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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 (NEAT)

Statistical Analysis Plan

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SIGNATURE PAGE

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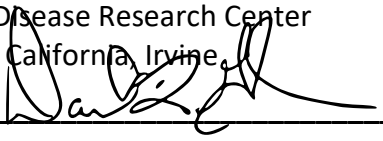
Author:	Daniel L. Gillen, Ph.D., Professor and Chair, Department of Statistics Institute for Memory Impairments and Neurological Disorders Alzheimer's Disease Research Center University of California, Irvine Signature:  Date: 07-Dec-2022

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Investigator Approval
By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, will be updated within any publications related to the study.

Project Director Signatory:

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Date: 12-4-2022

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Date: Nov 30,2022

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ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BQL	Below limit of quantification
BUN	Blood urine nitrogen
C-SSRS	Columbia-Suicide Severity Rating Scale
CI	Confidence interval
CPK	Creatine phosphokinase
CRF	Case Report Form
CSR	Clinical study report
DILI	Drug induced liver injury
ECG	Electrocardiogram
HDL	High-density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model for repeated measures
MMSE	Mini Mental State Exam
PID	Participant Identification Number
PK	Pharmacokinetic
PP	Per protocol set
PT	Preferred term
QD	Quaque die (once daily)
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
ULN	Upper limit of normal
WHO Drug	World Health Organization drug reference dictionary

1 INTRODUCTION

1.1 Introduction

This document presents the statistical analysis plan (SAP) for the protocol titled: 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 (NEAT). This study is a multisite, randomized, double-blind, placebo-controlled Phase 2 trial comparing efficacy and safety of nicotinamide versus placebo in patients with MCI due to AD and Mild AD Dementia.

This SAP is based on Version 4.0, Amendment 3, of the NEAT Protocol dated 28Dec2017. It contains the analysis details and methodology to answer the study objectives, including planned summary tables, by-subject listings, and figures, which will provide the basis for the results section of the clinical study report (CSR). Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analyses.

1.2 Randomization Methodology

The ADCS Safety Biostatistics group oversaw the randomization scheme, which was administered through the EDC. Randomization incorporated blocking to maintain temporal balance between arms and was stratified to ensure balance across likely correlates of the study outcome. Specifically, randomization was stratified by site, diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers).

1.3 Power and Sample Size Determination

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. 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).

2 OBJECTIVES AND OUTCOMES OF STATISTICAL ANALYSIS

2.1 Primary Objectives and Outcomes

- **Primary Objective 1:** Determine if daily treatment with high dose nicotinamide reduces levels of phosphorylated tau (p-tau$_{231}$) over 48 weeks in individuals with MCI due to AD and mild probable AD dementia

- **Primary Objective 2**: Assess the safety and tolerability of high-dose nicotinamide treatment in individuals with MCI due to AD and mild probable AD dementia, as assessed by:
    - Vital signs,

- Treatment emergent adverse events,
- Serious adverse events
- Suicidality rating scale scores,
- ECG abnormalities.

2.2 Secondary Objectives and Outcomes

- **Secondary Objective 1**: Determine if daily treatment with high-dose nicotinamide in individuals with MCI due to AD and mild AD dementia can affect CSF levels of:
    - amyloid beta (Aβ42)
    - amyloid beta (Aβ40)
    - phosphorylated tau (p-tau$_{181}$)
    - total tau (t-tau)
    - the ratio of total tau/Aβ40
    - the ratio of total tau/ Aβ42

- **Secondary Objective 2:** Determine if daily treatment with high-dose nicotinamide in individuals with MCI due to AD and mild AD dementia reduces the rate of cognitive decline, as assessed by:
    - ADAS-Cog 13

- **Secondary Objective 3:** Determine if daily treatment with high dose nicotinamide in individuals with mild AD dementia or MCI due to AD reduces the rate of functional decline and everyday activities as assessed by:
    - ADCS-ADL-MCI
    - CDR-SB

2.3 Exploratory Objectives and Outcomes

- **Exploratory Objective 1**: Determine if daily treatment with high-dose nicotinamide reduces rate of brain volumetric changes atrophy in individuals with MCI due to AD and mild AD dementia, as assessed by:
    - whole brain volume,
    - ventricular volume,
    - hippocampal volume,
    - and cortical thickness

- **Exploratory Objective 2:** Determine the effect size of daily treatment with high dose nicotinamide over 48 weeks in individuals with mild AD dementia or MCI due to AD on:
    - phosphorylated tau (p-tau$_{181}$)

- **Exploratory Objective 3**: Estimate the association between blood levels of nicotinamide and change in CSF p-tau$_{231.}$

- **Exploratory Objective 4:** Describe efficacy data by the randomization factors of diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers)

3 STUDY DESIGN

3.1 Synopsis of Study Design

Forty-seven participants were randomized to 1500 mg BID nicotinamide or matched placebo at a 1:1 ratio, stratified by site, diagnostic category (MCI or dementia), and APOE genotype (ε4 carriers, ε4 non-carriers).

