1. CLINICAL RESEARCH PROTOCOL

Study Title:	A Phase 2, Proof-of-Concept, Double-Blind-Randomized, Placebo-Controlled Adaptive Design Trial of Nicotinamide in MCI due to AD and Mild AD Dementia
Short Title:	Nicotinamide as an Early AD Treatment (NEAT) Trial
IRB #:	UC Reliance # 2016-10797
Project Director:	Joshua Grill, PhD, UC Irvine
ADCS Principal Investigator:	Howard Feldman, MD, UC San Diego
Sponsor:	UC Irvine Institute for Memory Impairments and Neurological Disorders
Funding source:	UC Cures Initiative, University of California Office of the President
Protocol version:	Version 4.0; December 28, 2017

2. STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Project Director: _____
Print/Type Name

Signed: _____ Date:_____
Signature

Site Principal Investigator: _____
Print/Type Name

Signed: _____ Date:_____
Signature

3. Protocol Synopsis

Title of study: A Phase 2, Proof-of-Concept, Double-Blind-Randomized, Placebo-Controlled Adaptive Design Trial of Nicotinamide in MCI due to AD and Mild AD Dementia

Background: Nicotinamide, the amide of nicotinic acid (vitamin B3/niacin), is an oral therapy with a wealth of clinical data in a variety of therapeutic areas, including preliminary data supporting its safety in Alzheimer's disease (AD). Preclinical work in a mouse model that develops both plaques and tangles supports the hypothesis that nicotinamide can act as a histone deacetylase (HDAC) inhibitor to reduce phosphorylation of tau.[1, 2] In this mouse model of AD, treatment with nicotinamide reduced p-tau, increased microtubule stability and rescued cognitive function.

Developmental phase: Phase 2

Research objectives:
1: Determine if daily treatment with high dose nicotinamide can reduce levels of phosphorylated tau in individuals with mild AD dementia or MCI due to AD.
2: Assess the safety and tolerability of high dose nicotinamide treatment in individuals with mild AD dementia or MCI due to AD.

Study design: Group sequential design, incorporating a futility analysis with a go/no-go decision conditional on CSF biomarker outcomes at 48-weeks.

Duration of study:
Screening period: Up to 60 days
Treatment period: 48-weeks

Sample size: 48

Study population: Patients age 50 or older who meet criteria for MCI due to AD or Mild AD dementia (Mini-Mental State Exam [MMSE] $\geq$ 20) not otherwise excluded.

Dose and administration: Nicotinamide is an oral tablet that will be administered at 1500 mg (two 750 mg tablets) BID.

Primary outcome: Change in CSF p-tau$_{231}$

Secondary outcomes:

1. Change in CSF p-tau$_{181}$
2. Change in CSF total tau
3. Change in CSF Aβ_{42}
4. Change in CSF Aβ_{40}
5. Change in ADAS-cog13
6. Change in ADCS-ADL
7. Change in CDR-SB

Safety parameters: Adverse events, symptom checklist, vital signs, physical, neurological examination and laboratory tests.

Go/No-go decision: An additional 48-weeks treatment and follow-up period is planned, contingent upon a "go" decision based on the primary outcome (CSF p-tau$_{231}$) or one planned secondary outcome (CSF p-tau$_{181}$)

Statistical methods: The study will implement a seamless phase 2a-2b sequential design with a go/no-go futility analysis based upon change in CSF biomarkers after 48-weeks. The primary outcome for the trial is change in p-tau$_{231}$. Inference for the initial go/no-go decision will be based upon the difference in within-subject change in p-tau$_{231}$ or p-tau$_{181}$ over 48-weeks between treatment and control.

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5. List of abbreviations	
3xTg	Triple Transgenic mouse
AE	Adverse Event
Aβ	Amyloid Beta
AD	Alzheimer's Disease
ADAS-cog	Alzheimer's Disease Assessment Scale – Cognitive subscale
ADCS	Alzheimer's Disease Cooperative Study
ADCS-ADL-MCI	Alzheimer's Disease Cooperative Study Activities of Daily Living Scale in Mild Cognitive Impairment
ADRC	Alzheimer's Disease Research Center
APOE	Apolipoprotein
BID	Twice daily
CBC	Complete Blood Count
CDR	Clinical Dementia Rating Scale
CDR-SB	Clinical Dementia Rating Scale Sum of Boxes
CFR	Code of Federal Regulations
CBIC	Clinician Based Impression of Change
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
C-SSRS	Columbia Suicidality Severity Rating Scale
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EKG	Electrocardiogram
FCSRT	Free and Cued Selective Reminding Task
GCP	Good Clinical Practice
GDS	Geriatric Depression Scale
GMP	Good Manufacturing Practices
HDAC	Histone Deacetylase
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IRB	Institutional Review Board
LFT	Liver Function Test
MCI	Mild Cognitive Impairment
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini-Mental State Exam
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
NAD	Nicotinamide Adenine Dinucleotide
PI	Principal Investigator
SAE	Serious Adverse Event
UA	Urinalysis
WNL	Within Normal Limits

6. INVESTIGATORS

UC Irvine:
Project Director, Joshua Grill, PhD
Associate Professor of Psychiatry & Human Behavior
Institute for Memory Impairments and Neurological Disorders
Alzheimer's Disease Research Center
Institute for Clinical Translational Science
3204 Biological Sciences III
Irvine, CA 92617
(949) 824-5905

Co-Investigator, Dan Gillen, PhD
Professor and Chair, Department of Statistics
Institute for Memory Impairments and Neurological Disorders
Alzheimer's Disease Research Center

Site Principal Investigator, Aimee Pierce, MD
Assistant Clinical Professor of Neurology
Medical Director, Memory Assessment and Research Center
Co-Leader, UCI ADRC Clinical Core

Site Co-Principal Investigator, Steven Schreiber, MD
Professor of Neurology

Site Coordinator, Beatriz Yanez, RN
Institute for Memory Impairments and Neurological Disorders

Site Coordinator, Robin Lawrence, RN, MS, AGPCNP-BC
Institute for Memory Impairments and Neurological Disorders

Consultant, Kim Green, PhD
Associate Professor, Neurobiology and Behavior
Institute for Memory Impairments and Neurological Disorders
Alzheimer's Disease Research Center

UCLA:
Site Principal Investigator, Sarah Kremen, MD
Staff neurologist
Co-Director, Katherine and Benjamin Kagan AD Treatment Development Program
Mary Easton Center for AD Research

Site Co-Investigator, Maryam Beigi, MD
Staff neurologist
Katherine and Benjamin Kagan AD Treatment Development Program
Mary Easton Center for AD Research

Site Coordinator, Celine Ossinalde, MA
Katherine and Benjamin Kagan AD Treatment Development Program

Consultant, Gregory Cole, PhD
Interim Director, UCLA Mary Easton Center for AD Research

UC San Diego/ADCS:

ADCS PI, Howard Feldman, MDCM, FRCP(C)
Professor of Neurosciences
Dean for Alzheimer's and Related Neurodegenerative Research

ADCS Biostatistics Core Director, Ron Thomas, PhD
Professor and Division Chief of Biostatistics and Bioinformatics
Department of Family and Preventive Medicine

ADCS Biomarker Core Director, Robert Rissman, PhD
Associate Professor of Neuroscience

ADCS Neuroimaging Core Director, James Brewer, MD, PhD
Professor of Neurosciences
Interim Director of the UCSD ADRC

ADCS Interim Medical Safety Core Director, Tilman Oltersdorf, MD
Department of Neurosciences

ADCS Medical Monitor, Branko Huisa, MD
Department of Neuroscience

7. BACKGROUND

Treatments capable of slowing the progression of Alzheimer's disease (AD) are desperately needed. Though a variety of promising candidates are in development,[3] there are no approved drugs able to slow AD.[4] Anti-amyloid therapies dominate the clinical pipeline but if shown to be effective will have great cost, may require routine infusions, and may be associated with substantial risk for serious adverse events.[5] Some experts argue that there is an over-emphasis on amyloid beta (Aβ)-targeting therapies in clinical development and most agree that there is a need to explore alternate hypotheses and therapeutic targets.[6] Safe and effective oral therapies that are capable of slowing the disease process represent a major therapeutic goal in AD research.

