit date and should take at least 80% of all doses in the last week prior to the confinement visit. Site personnel will count capsules and evaluate compliance from the blister packs at the pre-confinement visit.

Prior to the pre-confinement visit, site personnel will also contact the participant and study partner to remind them of the dose regimen, in particular to ensure that the necessary level of compliance has been achieved to continue with the confinement visit procedures.

Site personnel will assess compliance at the pre-confinement visit and again at the time of admission for the confinement visit. If participants are found to be < 80% compliant for all doses at home and in the last week there is an insufficient level of compliance, the site must consult with the Project Directors (and/or Medical Monitor) regarding timing and conduct of the confinement visit procedures. It is possible that subjects will be discontinued early from the study due to insufficient drug compliance.

During the leucine infusion, participants must comply with a low leucine diet – 1500 mg or less of leucine per day. The kitchen on-site will have the responsibility of preparing low leucine meals meals/snacks and maintaining a food journal for each individual. (Refer to Appendix 4 for a sample low leucine menu).

7.7 Breaking the Blind

Only in the case of an emergency, when knowledge of whether the participant has received the investigational product is essential for the clinical management or welfare of the participant, may the investigator request to unblind a participant's treatment assignment. If the investigator needs the blind to be unmasked for a subject for any reason, the investigator must contact the Medical Monitor (and/or Project Directors) to obtain an approval. The blind will need to be broken centrally at the ADCS Coordinating Center. If the blind is broken, whether it be by accident or for the welfare of the participant, the investigator MUST contact the Medical Monitor. Refer to the study procedures manual for detailed procedures related to breaking the blind and reporting.

7.8 Overdose

No specific antidote for the overdose of Posiphen is known; however, signs or symptoms of possible overdose should be noted and treated. The Medical Monitor and Project Directors should be notified as well. Because strategies for the management of overdose are continually evolving, it is advisable to contact a poison control center to determine the latest recommendations for the management of an overdose of any drug. Although Posiphen is a weak AChE inhibitor, based upon overdose information published in prescribing information for related products, overdose with AChE inhibitors can result in cholinergic crisis characterized by severe nausea, vomiting, salivation, sweating, bradycardia, hypotension, respiratory depression, collapse and convulsions. Increasing muscle weakness is a possibility and may result in death if respiratory muscles are involved. Tertiary anti-cholinergics such as atropine may be used as an antidote for overdosage. Intravenous atropine sulfate titrated to effect is recommended. Atypical responses in blood pressure (BP) and HR have been reported with other cholinomimetics when co-administered with quaternary anticholinergics such as glycopyrrolate. It is not known whether dialysis (hemodialysis, peritoneal dialysis, or hemofiltration) is an effective treatment for overdose.

7.9 Concomitant Medications

7.9.1 Prohibited Concomitant Medications

Current use of anti-coagulants (e.g., warfarin (Coumadin), dabigatran (Pradaxa) or anti-platelet drugs (e.g., aspirin, Plavix)), other than aspirin up to a dose of 325 mg per day, is prohibited. Prospective subjects who can safely discontinue these medications for 4 weeks before screening and remain off these medications throughout the study, can be considered for inclusion in the study.

Use of the following medications is prohibited within 4 weeks of screening and throughout the study except as noted in Section 7.9.2:

- specific psychoactive medications (tricyclic antidepressants, antipsychotics (except ≤50 mg quetiapine daily, risperidone ≤1.5 mg/day, olanzapine ≤5 mg/day, and aripiprazole ≤10 mg/day)
- mood-stabilizing psychotropic agents (e.g., lithium salts)
- psychostimulants
- opiate analgesics
- antiparkinsonian medications

- anticonvulsant medications
- systemic corticosteroids
- CNS active antihistamines for allergy or sleep (daily use)

Investigational agents are prohibited 4 weeks prior to entry and for the duration of the trial. Also, exclusionary, is previous treatment with an investigational small molecule with anti-amyloid properties or passive immunization against Aβ within 1 year of entry or previous treatment with an active immunization against amyloid. This will also include Aduhelm™, an Aβ-directed monoclonal antibody that received accelerated FDA approval for treatment of early AD but which is prohibited in this study.

Initiation of prohibited medications during the course of the study is discouraged, however, if an excluded medication is initiated after screening, the site should consult with the Project Directors and Medical Monitor for further guidance.

7.9.2 Permitted Concomitant Medications

This protocol allows concomitant treatment with cholinesterase inhibitors and/or memantine if on a stable dose (FDA approved drug ranges) for 12 weeks prior to screening. However, participants are only permitted to be on a maximum of 10 mg/day of Aricept (donezepil).

Initiation of or modifications to concomitant AD medications during the course of the study is discouraged; however, if a change occurs or a new medication is initiated, the site should consult with the Project Directors and Medical Monitor for further guidance. The addition of Aduhelm is not permitted during the trial.

Gabapentin and pregabalin for non-seizure indications, stable doses of bladder medication, and non-opiate analgesics for pain management are permitted.

Use of benzodiazepines for treatment on an as-needed basis for insomnia or daily dosing as anxiolytics is permitted. If they are being given chronically, use of sedatives or hypnotics should be avoided for 8 hours before administration of cognitive tests. If sedating medication is given for any short-term use, then all cognitive assessments must be administered at least 24 hours after administration of the sedative. Sedating medication should not be taken the night before the baseline visit, the day of the baseline visit, the night before the pre-confinement visit, or the day of the pre-confinement visit.

8 STUDY POPULATION

Up to 24 participants with early AD including those with a clinical profile consistent with MCI or mild AD will be enrolled in this study, in accordance with the criteria specified below. Subjects who do not meet all inclusion criteria, disease diagnostic criteria, or who meet any exclusion criteria may not be randomized into the study without prior approval from the Project Directors and Medical Monitor.

8.1 Inclusion Criteria

Subjects must meet the following criteria:

1. Male or female aged 55 to 89 years (inclusive), in good health, no frailty.

2. Female participants must be post-menopausal for at least 2 consecutive years or surgically sterile (bilateral tubal ligation, hysterectomy or bilateral oophorectomy) for at least 6 months prior to screening.
3. Female participants will be given a urine pregnancy test at the screening visit for which they should test negative.
4. Clinical profile consistent with MCI or mild AD, consistent with the core clinical criteria outlined in the NIA-AA Guidelines (2011).
5. MMSE score between 17 and 30 (inclusive).
6. CDR global score of 0.5 with a memory score of 0.5 or greater, or CDR global score of 1.0.
7. Participant likely to tolerate all study procedures per PI judgment.
8. To qualify for entry, subjects will have a CSF Aβ42/ Aβ40 ratio below 0.131 that is consistent with Alzheimer's disease as measured via mass spectrometry by C2N.
9. General cognition and functional performance sufficiently preserved that the subject can provide written informed consent.
10. A minimum of 6 years of education or good work history.
11. Study partner is available who has frequent contact with the subject (e.g., average of 10 hours per week or more), and can accompany the subject to most visits to answer questions about the subject. The study partner is required to attend the entire screening visit and the baseline visit. The study drug is dispensed to the participant at the baseline visit and the study partner (or other individual) should also oversee study drug administration if needed to ensure compliance with dose regimen. At a minimum, the study partner should stay for the first 3 hours of the confinement visit and return at the discharge to drive the subject home.
12. No evidence of current suicidal ideation or previous suicide attempt in the past month as evaluated in the Columbia Suicide Severity Rating Scale.
13. MRI scan within the 12 months prior to screening without evidence of infection, infarction, or other focal lesions and without clinical symptoms suggestive of intervening neurological disease. Lacunes that are not believed to contribute to the subject's cognitive impairment are permissible. If there is no MRI available within a 12-month timeframe, then an MRI must be performed as part of the screening procedures for eligibility.
14. Stability of permitted medications for 4 weeks prior to baseline. NOTE: Cholinesterase inhibitors and or memantine are allowable only if stable for 12 weeks prior to screen. If taking Arciept (donezepil), no more than 10 mg/day is permitted during the course of the study.
15. Adequate visual and hearing ability (physical ability to perform all the study assessments).
16. Good general health with no disease expected to interfere with the study. Subjects may have common age-related disorders (i.e., hypertension, type II diabetes, dyslipidemia, and hypothyroidism) as long as these disorders are being controlled by diet or medication.
17. Must speak English, Spanish, or Korean fluently.