There was a screening period of up to 60 days, during which each participant completed 3 screening visits.

The ADAS-Cog 13, ADCS-ADL-MCI and CDR-SB were collected at 3 timepoints: baseline, week 24, and week 48. The MMSE was collected at screening visit 1, baseline, week 24, and week 48. the Free and Cued Selective Reminding Task (FCSRT) was only done at screening visit 1.

Lumbar punctures and MRI scans were performed at screening and week 48. Safety measures including the C-SSRS, EKG, and safety laboratories were collected at screening or baseline, week 24, and week 48. Vital signs, blood collection for biomarker banking, physical exams, and neurological exams were done at screening or baseline, week 24, week 36, and week 48. Concomitant medications and adverse events were collected at every visit.

4 PARTICIPANT POPULATIONS

4.1 Population Definitions

The following populations will be evaluated and used for presentation and analysis of the data:

- **Enrolled Participants**: Participants who signed an informed consent form and were assigned a Participant Identification number (PID)
- **Intent to Treat (ITT) Population**: All enrolled participants who received a randomized treatment assignment.
- **Safety Population**: Enrolled and randomized participants who received at least 1 dose of blinded study therapy (nicotinamide or placebo). This will be the primary analysis population used for the safety analyses.
- **Modified Intent-to-Treat (mITT) population:** All randomized participants who have a baseline assessment of the endpoint, and who have at least one evaluation of the endpoint following baseline.

- **Per-protocol population:** All randomized participants who completed trial activity through week 48 including lumbar puncture.

5 STATISTICAL METHODS

5.1 Primary Efficacy Analysis

The primary outcome measure of phase 2a is the within-participant change in p-tau$_{231}$ from baseline to week 48. The primary analysis will be conducted on the ITT population. No missing data will be imputed

The change in p-tau$_{231}$ will be modeled 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 using a linear regression model of the form:

$$E\left[ptau_{231,post}\right] = \beta_0 + \beta_1 I_{Nic} + \beta_2 ptau_{231,pre}.$$

β_1 represents the parameter of interest. The Huber-White robust variance estimator[1] will be used to estimate the variance of the estimated difference in the mean change in p-tau$_{231}$ comparing treatment to placebo. Specifically, the variance estimator for the estimated treatment effect will be the (2,2) element of

$$(X^T X)^{-1} X^T \hat{\Sigma} \mathrm{X} (X^T X)^{-1},$$

where X denotes the $n \times 3$ design matrix from regression model, $\hat{\Sigma}$ is the $n \times n$ diagonal matrix with the squared model residuals on the diagonal and 0 on the off-diagonal, and n the number of observations included in the analysis. 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.

Sensitivity analyses for the primary outcome

An exploratory sensitivity analysis will investigate compare within-patient change in pTau$_{231}$ in the per-protocol population. Sensitivity analyses for the impact of potential missing data will be conducted for the primary outcome as described in Section 5.8.

5.2 Analysis of Safety Outcomes

Safety analyses will be conducted on the Safety Population.

The safety and tolerability of high-dose nicotinamide treatment in individuals with MCI due to AD or mild AD dementia will be assessed by the following, for each arm separately:

- Treatment emergent Adverse Events (TEAEs) summarized:
    - Overall and by number of subjects with an AE
    - By severity and relation to investigational product

    - By number of events and by subjects with an event by System Organ Class (SOC)
    - By Preferred Term (PT) within SOC
- Serious adverse events (SAEs) including deaths summarized as for TEAEs
- Vital signs (weight, BMI, blood pressure, pulse) will be presented with means and standard errors at each study visit
- Descriptive statistics for ECG interval data will also be reported across visits including baseline, week 24 and week 48 or early termination.
    - Proportion of subjects with at least 1 abnormal ECG by treatment arm
    - Proportion of subjects with at least 1 abnormal QTC by treatment arm
        - The QTC will be computed using the Friderica formula. Abnormal will be defined as above 460 for men and above 470 for women.
    - Average within subject change in QTC by treatment arm (baseline to latest available)
- Adverse events (AEs) leading to discontinuation

5.2.1 Columbia-Suicide Severity Rating Scale (C-SSRS)

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.

The number and proportion of subjects with treatment emergent Suicidal ideation or behavior will be reported overall and by study arm.