Neurofibrillary tangles represent the pathological hallmark of AD that is most strongly correlated with cognitive decline.[7, 8] Neurofibrillary tangles are composed of hyperphosphorylated tau, a microtubule associated protein critical to neuronal viability. Elevated levels of tau phosphorylated at threonine 231 (p-tau$_{231}$) in the cerebrospinal fluid (CSF) can be used to distinguish AD from normal controls and other neurodegenerative conditions,[9, 10] are correlated with neurofibrillary tangle burden at autopsy,[11] and are predictive of progression to dementia among patients with mild cognitive impairment (MCI).[12] Therefore, this biomarker substrate of AD pathology may be a meaningful target for candidate disease-modifying therapies.

Nicotinamide, or niacinamide, is a key component of nicotinamide adenine dinucleotide (NAD), a co-enzyme involved in many cellular oxidation-reduction reactions and vital for cellular respiration. Nicotinamide also acts as a free radical scavenger, and modulates both immune cell function and apoptosis. In addition to these crucial roles, high doses of nicotinamide function to inhibit the class 3 histone deacetylases (HDACs), or sirtuins. Sirtuins are a unique class of NAD(+)-dependent deacetylases required for diverse biological processes including autophagy, transcription, and apoptosis. Sirtuins function to remove acetyl groups from their substrate proteins, and in doing so regulate cellular pathways. In this process the acetyl group is passed from the substrate to an NAD molecule. The presence of nicotinamide within the cell competes with free pools of NAD, however, and thereby prevents this process from occurring. It has been found that regulating protein acetylation can be broadly protective and as such HDAC inhibitors are being pursued as potential treatments for numerous neurodegenerative diseases.[13] Four months of nicotinamide treatment (200 mg/kg/day) in triple transgenic AD mice (3xTg), which develop both amyloid plaques and neurofibrillary tangles,[14] selectively decreased the accumulation of p-tau$_{231}$ by 60% in AD-vulnerable brain regions and significantly ameliorated cognitive decline, without affecting levels of Aβ.[1] Treatment also increased acetylated alpha-tubulin and microtubule-associated protein 2c levels, enhancing microtubule stabilization.[1] Importantly, laboratories at the National Institute on Aging have replicated the effects of nicotinamide on tau phosphorylation in 3xTg mice. This group also found beneficial effects on Aβ pathology as well as improved brain bioenergetics with preserved functionality of mitochondria and the autophagy system.[2] Thus, there is a strong pre-clinical rationale for testing the hypothesis that nicotinamide can act as an HDAC inhibitor to reduce p-tau in AD.

Notably, trials in other neurologic conditions support the hypothesis that nicotinamide can act as an HDAC inhibitor. In Friedreich's ataxia, high dose nicotinamide (3.5-6g/day) resulted in a dose-response upregulation of expression of frataxin mRNA and overall increases in frataxin protein levels,[15] presumably due to HDAC inhibition, as protein changes were accompanied by decreased heterochromatin modifications in the *FXN* gene.[15, 16] Nicotinamide has also been shown to be beneficial/protective in animal models of stroke,[17] traumatic brain injury,[18-20] Parkinson's disease,[21, 22] and Huntington's disease,[23] emphasizing the rationale that nicotinamide may be therapeutic in CNS diseases, and that it modulates specific pathways implicated in AD pathogenesis.

Large human clinical trials demonstrate the safety of high dose nicotinamide. For example, in a study of more than 550 participants with a first-degree family history of Type I diabetes, no differences between nicotinamide and placebo were observed for any adverse event.[24] The daily dose in this study was mean 1.2 g/m^2 up to a maximum of 3g/day for five years. Similarly, in a phase 3 study of twice daily 500 mg

nicotinamide to prevent recurrent non-melanoma skin cancer (n=386), no differences were observed in adverse event profiles between those randomized to nicotinamide and those randomized to placebo. Treatment with nicotinamide also reduced the occurrence of new basal cell and squamous-cell carcinomas by 20% and 30%, respectively.[25]

We previously performed a single-site safety trial in humans with mild-to-moderate AD (Schreiber et al., *in preparation*). Participants age 50 or older with a diagnosis of probable AD and Mini-Mental State Exam (MMSE) scores 13-26, inclusive, were enrolled. Exclusion criteria were a diagnosis of non-AD dementia, unstable psychiatric disease or medical illness, history of alcoholism, drug abuse, liver disease or peptic ulcer disease, and Hachinski Ischemic Score >4 or evidence of stroke on magnetic resonance imaging (MRI) or computed tomography scan. Participants were required to be on stable medications for at least 30 days, including FDA-approved therapies for AD. Participants were not permitted to take supplements containing nicotinamide. Participants were required to have a study partner who could accompany them to visits and ensure compliance with the study treatment.

We randomized 31 participants to receive nicotinamide (Endur-amide [Niacinamide, vitamin B3] 1500 mg orally bid, Endurance Products Company, Tigard, OR), or a matching placebo for 24 weeks. The randomization was 1:1, with no stratifications. The dose of nicotinamide approximated that used in preclinical studies and was comparable to other human trials.[24, 25] Adverse events were recorded at weeks 6, 12, 18 and 24. Safety laboratories (tests of liver function [LFTs], serum electrolytes and complete blood count [CBC]) were assessed at week 4. Vital signs, physical and neurological examinations were assessed at baseline and week 24. Pill counts assessed compliance. In addition to safety assessments, we completed the 11-item cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog11), Clinician's Interview-Based Impression of Change Plus Caregiver Input (CIBIC-Plus), Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale (ADCS-ADL), and the Clinical Dementia Rating Scale (CDR) at baseline and weeks 6, 12, 18, and 24.

Participants in the active and placebo groups were mean age 79.7±6.9 and 79.0±8.4, respectively, and had mean MMSE scores of 22.1±3.17 and 21.3±3.6. Fifty-six percent of subjects in the treatment group were male, compared to 80% in the placebo group. Twenty-eight participants (90%) completed the study. Treatment compliance was 72% in both groups.

No adverse events resulted in participant withdrawal from the study. We observed no adverse effects of nicotinamide on serum electrolytes, CBC or LFTs. The most frequent adverse event was visual hallucination or delusions (n=4 nicotinamide, n=4 placebo). There was no difference between the groups on clinical outcome measures. The conclusion of this Phase 2a study is that nicotinamide can be safely administered at a dose of 3000 mg/day in patients with mild-to-moderate AD.