8.2 Exclusion Criteria

Subjects meeting any of the following criteria must not be included in the study:

1. Has a history of a psychiatric disorder such as schizophrenia, bipolar disorder or major depression according to the criteria of the most current version of the Diagnostic and Statistical Manual of Mental Disorders (DSM). Mild depression or history of depression that is stable on treatment with a tricyclic antidepressant SSRI or SNRI medication at a stable dose is acceptable.
2. Other neurodegenerative diseases, including Parkinson's disease and Huntington's disease, or cerebral tumor.
3. Dementia other than AD, such as Acquired Immunodeficiency Syndrome (AIDS), Creutzfeldt-Jakob disease (CJD), Lewy Bodies dementia (LBD), Cerebrovascular dementia (CVD), Progressive Supranuclear Palsy (PSP), or normal pressure hydrocephalus (NPH).
4. History of a seizure disorder.
5. Clinically significant abnormalities in screening laboratory or ECG results.
6. Has current serious or unstable illness including cardiovascular, hepatic, renal, gastroenterologic, respiratory, endocrinologic, neurologic, psychiatric, immunologic, or hematologic disease or other conditions that, in the investigator's opinion, makes them ineligible for participation in this study.
7. Has four or more microinfarcts as noted in the MRI scan.
8. Has cancer or has had a malignant tumor within the past 3 years, except patients who underwent potentially curative therapy with no evidence of recurrence. (Patients with stable untreated prostate cancer or skin cancers are not excluded).
9. According to the criteria of the most current version of the DSM, alcohol abuse, alcohol dependence, or drug abuse in the past 5 years.
10. Participation in another clinical trial with an investigational agent and have taken at least one dose of study medication, unless unblinded on placebo, within 16 weeks prior to screening. (The end of a previous investigational trial is the date the last dose of an investigational agent was taken).
11. Resides in a skilled nursing facility.
12. Subjects with infection or inflammation of the skin or skin disease at or in proximity to the lumbar puncture site.
13. History of lumbar spine surgery or chronic low back pain (CLBP).
14. Subjects whom the site PI deems to be otherwise ineligible.
15. Has a deep brain stimulator (DBS).

9 DESCRIPTION OF STUDY VISITS

Each subject will have a 42 day screening period followed by approximately 21-23 days of treatment at home. The study visits are described below and outlined in the Schedule of Events in Appendix 1 as well as in the Study Schematic Diagram in Appendix 2.

9.1 Pre-screening

During the prescreen phase, sites will identify potential participants through a variety of mechanisms (i.e., reviewing participants enrolled in ADRC centers, de novo recruitment, and referrals) and assess if they may potentially qualify for the study following the site's standard practice.

9.2 Screening Visit

The screening visit should occur up to 42 days before the baseline visit. The screening visit procedures may be completed over multiple days and will typically require at least two in-clinic visits, one of which will be to perform the lumbar puncture.

Potential participants, or legally authorized representatives, and their study partners must sign an informed consent form prior to administration of any study-related procedures. Information regarding the subject's demographics, concurrent medications, and medical history will be collected along with cognitive assessments, physical examination, and neurological examination.

Safety assessments will include an ECG and MRI scan that will need to be read locally and reviewed by the site investigator to confirm eligibility. The clinical safety laboratory blood and urine tests will be sent to a central lab for analysis. The screening safety laboratory tests will be comprised of a metabolic panel, complete blood count, coagulation panel, and urinalysis as listed in Section 12.1.3. Coagulation panel results must be obtained prior to performing the lumbar puncture. The urinalysis will include a pregnancy test for female participants.

A lumbar puncture will be performed as part of the screening visit, in the morning (before 10 am is recommended), after an overnight fast. 16-20 mL CSF will be typically obtained. 2 mL CSF will be sent for analysis by the site (total protein, glucose, cell count) to their local lab and the remainder will be shipped to the ADCS Biomarker Core. The CSF sent to the ADCS Biomarker Core should be placed into a 14 ml conical polypropylene tube, inverted gently 3-4 times, then centrifuged at 1500 x g for 10 minutes at room temperature. Next, the supernatant should be aliquoted into 2.0 ml tubes, frozen at -80ºC, and shipped overnight to the ADCS Biomarker core. If CSF cannot be shipped on the day of draw, it will be frozen and stored at -80 ºCby the site until it can be shipped on dry ice. The ADCS Biomarker Core will send CSF samples to C2N and C2N will analyze the screening CSF for Aβ42/ Aβ40 levels. All LP supplies will be provided to sites by ADCS.

Blood will be drawn at the time of the LP for research procedures. This will consist of 3 mL of plasma, and 10 mL of blood for APOE genotyping and storage of DNA. Blood samples should be shipped overnight at ambient temperature to the ADCS Biomarker Core. For a complete list of procedures, refer to the Schedule of Events (Appendix 1).

Cognitive assessments must be done either prior to the lumbar puncture or within 48 hours after the lumbar puncture. Cognitive assessments should not be administered when the subject is in

a fasted state. Given the timing of the MRI and the LP, during the screening visit the cognitive testing can be scheduled up to 7 weekdays prior to the LP visit date.

Once all screening visit procedures are completed, all information related to eligibility, including screening lab results, must be reviewed by the site investigator to assess the subject's eligibility before proceeding to the baseline visit.

9.2.1 Screening LP Exception

If a participant has undergone an LP for another study at your site within 90 days prior to the baseline visit, this LP and collected CSF samples may be used in place of the screening LP with prior approval from ADCS Clinical Operations. ADCS approval may be requested by email (csf@ucsd.edu)

9.2.2 Re-screens

Unless otherwise approved by the Project Directors, only one re-screen is allowed per participant, should the original screen be a failure. The re-screen should typically occur at least 3 months after the original screen failure. Individuals who fail screening due to ineligible results from the CSF testing may not be re-screened.

9.3 Baseline Visit

Baseline procedures include cognitive assessments, behavioral assessments, Research Satisfaction Survey, safety assessments, and review of concurrent medications and adverse events that occur in clinic.

For a complete list of all visit procedures, refer to the Schedule of Events (Appendix 1).

Following completion of all baseline procedures, subjects who continue to meet all protocol inclusion criteria and no exclusion criteria, should be scheduled for the confinement visit. The person should then be randomized, dispensed study drug and instructed on proper storage and dosing. The subject should be instructed on 1) when to take the first dose of the study drug and 2) the duration of study drug administration (21-23 days at home) based on the scheduled date of the confinement visit.

9.4 Day 14 Telephone Contact

A telephone call will be conducted at 14 ± 4 days from the baseline visit to assess for any changes in status, adverse events, or concomitant medications. Study medication compliance will be discussed and reminders given about the upcoming confinement visit. If there is an insufficient level of drug compliance (less than 80% overall and or less than 80% in the last week), the site must consult with the Project Directors and Medical Monitor regarding timing and conduct of the remaining confinement visit procedures. It is possible that subjects will be discontinued early from the study due to insufficient drug compliance.

9.5 Confinement Visits

Admission for the confinement visit should occur 21-23 days (±2 days) following the first dose of study medication. A pre-confinement visit will be conducted 1-3 days prior to admission and a

post-confinement phone follow-up will be conducted approximately 24 hours following discharge. The confinement visit is outlined in Appendix 3, Confinement Visit Diagram.

9.5.1 Pre-Confinement Visit

The Pre-Confinement Visit procedures include a physical and neurological examination, cognitive and behavioral assessments, certain safety assessments (including clinical safety labs), and a review of compliance, concurrent medications and adverse events. The clinical safety labs will be sent to a central lab for analysis. For a complete list of all visit procedures, refer to the Schedule of Events (Appendix 1).

If there is an insufficient level of drug compliance, the site must consult with the Project Directors and Medical Monitor regarding timing and conduct of the Confinement Visit procedures. It is possible that subjects will be discontinued early from the study due to insufficient compliance.

9.5.2 Confinement Visit

The confinement visit will last just over 2 days and will consist of four phases: admission, 36-hour sampling, 12-hour observation, and discharge. Subjects will be provided with meals (note: some restrictions are specified below) and sleeping accommodations during this visit. For a complete list of all visit procedures, refer to the Schedule of Events (Appendix 1) and to the Confinement Visit Diagram (Appendix 3).

Phase 1: Admission

Subjects should arrive fasted in the early morning after an overnight fast. Clinical safety labs will be drawn and sent to the local lab for analysis.

Study drug compliance over the preceding days will be assessed. If there is an insufficient level of drug compliance (less than 80% overall and or less than 80% in the last week), the site must consult with the Project Directors and Medical Monitor regarding timing and conduct of the remaining confinement visit procedures. It is possible that subjects will be discontinued early from the study due to insufficient study drug compliance.

Admission visit procedures include conducting a brief Physical and Neurological Exam, collecting vital signs, reviewing and recording adverse events and concurrent medications. Please refer to Appendix 3.1, Confinement Visit Phase 1: Admission Diagram.

Phase 2: 36-Hour Sampling

For an overview of the 36-Hour Sampling time points, please refer to the Confinement Visit Schedule of Events (Appendix 3.2b).