Treatment emergent suicidal ideation or behavior is defined as a "yes" answer at any time during treatment to any one of the questions in the ten suicidal ideation and behavior categories (Categories 1-10) on the C-SSRS[2]. Self-injurious behavior without suicidal intent, while assessed on the C-SSRS, does not form part of this outcome.

5.3 Analysis of Secondary Efficacy Outcomes

Mean differences in the within subject change in CSF p-tau181, CSF total tau, CSF Aβ40, CSF Aβ42, ADAS-cog13, ADCS-ADL-MCI, and CDR-SB will be tested between treatment arms, as described below. To control the familywise type I error rate at level 0.05 (two-sided) within each objective, the Holm-Bonferroni closed testing procedure will be utilized. Secondary analyses will be conducted on the mITT population.

5.3.1 Secondary Objective 1

The efficacy of nicotinamide will be assessed by the mean within-participant changes from baseline to week 48, compared between the treatment group and the placebo group, on the following CSF biomarker measures:

- amyloid beta (Aβ42),

- amyloid beta (Aβ40),
- phosphorylated tau (p-tau$_{181}$),
- total tau (t-tau),
- the ratio of total tau/Aβ40,
- the ratio of total tau/ Aβ42

Analyses will be the same as the primary efficacy outcome analysis. The Holm-Bonferroni closed testing procedure will be utilized to control the family-wise error rate at the 5% level across the 6 hypothesis tests.

5.3.2 **Secondary Objective 2** Determine if daily treatment with high-dose nicotinamide in individuals with mild AD dementia or MCI due to AD reduces the rate of cognitive decline, as assessed by ADAS-Cog 13

5.3.3 Secondary Objective 3

Alzheimer's Disease Cooperative Study-Activities of Daily Living - MCI (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.

CDR-Sum of Boxes

The CDR uses patient and informant interviews to assess memory, orientation, judgment and problem solving, community affairs, home and hobbies, and self-care. 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.

The within-participant change in each of these three measures (ADAS-Cog 13 ADCS-ADL-MCI and CDR) will be evaluated with a longitudinal mixed effects model with repeated measures. Terms in the model will be: age, baseline score, treatment, time defined categorically as visit, and time by treatment interaction.

A random effect will be included for participant. The within-participant covariance matrix will be unstructured. If the model does not converge a compound symmetry covariance matrix will be used. If the model still does not converge, the ANCOVA approach of the primary analysis will be used, using the last available measurement for each subject. Each endpoint will be tested using model-adjusted least squares means at the week 48 visit. A point estimate, standard error and two-sided 95% Wald confidence interval and a p-value will be presented for the difference between arms at 48 weeks. The Holm-Bonferroni closed testing procedure will be utilized to control the family-wise error rate at the 5% level across the 3 hypothesis tests.

Point estimates, standard errors and two-sided 95% confidence intervals for the mean within patient change at each time point will be presented by arm, as descriptive statistics unadjusted for multiple confidence intervals.

5.4 Analysis of Exploratory Outcomes

The efficacy and safety of nicotinamide will be further assessed for treatment effects between the treatment group and the placebo group, through the following exploratory analyses. As these analyses will be for descriptive purposes, no adjustment for multiple comparisons will be made when presenting subgroup analyses.

5.4.1 Exploratory Objective 1

MRI QUARC

Whole brain volume, ventricular volume, hippocampal volume, and cortical thickness will be measured as volumetric MRI. MRI imaging of the brain will be performed in order to measure brain atrophy over time. Results from vMRI studies suggest that the patterns of atrophy in AD can reliably be detected and tracked across time. Atrophy of the medial temporal lobe, including hippocampus and entorhinal cortex, has long been described in vMRI studies of AD. Hippocampal volume derived from MRI correlates with histological hippocampal volume and degree of neuronal loss and AD pathology, and entorhinal cortical thickness change appears to be an early and sensitive indicator of neurodegeneration associated with AD. Longitudinal MRI measures of regional and whole brain volumetric change provide a valuable complement to cognitive measures in that they are not influenced by temporary symptomatic improvements, and they provide an early index of the study drug's ability to reach the target organ and have an effect on AD-related atrophy.

The analysis of structural change will be performed through QUARC nonlinear registration comparing each participant's follow-up scan to the initial baseline scan. The procedure provides percent deformation within regions of interest (ROIs) obtained through segmentation of the baseline scan. The registration of serially-acquired brain volumes yields a deformation field that represents the volumetric shift of internal structure required for optimal overlaying of the two scans. The anatomically-based segmentation is overlaid on the smoothed voxel-wise deformation field and an average percent deformation is created for each ROI. Visual QC of registration is performed and values from scans passing QC are returned to the statistical team for analysis.