In summary, nicotinamide is a promising intervention for neurodegenerative disease that may specifically target neurofibrillary pathology in AD. Nicotinamide is safe at high doses and over long durations of treatment in humans. No study has tested the hypothesis that nicotinamide can inhibit HDAC to reduce phosphorylation of tau in people with AD.

8. STUDY OBJECTIVES

8.1. Primary Objectives

Objective 1: Determine if daily treatment with high dose nicotinamide reduces levels of phosphorylated tau (p-tau$_{231}$) in individuals with mild AD dementia or MCI due to AD.

Objective 2: Assess the safety and tolerability of high dose nicotinamide treatment in individuals with mild AD dementia or MCI due to AD.

8.2. Secondary Objectives

Secondary Objective 1: Determine if daily treatment with high dose nicotinamide can affect CSF levels of amyloid beta ($A\beta_{42}$), phosphorylated tau ($p\text{-}tau_{181}$), total tau (t-tau), or the ratio of total tau/Aβ in individuals with mild AD dementia or MCI due to AD.

Secondary Objective 2: Determine if daily treatment with high dose nicotinamide reduces the rate cognitive decline in individuals with mild AD dementia or MCI due to AD.

Secondary Objective 3: Determine if daily treatment with high dose nicotinamide reduces the rate of functional decline in individuals with mild AD dementia or MCI due to AD.

8.3. Exploratory Objectives

Exploratory Objective 1: Determine if daily treatment with high dose nicotinamide reduces the rate of brain volume atrophy in individuals with mild AD dementia or MCI due to AD.

Exploratory Objective 2: Determine the effect size of 48-weeks change in phosphorylated tau in individuals with mild AD dementia or MCI due to AD.

9. INVESTIGATIONAL PLAN

9.1. Overall Study Design and Plan

This is a Phase 2a group sequential design, incorporating a futility analysis with a go/no-go decision conditional on CSF biomarker outcomes at 48-weeks. With a go-decision, follow-up will be extended to 96-weeks in a Phase 2b trial, with dual co-primary outcomes of the 13-item cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog13) and the Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale (ADCS-ADL). The purpose of the Phase 2b trial is to provide preliminary measures of efficacy to facilitate the design of a larger registration trial.

The initial trial primary outcome is CSF $p\text{-}tau_{231}$. We will randomize 48 participants to 1500 mg BID nicotinamide or matched placebo at a 1:1 ratio. Additional CSF analyses ($p\text{-}tau_{181}$, *total tau,* $A\beta_{40}$, $A\beta_{42}$) will be performed as secondary outcomes in the proposed Phase 2a trial. A portion of the CSF sample will be stored for future biomarker analyses. Clinical measures will be collected every 24-weeks throughout the study and will be considered secondary outcome measures in the initial trial. Cognitive and functional outcomes will include ADAS-cog13, ADCS-ADL, and CDR sum of boxes (CDR-SB). Additionally, we will examine change in brain volume (whole brain volume, ventricular volume, hippocampal volume, and cortical thickness) as exploratory secondary outcome measures.

9.2. Study Procedures

9.2.1. Visit schedule (see Appendix 1).

- Screening period: Three visits will determine eligibility. An initial clinical visit will include completion of informed consent, MMSE, Wechsler Logical Memory II, FCSRT, CDR-SB, GDS, physical and neurological examinations including vital signs, medical history and collection of concomitant medications, and assessment of inclusion and exclusion criteria. This visit will also include a blood draw for apolipoprotein E (APOE) genotyping, safety labs, urinalysis (UA), and electrocardiogram (EKG). Assuming that participants have had clinical neuroimaging within the previous 12-months to exclude the possibility of space-occupying lesion, the second visit will include lumbar puncture (LP) and fasting blood draw for future biomarker research. A third visit will include MRI for screening and baseline volumetric assessment. For participants lacking clinical neuroimaging in the previous 12-months, the order of LP and MRI visits may be reversed. If needed, redraw of blood can be performed to check platelet level and coagulation parameters within 30 days prior to LP.

- Active period: Eligible participants will have a brief physical and neurological exam including vital signs, collection of adverse events and concomitant medications, cognitive testing (MMSE and ADAS-cog), be administered the C-SSRS, GDS, and ADCS-ADL-MCI, will receive study medication, and undergo safety labs (blood and urine) at the baseline exam. Telephone follow-up at 2, 4, and 36-weeks will assess safety. At 12-weeks, an in-person visit will include a brief physical and neurological exam including vital signs, collection of adverse events and concomitant medications, blood collection for drug levels, fasting blood collection for future biomarkers, EKG, assessment of treatment compliance and administration of study medication. At 24 and 48-weeks, participants will undergo blood safety labs, urinalysis (UA), blood collection for drug levels, blood collection for future biomarkers, and EKG. Participants will complete the MMSE, ADAS-cog13, ADCS-ADL-MCI, CDR-SB, GDS, C-SSRS physical and neurological examinations, including vital signs, collection of adverse events and concomitant medications. MRI, LP, and fasting blood collection for future biomarkers will be performed at 48-weeks.
- End of study: At 48-weeks, participants will complete all study assessments. Participants will be provided additional study drug (6-months supply) for the period of time for go/no-go analysis.
- Early termination: In the event of early termination due participant withdrawal of consent or removal by the investigator from the trial, the 48-weeks/early termination visit should be completed in all possible cases, if the participant has been randomized to therapy or placebo. No new investigational product will be administered.
- Unscheduled visits: If needed, unscheduled visits can be performed at the discretion of the Site Principal Investigator. Unscheduled visits should include collection of adverse events, concomitant medications, brief neurological and physical examination, and clinical blood laboratories, if indicated.
- Go/no-go: At the completion of the go/no-go analyses, participants will complete an additional visit to assess safety and collect study medication (no-go) or to be enrolled in the 48-weeks extension study (go). In the event of a "go" decision, trial duration will be extended to 96-weeks. Participants will remain blinded to randomization and group assignment will be unchanged. A safety visit will be performed at 74-weeks. The end of study visit will be performed at 96-weeks, and will include the co-primary outcome measures of the ADAS-cog13 and the ADCS-ADL-MCI, as well as the CDR-SB, neurological and physical exams, blood laboratories, assessment of adverse events, MRI, and lumbar puncture for CSF protein analysis.

9.2.2. Safety assessments. Participants will undergo brief physical and neurological examinations, laboratory testing, and EKG at all follow-up visits (12, 24, and 48-weeks /early termination). Adverse events will be assessed by the study site clinician. Adverse events include signs or symptoms that may or may not be related to study medication, abnormalities detected during physical examination, or clinically significant laboratory abnormalities. The Columbia Suicidality Severity Rating Scale (C-SSRS) will be performed at the baseline, 24-week, and 48-week visits. Adverse events will be graded as mild, moderate, or severe according to section 13.1.1, and recorded on case report forms. Regular safety reports will be prepared by the ADCS and submitted to an independent Data and Safety Monitoring Board (see section 13.3.3).