If no medical or compliance concerns are identified during the admission procedures, the subject will move on to the 36-Hour Sampling Phase, which entails the following procedures:

1. Place an intravenous line (for infusion) as well as a second venous line for blood sampling
2. Place the CSF catheter
3. Collect initial samples of blood and CSF

4. Administer assigned study drug (will vary based on blinded treatment assignments) at 0 hours
5. Begin the $^{13}C_6$-labeled "heavy" leucine infusion. Subjects will receive an initial bolus of 3 mg/kg over 10 minutes, followed by 8 hours and 50 minutes of continuous intravenous infusion at a rate of 2 mg/kg/hour. The total leucine administration with the bolus is 9 hours
6. Provide a low leucine meal (NOTE: all food provided during the heavy leucine infusion must conform to low leucine dietary guidelines; refer to Appendix 4, Sample Low Leucine Menu and to the Procedures Manual for more details)
7. Continue with the following for 36 hours:
    a. Collect CSF (6 mL) and blood (10mL) every 2 hours at the following time points: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36 hours; all collection times should be calculated from the start of the heavy leucine infusion. Samples should be in separate tubes and sent to the ADCS Biomarker Core

       NOTE: If unable to obtain a total of 6 mL CSF at any given collection time (e.g., due to participant headache or participant's CSF production level) please obtain the maximum amount possible and notify ADCS Clinical Operations
    b. At 0 hours, an additional 1-2 ml CSF (or volume per Local laboratory requirements) should be sent ambient to the local laboratory for protein, glucose, and cell counts
    c. Perform safety monitoring every 4 hours while the subject is awake. This includes questions about any symptoms in relation to the CSF catheter (including potential adverse events such as leg or back pain, dizziness or headaches) and vital sign monitoring. Participants also should be asked about pain, redness or swelling at the IV site in the arm
    d. Administer assigned study drug (will vary based on blinded treatment assignments) every 8 hours – at 8, 16, 24, and 32 hours
    e. Provide low leucine meals and snacks as needed during the duration of the heavy leucine infusion; return to normal diet after completion of the infusion (Refer to Appendix 4, Sample Low Leucine Menu)
    f. Subjects remain at bedrest throughout the CSF sampling period of 36 hours. They are allowed to use a bedside commode for toileting
8. 36 hours after the start of the heavy leucine infusion, collect the final blood and CSF samples. An extra 9mL of whole blood should be sent to the central lab for the metabolic panel and a CBC/Diff. An extra 2mL of CSF should be sent to the local lab for protein, glucose, and cell counts
9. Remove the blood and LP catheters
10. Perform final safety monitoring, a brief physical and a brief Neurological Exam

If the subject experiences intolerable AE(s) that require early discontinuation of the sampling procedures and removal of the LP catheter, contact the Medical Monitor and Project Directors as soon as possible to discuss the situation. If feasible, the standard 12-hour observation and discharge procedures should be followed to allow for management and follow-up on the adverse events.

Phase 3: 12-Hour Observation

The subject should be observed for at least 2 hours following removal of the LP catheter. Subjects are allowed to be up and out of bed during this period. The Research Satisfaction Survey should also be administered.

Any clinical safety labs from the pre-confinement visit that were clinically high should be repeated and sent to the local laboratory. A blood patch will be administered as required if a subject exhibits signs of a post-spinal tap headache that is not managed with bed rest, oral and intravenous fluids, caffeine, and/or mild analgesics (e.g., Ibuprofen, Naproxen or Tylenol).

Do not continue administration of study drug after completion of the 36-Hour Sampling Phase. All used and unused study drug should be returned to the clinical site for final accountability. Please refer to Appendix 3.3, Confinement Visit Phase 3:12-Hour Observation Diagram.

Phase 4: Discharge

Prior to discharge, vital signs should be recorded and a review of AE(s) and Concurrent Medications should be conducted. Upon determination by the investigator (or qualified designee) that the subject is stable, the participant will be discharged. If a participant experiences an unstable AE, please contact the Medical Monitor and Project Directors for guidance on the appropriate course of action to be taken. Please refer to Appendix 3.4, Confinement Visit Phase 4: Discharge Diagram.

9.5.3 Post-Confinement 24-Hour Phone Follow-up

Approximately 24 hours following discharge from the Confinement Visit the subject or a person designated to speak for them will be contacted by phone to confirm the subject's well-being and to query about any new AEs.

10 EARLY TREATMENT/STUDY DISCONTINUATION

The investigators at each site will make every reasonable effort to maximize subject retention. However, if an investigator removes a subject from treatment or study, or if a subject declines further treatment or study participation, an early discontinuation visit will be completed as soon as possible following discontinuation. The early discontinuation visit will contain the same assessments as the pre-confinement visit. If an in-person visit is not possible, site personnel will complete as much of the early discontinuation visit as possible by telephone.

Subjects who discontinue from the study prior to completing all protocol procedures may be replaced at the discretion of the Coordinating Center, in consultation with the Sponsor.

10.1 Reasons for Early Discontinuation

Participants may withdraw from the study at any time as stated in the informed consent document given to the participant at the screening visit. Participants may also be discontinued from treatment/study for reasons such as the following:

- Adverse experience: The participant has experienced an adverse event that, in the opinion of the investigator, requires early termination. This may include abnormal laboratory values.

- Death.
- Safety risk: Any participant who becomes a safety risk to themselves during the trial will be withdrawn.
- Protocol violation: The participant fails to meet protocol entry criteria or does not adhere to protocol requirements.
- Non-compliance: The participant is non-compliant with completion of study-related evaluations and/ or intake of study drugs.
- In the investigator's judgment, it is in the participant's best interest to discontinue participation in the study.
- Development of suicidal or homicidal ideation requiring hospitalization or confinement.
- Participant has need for a medication prohibited by the protocol.
- Consent is withdrawn. The participant wishes to withdraw from the study, or the legally authorized representative wishes that the participant be withdrawn.
- The study is terminated by the Sponsor/Coordinating Center, alone or at the recommendation of the Data Safety Monitoring Board.
- Lost to follow up. Participant could not be recalled back to conduct follow up visits.
- Loss of informed study partner. The participant no longer has a responsible study partner to oversee participant visits and administration of study drug.
- Coordinating Center (ADCS) request. The Coordinating Center determines it is in the participant's best interest to discontinue participation from the treatment and or study.

11 STUDY-SPECIFIC INSTRUMENTS

11.1 Cognitive and Clinical Evaluations

11.1.1 Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog12)

The ADAS-Cog (Rosen 1984) is a structured scale that evaluates memory (word recall, word recognition), reasoning (following commands), language (naming, comprehension), orientation, ideational praxis (placing letter in envelope) and constructional praxis (copying geometric designs). Ratings of spoken language, language comprehension, word finding difficulty, and ability to remember test instructions are also obtained. The test is scored in terms of errors, with higher scores reflecting poorer performance and greater impairment. An ADAS-Cog12 version will be used in the current study that includes Delayed Word Recall – a measure of episodic memory (Mohs 1997). Total ADAS scores can range from 0 (best) to 70 (worse) on the standard 11 items, and then the delayed recall item is added with a score ranging from 0-10, for a maximum score of 80. The ADAS-Cog has been the primary cognitive instrument in previous and ongoing ADCS trials. Psychometrists at each site are required to complete an ADAS-Cog training and certification process prior to administering the evaluation.

11.1.2 Columbia Suicide Severity Rating Scale (C-SSRS)

Consistent with FDA regulatory guidance (FDA 2012), occurrence of suicide-related thoughts and behaviors will be assessed. The C-SSRS is a scale that captures the occurrence, severity,

and frequency of suicide-related thoughts and behaviors during the corresponding assessment period (Posner 2011). The scale includes suggested questions to elicit the type of information needed to determine if a suicide-related thought or behavior occurred.

If a suicide-related thought or behavior is identified at any time during the study, a thorough evaluation will be performed by a study physician, and appropriate medical care will be provided.

11.1.3 Logical Memory Test (Wechsler Memory Scale-Revised)

The Logical Memory Test I and II (Delayed Paragraph Recall) is a modification of the episodic memory measure from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler 1987). In this modified version, free recall of one short story that consists of 25 bits of information will be elicited immediately after it is read aloud to the participant and again after an approximate thirty-minute delay. The total bits of information from the story that are recalled immediately (maximum score = 25) and after the delay interval (maximum score = 25) are recorded. A retention or "savings" score can be computed by dividing the score achieved during delayed recall by the score achieved during immediate recall.

11.1.4 Mini-Mental State Examinations (MMSE)

The MMSE (Folstein 1975) is a brief, frequently used screening instrument in Alzheimer's disease drug studies. The MMSE scale evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two overlapping pentagons. The MMSE is scored as the number of correctly completed items with a lower score indicative of poorer performance and greater cognitive impairment. The total score ranges from 0 (worse) to 30 (perfect performance).