9.2.3. Laboratory evaluations. Routine safety laboratory studies will include CBC, chemistries and urinalysis at screening, and lipids and liver function tests at follow-up visits (24 and 48-weeks/early termination).

9.2.4. Treatment compliance. At each follow-up visit (12, 24, and 48-weeks/early termination), participants will bring their study medication diaries and their study medications for the purpose of standard pill counts. The over-the-counter availability of nicotinamide presents the challenge that participants could purchase and take the study intervention. We will assess blood levels of N1-

Methylnicotinamide, 2-Pyridone (a nicotinamide metabolite) and blood levels of NAD at the 12-, 24- and 48-week visits.[26, 27] For these visits, study medication should be held until in the clinic, to optimize measures of compliance. Eighty percent compliance is expected. If there is an insufficient level of drug compliance, the site must consult with the Project Directors and Medical Monitor regarding timing and procedures. It is possible that subjects will be discontinued early from the study due to insufficient compliance.

9.2.5. Lumbar puncture. Within 30 days prior to lumbar puncture, participants' coagulation panel and platelet count will be measured and verified within normal limits. Lumbar puncture (LP) will be performed under fasting conditions (minimum 8-hour fast). Only water (and any medications that can be taken without food) is permitted until LP is complete. LP will be performed as close to 08:00AM as possible. Participants will be in a seated position for the LP, if possible. LP will be performed using a small caliber atraumatic needle (e.g. 22 or 24 gauge Sprotte needle.) LPs will be permitted to be performed under guided fluoroscopy if CSF is unable to be obtained by bedside LP. CSF will be collected by gravity flow or aspiration method, at the discretion of the site clinician. All syringes, tubing, collection tubes, and transfer tubes will be polypropylene. A total of 18mL of CSF will be collected. The first 2mL fraction of CSF will be sent to local lab for measurement of cell counts and differential, glucose, and protein. The next 16mL of CSF will be immediately placed on ice during transfer and aliquot procedures, frozen at -80°C, then shipped overnight on dry ice on the day of collection to ADCS Biomarker Core. Time of collection, verification of fasting status, type and gauge of needle, method of collection, and total volume of CSF collected will be recorded.

9.2.6. Data Management. Data will be collected on Case Report Forms (CRFs), identifying subjects only by study ID number. Electronic data will be entered into the ADCS Electronic Data Capture (EDC) System.

10. STUDY POPULATION

10.1. Participants

Modern AD trials have moved toward patient samples that meet biomarker criteria for AD. This includes MCI due to AD[28] or prodromal AD,[29] as well as dementia due to AD.[30] The rationale for implementing these criteria include 1) the desire to ensure that participants enrolled in trials truly have AD, 2) the need to limit exposure of non-AD patients to drugs targeting AD pathology (e.g., giving amyloid-lowering therapy only to patients with elevated brain amyloid), and 3) the resultant implications to trial design and primary outcome assessments (i.e., trials enrolling patients who are not likely to benefit from therapy reduce power[31]). In this study, the safety of the treatment has been demonstrated through numerous human studies, reducing the implications to participant health. Thus, the need to characterize the disease of participants and the implications to trial design and power instructed the enrollment criteria. The criteria for enrollment in this study will be intentionally liberal, due to the limited study resources, the safety profile of the intervention, and to ensure maximal recruitment. We will enroll participants meeting criteria for MCI due to AD or dementia due to AD, provided that all other criteria are met. AD biomarker status will be assessed through lumbar puncture and CSF protein analysis, and an algorithm whereby multiple criteria can be used to include a participant (low Aβ or the ratio of total tau to Aβ) will be implemented. In a recent prodromal AD trial that permitted enrollment with a similar algorithm, only about half of participants would have been eligible based on Aβ criteria alone, whereas nearly 90% of enrolled participants were eligible based on the ratio of total tau to Aβ.[32] Nearly 75% of amnestic MCI patients may meet CSF eligibility requirements[33] and rates are anticipated to be even higher among those with probable AD dementia.[34]

10.1.1. Inclusion Criteria

1. Age 50 or older
2. MCI or dementia due to AD
   - MCI due to AD[28]:
     - Subjective memory complaint by the participant or an informant

- Memory impairment demonstrated by at least one of the following:
  - CDR memory box ≥0.5
  - Wechsler Logical Memory II ≥1.5 standard deviations below education adjusted mean
    - Less than or equal to 11 for 16 or more years of education
    - Less than or equal to 9 for 8-15 years of education
    - Less than or equal to 6 for 0-7 years of education
  - Free and Cued Selective Reminding Test (FCSRT) Total Recall score ≤ 39
  - FCSRT Free Recall score ≤24
  - FCSRT Delayed Free Recall score ≤ 8
- Preserved functional independence/not demented, based on investigator clinical assessment

▪ Probable AD dementia[30]:

- Cognitive impairment to a minimum of two domains that interferes with ability to function at work or at usual activities and represents a decline from previous levels
  - Insidious onset with gradual progression
  - Amnestic presentation or nonamnestic presentation (language, visuospatial, or executive dysfunction)
  - The presentation is not indicative of non-AD pathology (e.g., history of stroke or core features of Lewy body dementia, behavioral variant frontotemporal dementia, or non-AD primary progressive aphasia)

3. Biomarker criteria:
   - CSF $A\beta_{42}$ ≤600 pg/mL, or
   - A ratio of total tau to $A\beta_{42}$ ≥ 0.39
4. Mini-Mental State Exam (MMSE) ≥ 20
5. Blood laboratories (LFTs, CBC, etc), UA, and EKG are WNL or deemed clinically not significant by the site investigator
6. Stable medications (including approved AD therapies) for at least 4 weeks prior to baseline
7. At least 6 years of education
8. Able to swallow oral tablets
9. Speaks English fluently
10. Available qualified study partner (≥3 times per week in-person communication with the participant)

10.1.2. Exclusion Criteria

1. Active neurological or psychiatric diagnosis other than AD that may affect cognition and/or function. (Obstructive sleep apnea is permitted, if treated.)
2. Any medical condition likely to significantly interfere with the evaluation of safety and efficacy of the study drug.
3. Inability to undergo lumbar puncture, including use of Coumadin, novel oral anticoagulants, clopidogrel, or dipyridamole. Use of aspirin <= 325mg daily is permitted.
4. Hachinski ischemic scale > 4
5. Magnetic Resonance Imaging (MRI) incompatibility
6. MRI evidence of cortical stroke >1cm, superficial siderosis, or extensive white matter hyperintensity (Cardiovascular Health Study score 7-8+)[35]
7. Diagnosis of cancer in the previous 3 years (with the exception of basal or squamous cell carcinoma)
8. Geriatric Depression Scale (GDS) score >6
9. History within the past 5 years of alcohol or substance use disorder (DSM-5)

10. Laboratory evidence of a clinically significant abnormality that may interfere with study assessments
11. Active partial or total malabsorptive disease (e.g., celiac disease)
12. Resides in a skilled nursing facility
13. Use of concomitant medications likely to significantly interfere with the evaluation of safety and efficacy of the study drug.
14. Participation in a clinical trial of a potential disease-modifying therapy for AD in previous 6-months (time between last investigational drug administration and baseline for the current study)
15. Pregnant, lactating or of child bearing potential (that is, women must be 2 years post-menopausal or surgically sterile to be considered not child bearing potential).
16. Unwillingness to abstain from over-the-counter nicotinamide for the duration of the trial

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.

10.1.3. 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 without permission from the Project Directors.

10.2. Recruitment and Retention

10.2.1. Recruitment rates and methods. The planned enrollment rate is 2 baselines/month/site for this trial. We plan a number of critical activities related to participant enrollment:

1. We will enroll patients taking approved AD therapies and will reimburse participants for their time. The study will enroll participants with presumed underlying AD, including both patients with MCI and those with mild dementia.
2. The two recruitment sites in this trial, UCI and UCLA, both have potential participant registries that will facilitate participant recruitment.[36] These registries may enable rapid start-up of recruitment for the trial, by providing a bolus of participants who can be contacted about the study at initiation.
4. We will leverage relationships with organizations such as the local chapters of the Alzheimer's Association (California Southland and AAOC) and the newly formed independent organizations, Alzheimer's Greater Los Angeles and Alzheimer's Orange County. Community partners will be called upon to a) refer potentially eligible participants, b) share study materials with families and other advocates, and c) schedule and publicize community talks by investigators describing the study and encouraging participation.
5. Participant education will be critical to recruitment, including dispelling myths about the lumbar puncture. Both sites have outstanding track records of community and participant education, stemming from a more than 20-year history (at both institutions) of being NIA-funded Alzheimer's Disease Research Centers and California Department of Public Health Alzheimer's Disease Centers.
6. Both sites have outstanding media relations departments and a substantial track record of working with the ADCS Recruitment Core to secure local and national media stories covering new studies and participants in those studies. We will work with the ADCS Recruitment Core and the media relations offices at the two sites to generate local media stories on this trial.

10.2.2. Participant engagement. Participant engagement will be critical to maximizing completion rates in this trial. To maximize participant retention, we will use a variety of methods to communicate investigator gratitude and the critical value of participant contributions.

1. *Telephone follow-ups.* Study staff will call participants to complete safety assessments at 2, 4, and 36-weeks. Calls will also serve to ensure participant satisfaction, remind participants about upcoming visits, and thank participants for their contribution.
2. *Thank you events*. Each site holds annual Thank You Events (leveraging other sources of funding), honoring research participants in clinical studies. Events include faculty who deliver brief research updates.
3. *Cards and reminders*. Each site will send annual Holiday and birthday cards and quarterly electronic newsletters (printed versions will be provided at study visits). We will use appointment reminder cards that, in addition to indicating the date of the next study visit, reiterate the value of participants' contribution.
4. *Bi-annual newsletter.* The principal investigator will generate a twice-annual newsletter that can be mailed or shared with participants at study visits. The newsletter will communicate the value of the study and the progress-to-date. The newsletter will also share personal stories from site or ADCS staff and testimonials from trial participants who are willing to forego confidentiality (with IRB approval).
5. *Compensation.* Participants will be compensated for their time spent participating in the study. They will receive $250. This compensation value is below the annual minimum for reportable taxable income.

11. TREATMENT

11.1. Investigational product.

Endurance Products Company, Tigard, OR, will provide the active therapy and matching placebo. Patients will be randomized to receive 1500 mg (two 750 mg tablets) BID. Participants and their study partners will receive 3-months supply of study medication at visit 1 and 2 and a 6-month supply at visit 3 (see Appendix 1).

The investigational product is produced by Innovite, Inc, and distributed by Endurance Products company. Innovite, Inc, specializes in the production of advanced tableting technology, including the development of effective controlled release delivery systems. Endurance Products Company is bulk provider of nutritional products that has operated since 1976. Innovite, Inc, and Endurance Products Company operate within the FDA guidelines for Good Manufacturing Practice.

750 mg tablets

The study treatment is a nutritional product, produced in bulk. The active treatment, niacinamide (nicotinamide; 99%) is produced in a 750 mg sustained release tablet that also includes: Vegetable Waxes (Rice Bran and/or Carnauba), Stearic acid (veg), Magnesium Stearate, and Silica. Tablets have a mottled, off-white appearance, measure with 0.365" x 0.770" with a thickness of 0.290". The actual tablet weight is 1137 mg. Sustained release is anticipated at 5-7 hours (50±10% at hour 1; 70±10% at hour 2; 85±10% at hour 3.5; ≥90% at hour 5).

Matching placebo

Placebo tablets have a mottled, off-white appearance, measure with 0.365" x 0.770" with a thickness of 0.295". The actual tablet weight is 1124 mg. Ingredients include: Cellulose, Vegetable Waxes (Rice Bran and/or Carnauba), Stearic acid (veg), Magnesium Stearate, and Silica.

11.2. AE experiences across other trials.

Vitamin B3 deficiency results in pellagra and is therefore recommended as part of the daily diet (0.3 mg/kg/day). Alternatively, high dose nicotinamide has been investigated as a treatment or prevention for a number of health conditions.[37] Because it is a vitamin, nicotinamide has not been subject to the same rigorous safety assessment as a new investigational drug would be. Nevertheless, the various clinical studies of high dose nicotinamide has resulted in a small literature related to potential adverse events.[38]

As noted in the Section 7, a previous pilot study supported the safe administration of high dose (1.5g BID) nicotinamide in mild-to-moderate AD. Similarly, a large (n=552) clinical trial to test 1.2g/m^2 (up to 3g/day) nicotinamide as a prevention for diabetes,[24] and a large (n=386) trial of 500mg BID for recurrent basal and squamous cell carcinomas,[25] revealed no differences in AE frequencies or profiles between those randomized to placebo and those randomized to nicotinamide. Similarly, no human study has revealed adverse growth, teratogenic, or oncogenic effects at these doses.[38]

Alternatively, at doses greater than 3 g/day, nicotinamide may result in AEs. In a Phase I/II trial in ten Fragile X patients, each of the participants experienced nausea in the extended (8 week) treatment exposure phase with the individual participant's MTD (2-8 g/day). The other most common AEs in this trial were headache (n=5), lightheadedness (n=6), vomiting (n=5) and diarrhea (n=4). Increases in abnormal results from liver function tests in three out of ten patients occurred when taking high doses—in two cases these effects were mild to moderate and self-limiting and in one case they were severe, but all resolved with reduction of the nicotinamide dose.[39] A schizophrenic man developed acute hepatic toxicity after 9 g/day treatment. The toxicity resolved after treatment cessation, but resumed at re-challenge.[40]

Thus, the tolerability and safety of nicotinamide at the doses that will be used in this clinical trial appear acceptable. At high doses, however, nicotinamide treatment may elicit adverse events. Ongoing safety monitoring, including assessment of compliance, will be important to ensure the safety of participants.

Perhaps of note, the safety profiles of related but distinct treatments have been observed. High dose nicotinic acid, which can reduce serum cholesterol, frequently results in flushing.[41] This is not an anticipated effect of nicotinamide treatment, which is not a vasodilator.[42] In the ADCS trial of supplementation with Vitamin B6 and B12, higher occurrence of depression was observed in the active treatment, compared to the placebo arm.[43] This difference was deemed marginal and inconsistent, because results with the Neuropsychiatric Inventory lacked a consistent effect.[43] To our knowledge, no study has observed depression as an adverse event associated with nicotinamide treatment.