11.1.5 Neuropsychiatric Inventory (NPI)

The NPI is a well-validated, reliable, multi-item instrument to assess psychopathology in AD based on interview with the study partner (Cummings 1997). The NPI evaluates both the frequency and severity of 12 neuropsychiatric features, including delusions, hallucinations, agitation/aggression, dysphoria, anxiety, euphoria, apathy, disinhibition irritability/lability, and aberrant motor behavior, as well as sleep and appetite/eating disorders. Frequency ratings range from 1 (occasionally, less than once per week) to 4 (very frequently, once or more per day or continuously). Severity ratings range from 1 (mild) to 3 (severe). The score for each subscale is the product of severity and frequency and the total score is the sum of all subscales.

11.1.6 Clinical Dementia Rating (CDR)

The CDR (Berg 1998) describes five degrees of impairment in performance on each of six categories of cognitive functioning including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. The ratings of degree of impairment obtained on each of the six categories of function are synthesized into one global rating of dementia (ranging from 0 to 3), with more refined measure of change available by use of the Sum of Boxes. Reliability and validity has been established, as has high inter-rater reliability. This will be used as a global measure of severity of dementia. Where a full CDR interview is not possible, the abbreviated CDR (Davis 1991) can be utilized.

11.1.7 Research Satisfaction Survey (RSS)

The Research Satisfaction Survey (RSS) was adapted from the work of Larsen (Larsen 1979) to measures the satisfaction of elderly volunteers in clinical trials to treat or prevent cognitive loss or dementia. The RSS builds on the Client Satisfaction Questionnaire (CSQ-8) which demonstrates a high degree of validity and internal consistency and measured consumer satisfaction with health and human service programs. Higher scores on the CSQ indicate a greater level of satisfaction in service programs[102]; conversely, lower CSQ-8 scores have been associated with higher dropout rates (Lunnen 1998) and missed appointments in treatment settings (Larsen 1983) and in urban Community Mental Health programs (Larsen 1979). Open-ended questions about satisfaction are part of the CSQ, and allow participants to provide feedback about specific operational procedures of a particular service program (Larsen 1979). The RSS is similarly designed with a 7- item survey and may include additional items to inquire about the specific aspects of a given trial. Four items are designed to be answered with a modified Likert scale and can be used to create a summary score with a range of 4-16, which can be analyzed with parametric statistics; three items provide for open ended responses about the intervention, the assessments and the frequency of visits and may be used to understand retention rates.

12 STUDY-SPECIFIC PROCEDURES

12.1 Safety Assessments

Safety will be evaluated by monitoring for changes in the parameters summarized below, including any AEs/SAEs as reported by subjects or observed by the clinical staff, or by the use of concomitant medication during the study.

12.1.1 Physical and Neurological Examination

A medically qualified professional will perform a brief physical examination that consists of a review of the major body systems (i.e., skin, head/ears/eyes/nose/throat (HEENT), cardiovascular, pulmonary, abdomen, musculoskeletal, and extremities) and a brief neurological examination which will include an assessment of cranial nerves, strength, coordination, reflexes, sensation, tremor, gait and mental status. Assessments of height, weight, and vital signs (systolic and diastolic blood pressure, pulse, temperature, and respiration) are included.

12.1.2 Electrocardiogram (ECG)

An appropriately qualified individual will conduct a standard 12-lead resting ECG. The ECG report must be reviewed, signed, and dated by the site PI (or a medically-qualified individual delegated by the site PI). Those with clinically significant ECG findings will be referred for follow-up as deemed appropriate by the investigator and may be excluded from the study.

12.1.3 Clinical Laboratory Evaluations

Blood and urine samples will be obtained for clinical safety lab assessments as described in the Schedule of Events (Appendix 1). The following table lists the clinical safety lab tests that will be assessed by the central lab at three time points: the screening visit, the pre-confinement visit, and the end of the 36-hour sampling period.

Table 4. Clinical Safety Lab Tests

CLINICAL SAFETY LAB TESTS		
METABOLIC PANEL	**COMPLETE BLOOD COUNT**	**URINALYSIS**
Sodium (Na) Potassium (K) Chloride (Cl) Carbon Dioxide (CO2) Blood Urea Nitrogen (BUN) Glucose Calcium (Ca) Creatinine (Crn) Bilirubin Total Albumin Protein (NOS) Total Glutamic-OxaloaceticTransferase (AST, SGOT) Glutamic-PyruvateTransferase (ALT, SGPT) Alkaline Phosphatase NOS	White Blood Cell Count (WBC) Red Blood Cell Count (RBC) Hemoglobin (Hb) Hematocrit (HCT) Mean Corpuscular Volume (MCV) Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Hemoglobin Concentration (MCHC) Red Blood Cell Distribution Width (RDW) Mean Platelet Volume (MPV) Platelet Count (PLT) COAGULATION PANEL PPT/PTT [Screening Visit ONLY]	Color Appearance Specific Gravity pH Blood Glucose Protein Ketones Leukocyte Esterase Nitrite Urobilinogen Bilirubin Pregnancy Test (only screening visit)

Routine laboratory samples (including CSF) that are collected at the admission before the LP, the post-confinement visit, and the 12-hour observation (if necessary) should be analyzed by a CLIA/CAP (or equivalent) certified local laboratory. Lab reports will be reviewed, signed and dated by the site PI (or a medically qualified individual delegated by the PI). If a value is outside of the laboratory's normal range, the clinician will indicate if the value has clinical significance. Those results that are deemed clinically significant may need to be repeated and may require follow up with the subject's primary care physician for further evaluation.

12.2 Biofluids

12.2.1 CSF for Biomarkers

Standard Lumbar Puncture:

A lumbar puncture will be performed as part of the screening visit, in the morning (before 10am is recommended), after an overnight fast. 2ml CSF will be sent for analysis by the site (total protein, glucose, cell count) to a local lab. The remaining CSF will be placed into a 14 ml conical polypropylene tube, inverted gently 3-4 times, then centrifuged at 1500 x g for 10 minutes at room temperature. The supernatant should be aliquoted into 2.0 ml tubes, frozen at -80^{o}, and shipped overnight to the ADCS Biomarker Core. The ADCS Biomarker Core will subsequently send these samples to C2N for processing and analysis according to Biomarker core SOPs. Analyses will include AD biomarkers (e.g., Aβ, sAPPs, tau).

CSF samples will be banked in the biospecimen repository of the ADCS Biomarker Core for various future research studies; access to samples will be granted according to SOPs established by ADCS Biomarker Core.

Prior to the LP, a coagulation panel will be obtained to rule out a clotting disorder. Participants taking an anti-platelet agent (e.g., Plavix) must be discontinued from that agent for a minimum of 5 days prior to the lumbar puncture. These participants may continue with the agent after a minimum of 24 hours post LP. Participants who are taking anticoagulants (e.g., warfarin (Coumadin) and/or dabigatran (Pradaxa) may not undergo an LP and are not suitable to participate in this study.

When done at the same visit, the LP should occur after the MRI scan. When done at the same visit, if the MRI scan can only be scheduled prior to the LP, the site must contact ADCS Clinical Operations for permission before proceeding. In the unplanned event that the MRI does not precede the LP, there should be a minimum window of 72 hours between the MRI and the LP. LP under fluoroscopy is permitted; however, the site must contact ADCS Clinical Operations for permission before proceeding. Site personnel should advise the subject that use of fluoroscopy (x-rays) involves exposure to radiation.

Each study participant or a person designated to speak for them will be contacted by phone approximately 24 hours after the LP to confirm participant well-being and any adverse events.

CSF Sampling:

A total volume of 116 mL of CSF should be collected during the CSF catheter sampling during the confinement visit. At hours 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36, 6 mL of CSF is collected.

NOTE: If unable to obtain a total of 6 mL CSF at any given collection time (e.g., due to participant headache or participant's CSF production level) the maximum amount will be obtained and ADCS Clinical Operations must be notified.

To clear blood associated with needle insertion, the first 1-2 mL (or more if needed) of CSF should will be discarded. In addition, at 0 hours, 1-2 ml CSF (or volume per Local laboratory requirements) will be sent ambient to the local laboratory for protein, glucose, and cell counts. At the 36 hours, an extra 2mL of CSF should be sent to the local lab for protein, glucose, and cell counts. The remaining CSF should be placed into a 14 ml conical polypropylene tube, inverted gently 3-4 times, and then centrifuged at 1500 x g for 10 minutes at room temperature. Then the supernatant should be aliquoted into 2.0 ml tubes and frozen at -80^{o} and shipped overnight to the ADCS Biomarker Core.

Each study participant or a person designated to speak for them will be contacted by phone one day after being discharged from the confinement visit. The participant will be asked about their well being and the occurrence of any adverse events.

12.2.2 APOE Genotype and other DNA Markers

DNA will be extracted from participant blood samples at the screening visit and will be analyzed for APOE genotype. This will allow secondary analyses of data on the impact of the APOE genotype on putative biomarkers of AD, clinical outcome measures, and adverse events. APOE polymorphisms are an important genetic risk factor for AD. Individuals with one or two Apolipoprotein epsilon 4 (APOE4) alleles have a higher risk of developing AD and an earlier age of onset. Furthermore, those with one or two APOE4 alleles exhibit a more rapid rate of clinical change in some, but not all studies.