11.3. Access to therapy and trial closure.

There is no plan to offer open label access to nicotinamide at the closure of trial, regardless of the go/no-go decision. Upon completion of the 48-week end of study visit, participants will be administered blinded treatment until such time as the go/no-go decision outcome has been determined. In the event of a no-go decision, participants will return for an additional visit to assess safety and collect study medication (no-go).

12. OUTCOME MEASURES

12.1. Primary outcome measure

The primary outcome of this 48-week go/no-go trial will be change in CSF p-tau$_{231}$. As described in the Significance section of this proposal, CSF p-tau$_{231}$ levels are elevated in AD dementia[9, 10] and MCI due to AD.[12] Preclinical data are supportive of this biomarker primary outcome, as nicotinamide treatment in 3xTg mice decreased p-tau$_{231}$ by 60% and this biomarker change was associated with improved microtubule stabilization and behavioral improvements.[1]

12.2. Secondary outcomes measures

12.2.1. CSF analyses

1. CSF p-tau$_{181}$. CSF p-tau$_{181}$ is a well-replicated biomarker for AD and is used frequently in large AD natural history studies and clinical trials.[34, 44]
2. CSF total tau. Increased total tau in the CSF is believed to be a marker of neuronal injury or degeneration, though this change is not necessarily unique to AD. Total tau may increase by 1.5-2 fold in AD, suggesting that treatment-related reduction in total tau would be strongly indicative of a disease modifying effect.[34]

3. CSF $A\beta_{42}$. Reduced CSF $A\beta_{42}$ is strongly associated with AD.[34] Although preclinical work at our institution did not find an effect of nicotinamide treatment on brain $A\beta_{42}$ levels, work from another laboratory, in which experiments involved longer exposure to nicotinamide did see treatment-induced amelioration of Aβ burden.[2]
4. CSF $A\beta_{40}$. We will also examine CSF levels of the shorter (40 amino acid) peptide fragment of Aβ.

CSF biomarker and APOE genotype analyses. Analyses of p-tau_{231} levels will be performed by Quanterix (www.quanterix.com). Quanterix uses proprietary technology, called the Accelerator, and their Simoa Discovery kits to assay p-tau_{231} levels. Preliminary analyses reveal low variability among control samples, adequate differential between cases and controls, and strong correlation between this assay and p-tau_{181} (unpublished results), justifying selection of this assay.

The ADCS Biomarker Core will perform the remaining CSF protein analyses. Validated assay platforms from Meso Scale Discovery (Rockville, MD) will be used to assess total tau, $A\beta_{42}$ and $A\beta_{40}$ levels. Fujirebio INNOTEST® protein assay kits will be used to assess p-tau_{181} levels.

Real-time PCR restriction fragment length polymorphism analysis will be performed to identify participant APOE genotypes. DNA will be extracted from blood using QIAamp DNA blood maxi kit (Qiagen, Venlo, Netherlands) and genotyping performed using Applied Biosystems (Foster City, CA) TaqMan SNP Genotyping Assay. The amplification reaction will contain 5 μL genomic DNA, 2.5 units of Taq DNA Polymerase (New England Biolabs, Inc, Ipswich, MA, USA), 1 × ThermoPol Reaction Buffer (New England Biolabs), 0.3 mmol/L dNTPs, 10% DMSO, and 0.3 μmol/L of each primer (forward primer: 5′-ACGCGGGCACGGCTGTCCAAGGA-3′; reverse primer: 5′-GCGGGCCCCGGCCTGGTACAC-3′). The PCR cycling conditions will be: initial denaturation at 94°C for 3 minutes followed by 30 cycles of 94°C for 30 seconds and 72°C for 90 seconds with a final extension at 72°C for 4 minutes. The assays will be run on a Bio-Rad (Hercules, CA) CFX96.

12.2.2. Clinical outcomes

1. ADAS-cog13. The ADAS-Cog is the most commonly used cognitive outcome measure in AD trials. The 11-item version has a range of 0 to 70 and incorporates tests of memory, language, attention, and praxis.[45] A 13-item version includes a delayed recall item and a number cancellation task, increasing the maximum score to 85, with higher scores indicating poorer performance.[46] Extended versions of the ADAS-cog may increase sensitivity and statistical power in early AD.[31, 47]
2. ADCS-ADL-MCI. The ADCS-ADL-MCI is a measure of patient functional performance in AD and MCI trials. The informant-based questionnaire assesses conduct of basic and instrumental ADLs. A total of 24 ADLs are evaluated. Scores range from 0 to 53, with higher scores representing more maintained function.[48-50]
3. CDR-SB. The CDR uses patient and informant interviews to assess memory, orientation, judgment and problem solving, community affairs, home and hobbies, and self-care.[51] The CDR can provide a global score of 0, 0.5, 1.0, 2.0, or 3.0 relating to not demented, very mild dementia, mild dementia, moderate dementia, and severe dementia, respectively. Alternatively, a sum of the boxes can be assessed, providing a wider range and greater sensitivity to change.
4. MMSE. The MMSE[52] evaluates orientation, memory, attention, concentration, naming, repetition, comprehension, and ability to create a sentence and to copy two intersecting pentagons. The range is 0-30, with lower scores indicating greater cognitive impairment. Participants must demonstrate MMSE scores $\geq$20 to be included in the study.
5. Free and Cued Selective Reminding Task (FCSRT). The FCSRT[53] is a 16-word list memory task that incorporates auditory and visual cueing. The FCSRT Total score is the sum of three trials – the total recall, the free recall, and the delayed recall of items across the 3 trials (Range: 0-48). The FCSRT will be used only as an inclusion criterion in this study and only for participants with MCI.
6. C-SSRS: Consistent with FDA guidance, the C-SSRS will be implemented throughout the study. The C-SSRS captures the occurrence, severity, and frequency of suicide-related

thoughts and behaviors during the corresponding assessment period. The scale includes suggested questions to elicit the type of information needed to determine if a suicide-related thought or behavior occurred. The first time the scale is administered in this study, the C-SSRS "Screening/Baseline" version will be used, and the findings will constitute the baseline assessment. The C-SSRS "Since Last Visit" scale will be used for all subsequent assessments. If a suicide-related thought or behavior is identified at any time during the study, a study physician will perform a thorough evaluation, and must immediately contact ADCS Medical Safety. Appropriate medical care will be provided.

12.3. Exploratory outcomes

12.3.1. Neuroimaging. The ADCS Imaging Core will oversee all MRI procedures. MRI will be performed on a 1.5T or 3.0T scanner. Each site's scanner will have an identifying number, which will be appended to the image header of all MRI data. Each scanner will be subjected to a qualifying process that includes an evaluation for excessive vibration or other image artifact revealed through submitted MR images obtained through a standard protocol of an American College of Radiology phantom. In addition, 3D T1 MPRAGE or IR-FSPGR images of a human brain obtained through a standardized imaging protocol of a volunteer at each site will be submitted to the ADCS Imaging Core for automated segmentation, and the resulting segmentations will be examined by ADCS Imaging Core for anatomical accuracy. Once the phantom and volunteer scan passes quality control by the ADCS Imaging Core, the scanner will be considered certified for the study. Local reads will be used at screening to confirm participant eligibility. MRI will be performed only on participants deemed eligible based on CSF criteria. Because longitudinal analysis of brain volumetry will be used in this study, sites will use the same scanner for both scans (screening, 48-weeks) of a particular participant.

Volumetric analyses. The ADCS Imaging Core will analyze MRI data and secondary outcomes will assess changes in hippocampal volume, total brain volume, and lateral ventricular volume. Volumetric measures will be made using 3D T1-weighted sequences analyzed longitudinally. Methods based on FreeSurfer will be used to segment images, and Quarc, an inverse-consistent nonlinear registration method[54] will be used to assess regional deformation between screening and 48-weeks within regions of interest.

12.3.2. In order to bank tissue (including blood and CSF) for future experiments and analyses to be determined, participants will need to provide consent for specimen banking to the study investigators.

13. SAFETY ASSESSMENTS

13.1. Definition of adverse event (AE)

Any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related. Included in this definition is the worsening of a preexisting medical condition. AEs will be collected for the period beginning with the signing of informed consent through the end of the study follow-up period.

13.1.1. Adverse events will be categorized based on the following taxonomy:

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment.

13.1.2. The site investigator will assign each adverse event as being:

- Related to therapy. There is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent and the AE.

- Not related to therapy. There is not a reasonable possibility that the administration of the study agent caused the event, there is no temporal relationship between the study agent and event onset, or an alternate etiology has been established.

13.2. Definition of serious adverse event (SAE)

An AE 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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

13.3. AE and SAE collection and reporting

13.3.1. AE and SAE collection. Adverse event information will be collected at every planned and unplanned in-person and telephone visit until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be documented using an AE log eCRF (EDC entry) and followed for outcome information until resolution or stabilization.

13.3.2. AE and SAE reporting. All AEs will be recorded in the eCRF. SAEs must be reported on a SAE report form and on the AE pages in the eCRF. All SAEs must be reported immediately by the site investigator, within one calendar day (24 hours) from time of awareness by email to the Project Director and the ADCS.

All safety related information will be collected and processed promptly, to comply with regulatory requirements. An adverse event or suspected adverse reaction is considered “unexpected” if specificity or severity exceeds what that has been observed in previous studies. “Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Suspected Unexpected Serious Adverse Events (SUSARs) will be reported to the FDA and IRB in an expedited manner as follows: fatal or life-threatening SUSARs – as soon as possible but no later than 7 calendar days after the sponsor’s initial receipt of the information: non-fatal and non-life threatening SUSARs—as soon as possible but no later than 15 calendar days after the sponsor determines that the information qualifies for reporting. Relevant follow-up information for fatal or life-threatening SUSARs will be provided to the FDA and IRB.

The UC Irvine regulatory team will report events meeting reporting criteria to the UCI IRB (IRB of record) within 5 business days. Once the SAE is reported, the ADCS DSMB liaison will communicate the information to the DSMB. In the event that the Project Director, ADCS, DSMB, or the IRB determine that the safety information related to the study has changed, site investigators and the remaining parties will be notified as soon as possible, but in no case later than 15 calendar days from said determination.

13.3.3. Data Safety Monitoring Board (DSMB). This trial will utilize the ADCS DSMB. The DSMB will be provided with regular safety reports approximately every 3 months (see DSMB Charter). Life-threatening events and deaths will be reported to the DSMB immediately. In addition the DSMB will be notified of unexpected significant new safety data on an ad hoc basis as deemed necessary by the Project Director and the ADCS.

14. SAMPLE SIZE AND STATISTICAL METHODS

14.1. Overview of the statistical design

The study will implement a seamless phase 2a-2b sequential design with a go/no-go futility analysis occurring after one year of follow-up. While the primary efficacy endpoint for the first phase of the study is p-tau$_{231}$, the futility analysis occurring at the end of phase 2a will be based upon change in p-tau$_{231}$ or p-tau$_{181.}$ Regardless of the decision to continue onto phase 2b, unadjusted inference for the change in p-tau$_{231}$ comparing treatment to control will be reported at the end of phase 2a. In the event that the trial does continue on to phase 2b, final inference will be adjusted for the futility analysis based on p-tau$_{231}$ and p-tau$_{181}$.

14.2. Analysis of the primary outcome

The primary analysis of phase 2a will focus on the change in p-tau$_{231}$. The primary analysis will be based on the Intention-to-Treat population, defined as all participants who were randomized to study drug or treatment. We will model the change in p-tau$_{231}$, using an analysis of covariance model (ANCOVA). That is, 48-week p-tau$_{231}$ will be regressed upon baseline p-tau$_{231}$ and an indicator for treatment vs. placebo. The Huber-White robust variance estimator will be used to estimate the variance of the estimated difference in the mean change in p-tau$_{231}$ comparing treatment to placebo. Along with the estimated difference in mean change in p-tau$_{231}$, a Wald-based 95% confidence interval and corresponding p-value will be computed and presented.

14.3. Implementation of the futility boundary

At the conclusion of phase 2a, a futility decision of whether to continue follow-up of patients for an additional year will be based upon either the change in p-tau$_{231}$ or p-tau$_{181}$ after 48-weeks. The decision boundary for continuing to an additional 48-weeks of follow-up will be based upon the observed mean differences in p-tau$_{231}$ or p-tau$_{181}$ after 48-weeks of treatment. Specifically, the trial will continue to the second phase if the observed relative reduction in change in p-tau$_{231}$ or p-tau$_{181}$ is greater than 0.5 pg/ml, corresponding to a 10% reduction based upon an anticipated change of 5.1 pg/ml in the control arm (a similar distribution is expected for the change in or p-tau$_{231}$ and p-tau$_{181}$).[44] Individually, this boundary decision corresponds to a Pocock futility boundary at 50% of the maximal statistical information, maintaining level 0.025 for a one-sided hypothesis test.[55, 56] While implementation of the futility boundary will not affect inference for the primary outcome in phase 2a, it will need to be accounted for in the inference corresponding to the outcomes of phase 2b. In the event that the trial does proceed to phase 2b, final inference will account for the futility decision by utilizing a Benjamini-Hotchberg adjustment[57] for multiplicity bias stemming from the use of two biomarkers in the decision to continue the trial. In this case, simulation will be used to adjust the final inference at phase 2b in order to maintain an overall familywise type I error rate of .05.

14.4. Statistical power for phase 2a.

Based upon prior reported data, it is expected that the change in p-tau$_{231}$ over one year will be approximately 5.1 pg/ml with a standard deviation of 1.5.[44] With a sample size of 48 participants, a 1:1 randomization scheme, and a 10% per year loss-to-follow-up rate, the proposed study will attain approximately 80% power for detecting a true absolute difference in mean within-subject change in p-tau$_{231}$ of 1.26 pg/ml (24.5% relative reduction). Further, the probability of successfully passing the "go/no-go" decision for a second year of clinical follow-up is 80% if the true absolute difference in the within subject change in either CSF p-tau$_{231}$ or p-tau$_{231}$ 1.06 pg/ml (20.9% relative reduction).