Blood is collected in a uniform fashion using EDTA as anti-coagulant, as described in Section 12.2.3. Once blood is collected into a 10 mL EDTA plastic tube, it is shipped overnight at ambient temperature to the ADCS Biomarker Core for APOE genotyping. Participants will be asked to consent to optional DNA banking for future research studies.

12.2.3 Plasma for Biomarkers and Pharmacokinetics

Plasma samples will be collected at screening visit and during the confinement visit every 2 hours for analysis of AD biomarkers (e.g., Aβ40 & 42 and exploratory biomarkers), cholinesterase inhibition, and drug level analyses.

Biomarker and PK analyses at the screening visit will be performed on the 10 ml EDTA plastic tube whose collection is described in Section 12.2.2.

In addition, 10 ml of blood will be collected in a uniform fashion into 10mL lavender top EDTA tubes at hours 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 during the confinement visit. Once blood is collected, it should be centrifuged at 1500 x g for 10 minutes at room temperature. 2 ml of plasma should be aliquoted into 2.0 ml freezer tubes, frozen at -80°, and shipped overnight on dry ice to the ADCS Biomarker Core.

At the last collection (36 hours), an extra 9mL of whole blood should be sent to the central lab for metabolic panel and CBC/Diff.

Plasma samples will be banked in the biospecimen repository of the ADCS Biomarker Core for future research studies; access to samples will be granted according to SOPs established by the ADCS Biomarker Core.

12.2.4 DNA/Genome Wide Association Studies (GWAS)/APOE Sample

A single 10 mL EDTA tube of whole blood will be collected for DNA/GWAS and APOE genotyping and epigenetic analysis at the screening visit. The EDTA tube will be shipped overnight at ambient temperature to the ADCS Biomarker Core and processed per SOP for APOE genotyping. If the sample condition is compromised or if there is poor sample yield, subjects will be asked to agree to re-sampling.

In addition, subjects will be asked to consent to optional DNA banking for future research studies. The DNA will extracted and banked in biospecimen repository of the ADCS Biomarker Core.

13 PERSONNEL REQUIREMENTS

The site PI is responsible for the overall conduct of the study at the site. The PI is to supervise project personnel and ensure that clinical raters maintain a high level of skill and accuracy in conducting assessments. Additionally, the PI will perform or supervise clinical evaluation of all subjects and ensure protocol adherence. Additional key personnel will be required, as outlined in the procedures manual.

14 ADVERSE EVENTS (AEs)

14.1 Definition

An AE is defined as per the US Code of Federal Regulation, Title 21, Part 312.32 [http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32]. An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Collection of adverse events will begin once informed consent is signed and will continue until the follow-up phone call performed on the day after discharge. Adverse events include but are not limited to: (1) worsening or change in nature, severity, or frequency of conditions or symptoms present at the start of the study; (2) Subject deterioration due to primary illness; (3) intercurrent illness; and (4) drug interaction. An abnormal laboratory value will only be reported as an AE if the investigator considers it clinically significant, or if it leads to the subject being withdrawn from the study.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and or other clinical information. In such cases, the diagnosis should be documented as the AE and not the individual signs or symptoms. Symptoms and conditions present at the beginning of the study will be characterized, so that AEs can be defined as any new symptom, or any increase in frequency or severity of an existing symptom. Adverse events should be described with medical terminology so that the event can be matched against a medical coding dictionary, such as MedDRA (Medical Dictionary for Regulatory Activities).

Investigators should report their assessment of the potential relatedness of each AE to the protocol procedure, and to the investigational product, and/or drug delivery system used in the protocol.

Following questioning and evaluation, all AEs, whether determined to be related or unrelated to the study drug by a medically qualified site PI or clinician must be documented in the subject's records, in accordance with the investigator's normal clinical practice, and on the AE electronic case report form (eCRF).

14.2 Following Up on AEs

The investigator is obliged to follow subjects with AEs until the events have subsided, the conditions are considered medically stable, or the subjects are no longer available for follow up. Subjects who discontinue due to adverse experiences will be treated and followed according to established medical practice. All pertinent information will be entered into the eCRF. Adverse events will be reported to the independent DSMB, per ADCS SOPs and study DSMB Charter.

Because of the potential of additive cholinergic adverse events caused by a combination of Posiphen and cholinesterase inhibitors in some subjects, the severity of such adverse events should be carefully evaluated taking the criteria in Appendix 5 into consideration (modified from CTCAE version 4.03.)

15 SERIOUS ADVERSE EVENTS (SAE)

15.1 Definition

A SAE is defined as per the US Code of Federal Regulation, Title 21, Part 312.32 [http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=312.32].

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or Sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

15.2 Reporting SAEs

Collection of serious adverse events will begin once informed consent is signed (regardless of study drug exposure) and will continue until 30 days following the last dose of study drug. All SAEs must be reported to the Medical Monitor, Project Directors and the Coordinating Center within 24 hours of learning of the event. This in turn will trigger an alert to the appropriate Coordinating Center personnel and Protocol Project Directors, which will lead to the initiation of the creation of the report. A notification will be sent to all participating sites and the DSMB once the report is available. Sites will inform their IRB of the event based on local IRB requirements. Annovis Bio, Inc. the IND holder is responsible for submitting any SAEs according to the FDA reporting requirements.

16 DATA AND SAFETY MONITORING BOARD (DSMB)

The Coordinating Center currently has a DSMB that reviews the safety of all subjects enrolled in trials on an ongoing basis. The initial task of the DSMB will be to review the protocol to identify any necessary modifications. If modifications are necessary, revisions will be reviewed by the DSMB prior to its recommendation on initiation of the project. The DSMB, based on its review of the protocol, will work with the Medical and Safety Core personnel to identify the study-specific data parameters and format of the information to be regularly reported. The DSMB will initially be provided with data blinded to treatment status, but they may request unblinded data if there is a safety concern. The DSMB and NIA representative will meet in person or by conference call on a quarterly basis.

Additionally, the DSMB will be informed of the occurrence of any serious adverse events within 7 days of being reported to the Coordinating Center. The DSMB may at any time request additional information from the Coordinating Center.

Based on the review of safety data, the DSMB will make recommendations regarding the conduct of the study. These may include amending safety monitoring procedures, modifying the protocol or consent, terminating the study or continuing the study as designed. Using the ADCS

Safety Review Process (review of lab data, vitals, and adverse events) and the DSMB, there is substantial oversight and case review to alert the investigators, in a timely manner, to any safety issues that may arise. For further details, please refer to the DSMB charter.

Lastly, the DSMB will evaluate safety and tolerability for each dosing group.

17 RECORDING AND COLLECTION OF DATA

17.1 Case Report Form

The PI or designee will record all data collected (either written or electronic record of data). Written or electronic data of record must be entered on the eCRF provided for that purpose. In some instances, no prior written or electronic record of data may exist and data recorded directly on the eCRF is considered source data. The site will be suitably trained on the use of the eCRF and appropriate site personnel will be authorized to provide electronic signatures. The PI is responsible to verify the integrity of the data and acknowledge as such by signature.

All site entries will be made in a secured web site and the PI will review the record for completeness. If corrections are necessary to the eCRFs, the PI or designee will update the eCRF and provide documentation for the reason for change.

Completed eCRFs will be submitted according to provided instructions, and reviewed by the Coordinating Center to determine their acceptability. If necessary, data correction requests will be generated for resolution by the study site.

17.2 Study Files and Source Documents

Subject confidentiality is strictly held in trust by the participating investigators, research staff, and the Coordinating Center and/or sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsoring institution. Authorized representatives of the sponsoring institution may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records. Any data, specimens, forms, reports, video/audio recordings, and other records that leave the site will be identified only by a subject identification number (Subject ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the subject, except as necessary for monitoring by the Institutional Review Board (IRB), the FDA, the NIA, and the Office for Human Research Protections (OHRP).

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Those regulations require a signed Subject HIPAA 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. Each site PI, under the guidance of his/her IRB, is responsible for ensuring that all applicable HIPAA regulations and State laws are met.

18 ETHICS AND REGULATORY CONSIDERATIONS

18.1 Good Clinical Practice

This study will be conducted in compliance with the protocol and accordance with Good Clinical Practice (GCP) guidelines, as defined by the International Conference on Harmonisation (ICH) Guideline, Topic E6, the United States Code of Federal Regulations (CFR), Title 21, Part 50 – Protection of Human Subjects, and Part 56 – IRBs, HHS Regulations (45 CFR part 46, 160, 164 HHS Regulations for the Protection of Human Subjects; HIPAA; 42 CFR part 50), Subpart F HHS Regulations for Responsibility of Applicants for Promoting Objectivity in Research for Which PHS Funding Is Sought HIPAA, State and Federal regulations and all other applicable local regulatory requirements and laws.

Study personnel involved in conducting this study will be qualified by education, training and experience to perform their respective task(s) in accordance with GCP.

No study document shall be destroyed without prior written agreement between the Coordinating Center and the investigator. Should the investigator wish to assign study records to another party or move them to another location, he/she may do so only with the prior written consent of the Coordinating Center.

18.2 Institutional Review Board (IRB)

Institutional Review Boards must be constituted and their authority delegated through the institution's normal process of governance according to applicable State and Federal requirements for each participating location. Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate IRB registered with the OHRP. Any amendments to the protocol or consent materials must also be approved before they are placed into use. In the United States, only institutions holding a current US Federal wide Assurance (FWA) issued by OHRP may participate. Refer to: http://www.hhs.gov/ohrp/assurances/.

The investigator must obtain approval from the IRB for all subsequent protocol amendments and, when warranted, changes to the informed consent document. Protocol and informed consent form amendments can be made only with the prior approval of the Coordinating Center. The investigator may not implement any protocol deviation without prior notification, review, and documented approval from the ADCS, except where necessary to eliminate an immediate hazard to study subjects, or when change(s) involve only logistical or administrative aspects of the trial. The investigator shall notify the IRB of deviations from the protocol or serious adverse events occurring at the site, in accordance with local procedures.

18.3 Informed Consent and Health Insurance and Portability and Accountability Act (HIPAA) Compliance

Informed consent will be obtained in accordance with 21CFR§50.25, and ICH Good Clinical Practice. Applicable HIPAA privacy notifications will be implemented and HIPAA authorizations signed before protocol procedures are carried out. Information should be given in both oral and written form as deemed appropriate by the Site's IRB.

Prior to the beginning of the trial, the investigator must obtain the IRB's written approval of the informed consent form and any other written information to be provided to subjects and be acceptable by ADCS Regulatory Affairs. Consent forms must be in a language fully comprehensible to the prospective subjects and/or their authorized representatives and study partners. Subjects, their relatives, guardians, or authorized representatives and study partners will be given ample opportunity to inquire about the details of the study. Prior to a subject's participation in the trial, the written informed consent form must be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion. Subjects will be provided a copy of the signed ICF.

The informed consent will not only cover consent for the trial itself, but for the genetic samples/data/storage and biomarker samples/data/storage as well. Consent forms will specify that DNA and biomarker samples are for research purposes only; the tests on the DNA and biomarker samples are not diagnostic in nature and subjects will never receive results.

18.4 Genetic Research and Storage of Genetic Material

Only DNA from consenting subjects will be banked and used to facilitate future research on aging and dementia, particularly in the discovery of genetic polymorphisms that may influence risk of developing AD. Collection of DNA will permit investigators to probe candidate genetic polymorphisms as predictors of outcome in future studies. The samples will be stored by the ADCS as long as funding is available from the National Institutes of Health (NIH). If funding should lapse completely, the UCSD ADRC will provide responsible custodianship of the ADCS Biomarker Core.

18.5 Storage of Biospecimen Samples

All biospecimens being banked for future AD biomarker research will be shipped to and stored by the ADCS Biomarker Core. Sample tubes will be barcoded and inventoried by subject ID number only and banked without personal identifiers, in accordance with ADCS Biomarker Core SOPs. The presence of the sample is recorded into a computerized inventory database that is encrypted and password-protected.

Only specimens from consenting subjects will be banked and used to facilitate future research on aging and dementia. The samples will be stored by the ADCS as long as funding is available from the NIH. If funding should lapse completely, the UCSD ADRC will provide responsible custodianship of the ADCS Biomarker Core.

19 STUDY MONITORING

The clinical monitor is responsible for inspecting the online case report forms and source documentation at specific time points throughout the study to verify adherence to the protocol,

completeness and accuracy of the data, and adherence to local regulations on the conduct of clinical research. The monitoring visits must be conducted according to the applicable ICH and FDA guidelines to ensure protocol adherence, quality of data, drug accountability, compliance with regulatory requirements and continued adequacy of the investigational site and its facilities. The Site Investigator will cooperate in the monitoring process by ensuring the availability of the eCRFs, source documents and other necessary documents at the time of the monitoring visits. The Site Investigator will promptly address any matters brought to his/her attention by the monitor. The Site Investigator may also be asked to meet in-person with the site monitor during certain visits.

20 AUDIT

In accordance with ICH E6 (Good Clinical Practices) representatives of the Coordinating Center and/or Sponsor and/or regulatory agency may select this study for audit. The investigator and study staff are responsible for maintaining the site master file containing all study-related regulatory documentation as outlined by ADCS Regulatory Affairs that will be suitable for inspection at any time by ADCS, Sponsor, its designees, and/or regulatory agencies. Inspection of site facilities (e.g., pharmacy, laboratories) to evaluate the trial conduct and compliance with the protocol may also occur.

21 RECORD RETENTION

Essential documents and study records must be retained for a minimum of seven years following primary publication of study results. The Coordinating Center will notify sites when retention of such documents is no longer required. Essential documents should be retained for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

22 PUBLICATION POLICY

The results of this study will be published. To coordinate dissemination of data from this study, a publication committee will be formed. The committee will consist of the The study PIs, interested site PIs, and appropriate ADCS and Coordinating Center personnel The committee will solicit input and assistance from the Sponsor and other investigators, as appropriate, and adhere to all ADCS Publications Policies.

23 SHARING OF FINAL RESEARCH DATA

Data from this research will be shared with other researchers pursuant to the 02/26/2003 “NIH Final Statement on Sharing Research Data”. The ADCS grant contains a data sharing policy consistent with the goals of the NIH but which also respects the rights of commercial partners. The NIH policy can be found at:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html

NIH believes that data sharing is important for further translation of research results into knowledge, products, and procedures to improve human health. The NIH endorses the sharing of final research data to serve these and other important scientific goals. To protect subjects' rights and confidentiality, identifiers will be removed from the data before they are shared.

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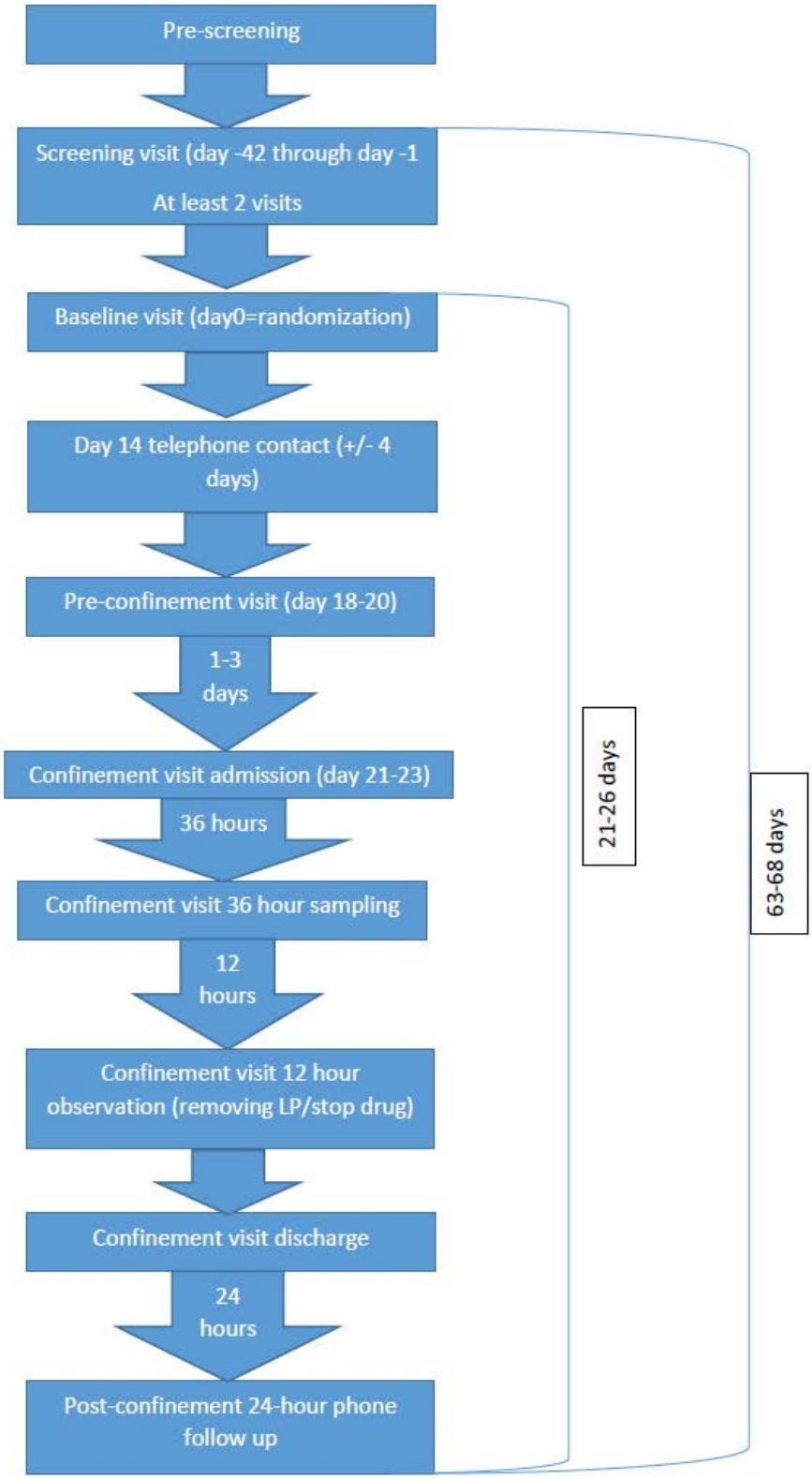
25 APPENDICES