14.5. Handling of missing data

In primary analyses, outcome values will not be imputed. Further sensitivity analyses for the impact of potential missing data will be conducted following study completion. These will include a multiple imputation analysis and a pattern mixture model analysis considering various departures from the estimated treatment effect using completely observed data.[58]

14.6. Analysis of secondary outcomes

Mean differences in the within subject change in CSF p-tau$_{181,}$ CSF total tau, CSF A$\beta_{40,}$ CSF Aβ_{42}, ADAS-cog13, ADCS-ADL, CDR-SB will be tested between treatment arms. To control the familywise type I error rate at level 0.05 (two-sided), the Benjamini-Hotchberg closed testing procedure will be utilized.[57]

14.6.1. Additional exploratory analyses. To assess sensitivity to patient compliance, the primary outcome will also be analyzed using only data on study completers and with adjustment for protocol compliance. In addition, we will examine for association between blood levels of nicotinamide and change in CSF p-tau$_{231}$, quantified via linear regression. Descriptive analyses of safety and efficacy data will be presented by site, diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers). The primary efficacy endpoint, as well as all secondary efficacy endpoints and safety outcomes will be presented by site, diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers). As these analyses will be for descriptive purposes, no adjustment for multiple comparisons will be made when presenting subgroup analyses.

14.6.2. Analysis of safety outcomes. Safety analyses will be conducted on the Full Analysis Population, defined as all subjects who were randomized into the study and received study intervention. Summaries of the number and percent of subjects with at least one AE/SAE will be provided. Comparisons of the proportion of subjects experiencing AEs/SAEs will be made between treatment arms. Summaries of the number or percent of AEs will be provided for each treatment arm.

14.6.3 Randomization. The ADCS Safety Biostatistics group will oversee the randomization scheme, which will be administered through the EDC. Randomization will incorporate blocking to maintain temporal balance between arms and will be stratified to ensure balance across likely correlates of the study outcome. Specifically, randomization will be stratified by site, diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers). The ADCS Biostatistics Core will perform analyses. Both enrollment sites have a long history of completing trials using the ADCS Electronic Data Capture (EDC) system, which will make the initiation and conduct of data entry and upload for this trial seamless.

15. ETHICS

15.1. Ethical Conduct of Study

This study will be conducted according to Good Clinical Practice, the Declaration of Helsinki,[59] the Belmont Report, and US 21 CRF Part 50 – Protection of Human Subjects – and Part 56 – Institutional Review Boards.

15.2. Institutional Review Board (IRB)

All materials will be submitted, reviewed, and approved by the UC Irvine Institutional Review Board. UCLA will utilize the reliance agreement available through the UC BRAID/UC Reliance Services mechanism to achieve approval for this multisite trial using a single IRB mechanism.

15.3. Human subject protection

The substantial clinical experience with nicotinamide in a variety of therapeutic areas, including doses higher than that proposed in this study, suggest that the overall risk for this study is low. We will nevertheless utilize the ADCS DSMB to ensure that the study has appropriate monitoring for safety. The DSMB will be notified immediately of any life-threating events or deaths. The DSMB will receive blinded reports of adverse events and serious adverse events on a quarterly basis and will be convened telephonically for the purpose of assessing trial continuation. At any time, the DSMB will be able to request additional data, including unblinded data, for the purpose of safety review. The DSMB may recommend halting of the study prior to conclusion or modification to the protocol at any time.

15.4. Informed Consent Document

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures are carried out. Informed consent will be obtained in accordance with US 21 CFR 50.25 and requires approval from the UCI IRB. Consent forms must be in

a language fully comprehensible to the prospective participant and the dated signature of the participant will document consent.

Informed consent will be performed in the private research setting by the site PI or study coordinator. The capacity to provide informed consent will be assessed using the University of California Post-Consent Assessment Instrument. Individuals lacking the capacity to provide informed consent may be enrolled provided that 1) a legally authorized representative (LAR) is able to give surrogate consent, and 2) the participant provides assent. The LAR must be certified as appropriate using the "Self-Certification of Surrogate Decision Makers for Potential Subject's Participation in University of California Research."

Each participant must have a qualified study partner (see section 10.1.1) and this individual must also provide written informed consent to participate.

15.4. Participant Confidentiality

All participants have a right to protection against invasion of privacy. After completion of the informed consent process, participants will be assigned a study identification number. Throughout this study, data will be identified in the Case Report Forms by the subject identification number only.

16. DATA HANLDLING AND RECORDING

16.1. Data collection and management responsibilities

Case report forms (CRFs) will be provided for use as source documents and used for primary data collection at the sites. These will be maintained for each participant enrolled in the study. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents must be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required. When making changes or corrections, cross out the original entry with a single line, and initial and date the change.

Participant visit data will be entered into the ADCS Electronic Data Capture (EDC) system within 72 hours of visit completion. EDC entry will include manual entering of data and the scanning and uploading of some specific CRFs (see procedures manual). Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record. Clinical data (including AEs, concomitant medications, and adverse reactions data) and clinical laboratory data will be entered into the EDC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

16.2. Study records retention

Study documents should be retained for a minimum of 2 years after the investigation is discontinued and FDA notified. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

16.3. Protocol deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents and reported in

the ADCS EDC system. Protocol deviations must be sent to the IRB of record, per IRB reporting guidelines.

16.4. Publication and data sharing

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. This trial will be registered through ClinicalTrials.gov (NCT03061474).

At a minimum, electronic item-level data from this trial will be made publicly available through the ADCS Data Sharing Process, one year after publication of the primary outcome results, in accord with the data-sharing policy of this NIH-funded trial organization.

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APPENDIX 1.

Table 1. Study schedule of events.										
Week	*	*	*	0	2	4	12	24	36	48 (or early termination)
Visit	SC v1	SC v2	SC v3	V1	@	@	V2	V3	@	V4^
ICF/HIPAA	X									
Demographics/medical history	X									
Concomitant medications	X			X	X	X	X	X	X	X
Adverse events	X			X	X	X	X	X	X	X
Physical/neurological exams	X			X			X	X		X
Vital signs	X			X			X	X		X
MMSE	X			X				X		X
CDR-SB	X							X		X
Wechsler Logical Memory II	X									
Free and cued selective reminding task (FCSRT)	X									
Assessment of inclusion/exclusion criteria	X									
Blood draw (safety laboratories)	X			X				X		X
Fasting blood draw (future biomarkers)		X					X	X		X
Blood draw (drug levels)							X	X		X
Urinalysis	X			X				X		X
Electrocardiogram (EKG)	X							X		X
Blood draw (APOE genotyping)	X									
GDS	X			X				X		X
Lumbar puncture		X[#]								X
MRI			X							X
ADAS-cog				X				X		X
ADCS-ADL-MCI				X				X		X
C-SSRS				X				X		X
Dispense study drug				X			X	X		X[π]
Distribute study medication diaries				X			X	X		
Collect completed study medication diaries							X	X		X
Assess treatment assignment blindness										X

* 60 days are allotted to complete the screening assessments

[#] Must be performed within 30 days of platelet level and coagulation parameters (SC v1 blood panel). In the event of inability to procure adequate sample, may be performed under guided fluoroscopy, which may require additional visits.

@ Telephone visits will assess safety and adverse events

^ In the event of early termination due to participant withdrawal of consent or removal by investigator, the end of study visit should be completed.

[π] Participants will be administered blinded study drug at the end of study visit as part of an extension study, until the go/no-go analyses are complete. If a go decision is achieved, participants will be enrolled in an extension study.