APPENDIX 1: SCHEDULE OF EVENTS

Visit Timing [a]	Day -42 to 0		Day 14 (+/- 4)	1-3 days prior to confinement	Days 21-23 (+/- 2)			
					Confinement			
	Screening	Baseline	Phone contact	Pre-confinement [e]	Admission	36-Hour Sampling	12-Hour Observation	Discharge
Informed Consent	X							
Demographics	X							
Medical and Psychiatric History	X							
CDR	X							
Logical Memory	X							
MMSE	X			X				
ADAS-Cog12		X		X				
NPI		X		X				
Physical and Neurological Examination	X	X		X	X	X		
C-SSRS	X			X				
Research Satisfaction Survey		X					X	
Vital Signs [b]	X	X		X	X	X		X
Height	X							
Clinical Safety Lab Tests[g]	X			X	X	X	PRN [f]	PRN [f]
12-Lead ECG	X							
MRI of Brain[c]	X							
Lumbar Puncture	X							
Blood Sampling for Research Labs[h]	X					X		
PK Sampling						X		
Adverse Events	X	X	X	X	X	X		X
Concomitant Medication	X	X	X	X	X	X		
Dispense Study Drug		X				X		
Dosing		X				X		
Study Drug Compliance			X	X	X		X	
LP Catheter Placement						X		
Heavy Leucine Infusion						X		
CSF Catheter Sampling						X		
24-Hour Phone Follow-Up [d]	X							X

[a] Refer to Section 9 for more details on visit timing and windows.
[b] Vital signs will include sitting blood pressure, pulse, temperature, respiration rate, and weight, except during the 36-hour Sampling Phase of the Confinement Visit when weight will not be included.
[c] An MRI of the brain will only be conducted if there is no brain MRI available from within the window specified in Inclusion Criteria.
[d] Phone Follow-up should occur approximately 24-hours after completion of the LP procedure at Screening and after discharge from the Confinement Visit.
[e] The Early Discontinuation Visit will contain the same assessments as the Pre-confinement Visit.
[f] If safety labs are clinically indicated during the Confinement Visit, they should be sent to a local laboratory rather than the central lab.
[g] Refer to Section 12.1.3 for the list of tests to be performed
[h] Refer to Section 9.2 for details

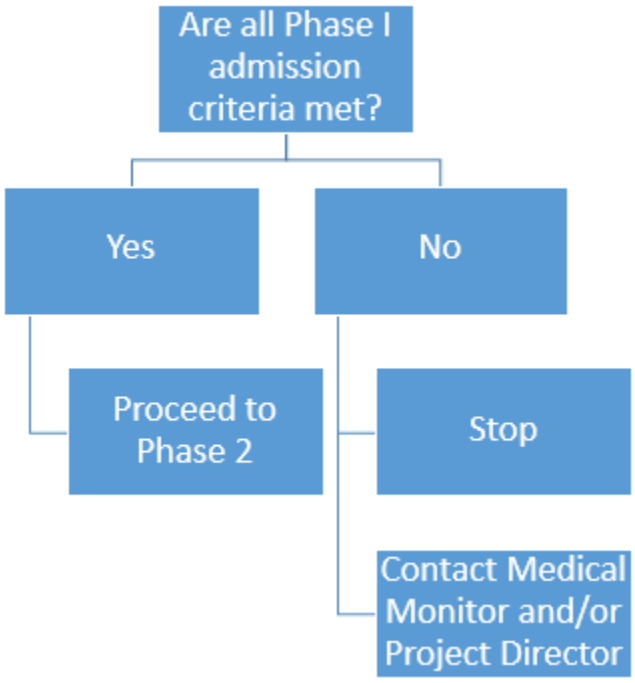
APPENDIX 2: STUDY SCHEMATIC DIAGRAM



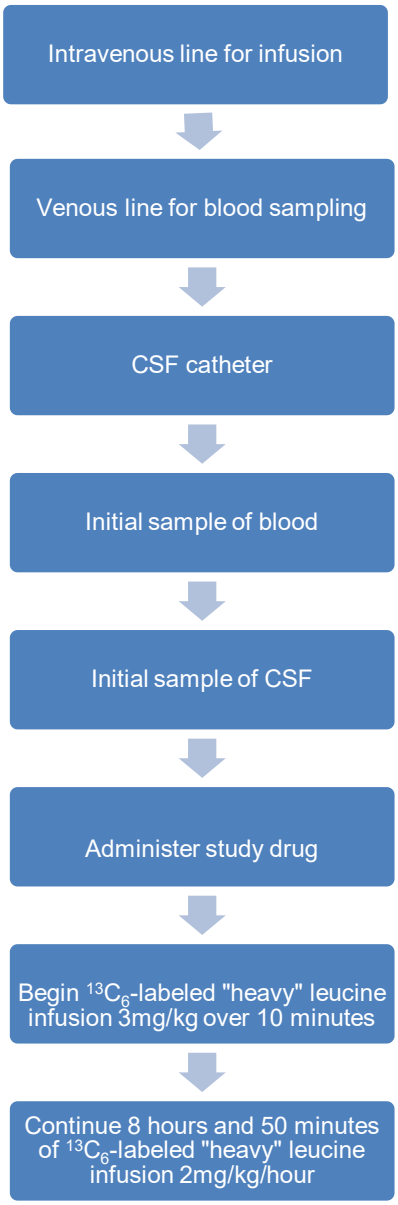
APPENDIX 3: CONFINEMENT VISIT DIAGRAM

Appendix 3.1 Confinement Visit Phase 1: Admission Diagram

Conditions	Check
Arriving early pre-lunch	
Overnight fasting	
Study Drug Compliance	
Physical exam	
Neurological exam	
Clinical safety labs	
Vital signs	
AEs reviewed and recorded	
Con Meds reviewed and recorded	



Appendix 3.2a Confinement Visit Phase 2: Sampling – 36-Hour Observation Diagram

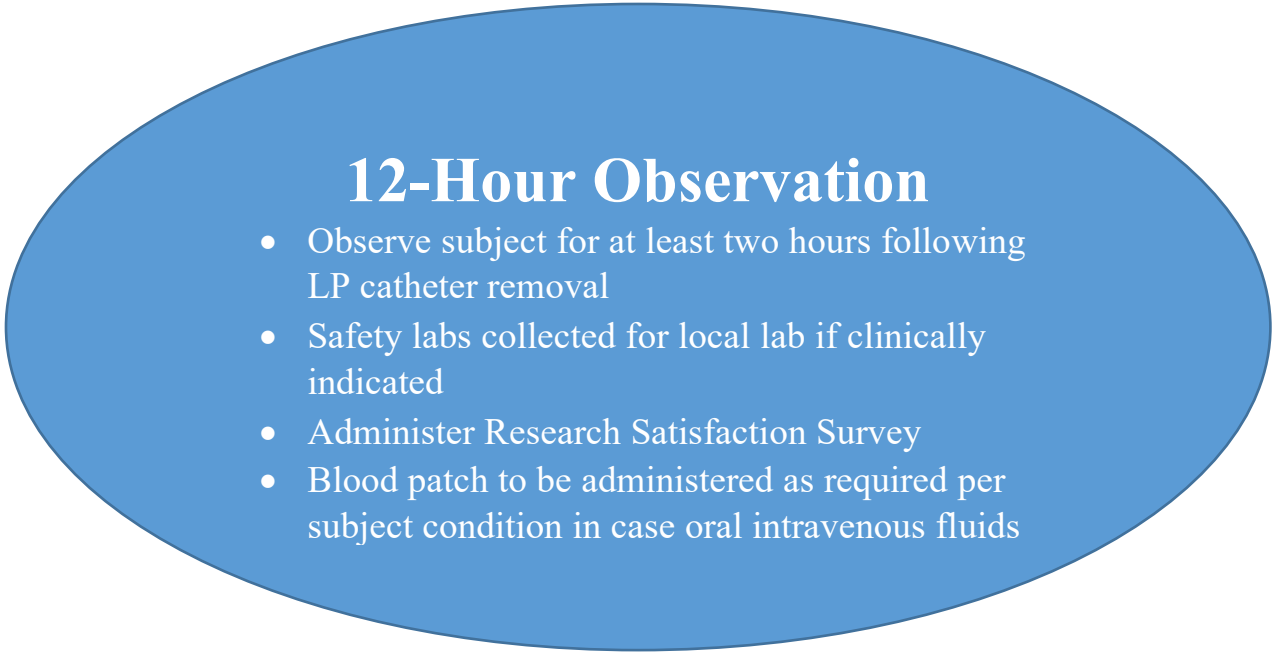


Appendix 3.2b Confinement Visit Phase 2: Sampling – 36-Hour Observation Schedule of Events

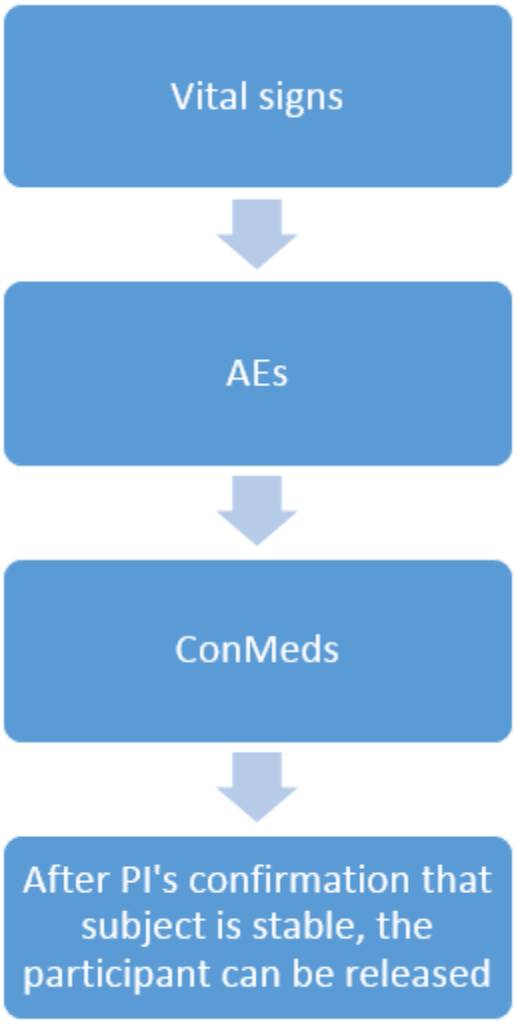
All sample collection times should be calculated from the start of the heavy leucine infusion

Hours	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Blood draw(10mL) includes PK sampling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Posiphen dosing	X				X				X				X				X		
CSF catheter sampling (6mL)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Extra blood 9mL (central labs)																			X
Extra CSF 2mL (local labs)	x																		X
Leucine infusion (3mg/kg–10min)	X																		
Leucine infusion (2mg/kg/hour – 8 hours 50min)	X	X	X	X	X														
Safety Monitoring including vital signs (when subject is awake)	X		X		X		X		X		X		X		X		X		X
Removing blood and LP catheters																			X
Low leucine meal	Includes meals, snacks, and beverages					Normal diet													
Physical and Neurological Exam																			X

Appendix 3.3 Confinement Visit Phase 3: 12-Hour Observation Diagram



Appendix 3.4 Confinement Visit Phase 4: Discharge Diagram



APPENDIX 4: SAMPLE LOW LEUCINE MENU

Low leucine meals are 1,500 mg of leucine or less a day. Individualized diet/menu is based on food preferences and coffee consumption since coffee contains leucine. Foods used for the menu are determined by availability in research kitchen and leucine values based on nutritional database values (i.e. Nutritionist Pro or Esha.com). Below is the sample of meals, beverages, and snacks menu.

	Amount	Unit	Kcal (kcal)	Protein (g)	Carb (g)	Fat (g)	Leuc (mg)
			2268.830	17.847	528.804	20.605	901.059
Breakfast			318.215	5.040	67.456	4.304	199.980
Juice, Orange, Unsweetened, Prepared from Frozen Concentrate	4.000	fl. oz.	56.025	0.847	13.421	0.075	16.185
Banana No peel (prep just before serving	101.500	g	90.335	1.106	23.183	0.335	69.020
Bread, Wheat	1.000	sl.	67.500	2.593	12.365	0.860	97.000
Jelly (includes Grape) with Leucine	25.000	g	66.500	0.038	17.488	0.005	0.000
Margarine, Spread, 60% Fat, Stick or Tub or Bottle w/ Leucine	5.000	g	26.300	0.030	0.000	2.959	0.000
Coffee, Brewed	12.000	fl. oz.	3.555	0.427	0.000	0.071	17.775
SWEET N LOW Sugar Substitute, Packet	2.000	item	8.000	0.000	1.000	0.000	0.000
Morning Snack			275.270	1.661	65.782	1.552	100.007
Crackers, Graham, Plain	14.000	g	59.220	0.966	10.752	1.414	66.500
Pear, Halves, Canned in Juice, Drained	197.100	g	98.550	0.670	25.505	0.138	33.507
Drink, Cranberry Juice Cocktail, Prepared from Frozen Concentrate w/ Leucine	8.000	fl. oz.	117.500	0.025	29.525	0.000	0.000
Lunch			477.229	3.660	114.135	3.677	199.994
Lettuce, Iceberg Leaves	50.000	g	7.000	0.450	1.485	0.070	12.500
Tomatoes, Red	15.000	g	2.700	0.132	0.584	0.030	3.750
Carrots	15.000	g	6.150	0.140	1.437	0.036	15.300
WISH BONE Salad Dressing, Italian, Light	20.000	g	23.333	0.000	2.000	1.667	0.000
Crackers, Saltine	18.000	g	75.780	1.710	13.381	1.593	117.360
Peach, Halves, Canned in Juice, Drained	141.900	g	62.436	0.894	16.418	0.043	51.084
Juice, Apple, Prepared from Frozen Concentrate w/ Leucine	8.000	fl. oz.	112.330	0.335	27.581	0.239	0.000
PEPSI Soda, Regular Cola	15.000	fl. oz.	187.500	0.000	51.250	0.000	0.000
Afternoon Snack			357.310	2.353	78.447	5.483	100.780
Cookie, Wafer, Vanilla	26.000	g	122.980	1.118	18.486	5.044	78.780
Mandarin Oranges, Canned in Light Syrup, Drained	200.000	g	122.000	0.900	32.380	0.200	22.000
Juice, Apple, Prepared from Frozen Concentrate w/ Leucine	8.000	fl. oz.	112.330	0.335	27.581	0.239	0.000
Dinner			538.445	3.264	129.832	4.007	200.294
Lettuce, Iceberg Leaves	50.000	g	7.000	0.450	1.485	0.070	12.500
Tomatoes, Red	15.000	g	2.700	0.132	0.584	0.030	3.750
Carrots	15.000	g	6.150	0.140	1.437	0.036	15.300
WISH BONE Salad Dressing, Italian, Light	20.000	g	23.333	0.000	2.000	1.667	0.000
Crackers, Saltine	22.200	g	93.462	2.109	16.503	1.965	144.744
Applesauce, Unsweetened, Canned	240.000	g	100.800	0.408	27.048	0.240	24.000
Drink, Cranberry Juice Cocktail, Prepared from Frozen Concentrate w/ Leucine	8.000	fl. oz.	117.500	0.025	29.525	0.000	0.000
PEPSI Soda, Regular Cola	15.000	fl. oz.	187.500	0.000	51.250	0.000	0.000
Evening Snack			302.360	1.870	73.153	1.582	100.004
Crackers, Graham, Plain	14.000	g	59.220	0.966	10.752	1.414	66.500
Pineapple, Canned in Juice, Drained	209.400	g	125.640	0.879	32.876	0.168	33.504
Drink, Cranberry Juice Cocktail, Prepared from Frozen Concentrate w/ Leucine	8.000	fl. oz.	117.500	0.025	29.525	0.000	0.000

APPENDIX 5: SEVERITY EVALUATION OF CHOLINERGIC ADVERSE EVENTS

Because of the potential of additive cholinergic adverse events caused by a combination of Posiphen and cholinesterase inhibitors in some subjects the severity of such adverse events should be carefully evaluated taking the criteria below into consideration (modified from "CTCAE" version 4.03.)

Adverse Event	Mild	Moderate	Severe
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>=6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated
Diarrhea	Increase of <4 stools per day over baseline	Increase of 4 - 6 stools per day over baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; limiting self care ADL
Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); tube feeding or TPN indicated
Fatigue	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self care ADL