The mean difference in QUARC change will be compared for each volumetric measure between the nicotinamide and placebo groups. Analyses will be the same as the primary efficacy outcome analysis. The Holm-Bonferroni closed testing procedure will be utilized to control the family-wise error rate at the 5% level across the 4 hypothesis tests.

The number of images which are not quantifiable due to scan alignment failure will be summarized by study arm.

5.4.2 **Exploratory Objective 2** Determine the effect size of daily treatment with high dose nicotinamide over 48 weeks in individuals with mild AD dementia or MCI due to AD on phosphorylated tau (p-tau$_{181}$)

This will be conducted in the same manner as the primary analysis.

5.4.3 **Exploratory Objective 3**: Estimate the association between blood levels of nicotinamide and change in CSF p-tau$_{231}$.

A linear regression model will be used to assess the association between blood levels of nicotinamide, as measured in the lab of Dr. Greg Brewer, and within-patient change in CSF p-tau$_{231}$.

5.4.4 **Exploratory Objective 4:** Describe efficacy data by the stratification subgroups of MCI or dementia, and APOE genotype (ε4 carriers, ε4 non-carriers)

Means and standard deviations will be presented by arm for the 48 weeks within subject change on each primary and secondary endpoint, separately for these two subgroup categories.

5.5 Enrollment and Participant Flow

5.5.1 Study Accrual Tables and Figures

Tables will summarize accrual by study site, and figures will summarize the overall rate of accrual over calendar time.

Participant Disposition

A description of participant flow per the CONSORT guidelines and checklist will be provided. The diagram will describe study status from screening to the end of the study and will include the following information:

- Number of participants enrolled and signed informed consent form
- Number of enrolled participants excluded from the study prior to randomization and reason for exclusion
- Number of participants randomized
- Number of participants treated
- Number of participants who completed a lumbar puncture
- Number of participants who prematurely withdrew from the study and reasons for withdrawal

The CONSORT diagram is separated by study arm after the randomization step.

5.5.2 Early Termination

Early termination from the study includes participants who leave the study and/or discontinue treatment prior to completing the week 48 visit. Proportions of participants who prematurely discontinued the study prior to the week 48 visit will be compared between the study arms.

5.6 Demographics and Baseline Characteristics

Tabulations of demographic and baseline characteristics will be made for all randomized participants.

Descriptive statistics will be presented as N, mean, standard deviation, minimum, 25th quartile, median 75th quartile and maximum for continuous variables, and frequency tables (row, column percentages) for categorical variables. Statistical comparisons will be performed between randomized arms using Wilcoxon Rank Sum Test (for continuous variables) or Fisher's exact test (for categorical variables).

The following variables will be reported:

- Baseline Demographics:
- Baseline Vital Signs: All variables collected in the Vital Signs Form

5.7 Protocol Deviations

Any significant event that does not comply with the inclusion/exclusion criteria, study conduct (e.g., inadequate informed consent, unreported SAEs), or study procedures (e.g., use of prohibited medications as defined by the protocol; improper breaking of the blind) will be documented as a protocol deviation.

All protocol deviations will be presented in a tabulation

5.8 Handling of missing data

No imputation of missing data will be performed in the primary analysis of the primary, secondary, or exploratory endpoints. Sensitivity analyses for the impact of potential missing data will be conducted for the primary outcome and the secondary clinical outcome measures, including CDR-SB, ADAS-Cog 13, and ADCS-ADL-MCI. In each case a multiple imputation analysis will be performed and compared to the non-imputed analysis results.

5.9 Data Handling

Tabulations will be produced by randomized treatment group and overall, unless otherwise specified.

Descriptive statistics will be presented as N, mean, standard deviation, minimum, 25th quartile, median 75th quartile and maximum for continuous variables, and frequency tables (row, column percentages) for categorical variables.

Change from baseline is calculated by subtracting the baseline value from the observed post-baseline value at any subsequent visit. Baseline will be defined as last available assessment on or before the 1st day the participant receives study medication for that phase.

Tabulations of the following endpoints will present the number of unique participants with an event: protocol deviations; non-study medications; AEs; and laboratory abnormalities. Thus, for these endpoints, multiple occurrences of the same event are counted only once per participant.

Unique Participant ID’s take the form “NEATsite#participant#”.

5.10 Coding of AEs and Concomitant Medications

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 21.1.) Concomitant medications will be coded with WHO Drug (Global March 2019) using the WHO Drug Insight tool.

5.11 Adjustments for Covariates and Stratification

No additional covariate adjustment or stratification will be made in the primary analysis.

5.12 Randomization

The ADCS Safety Biostatistics group has overseen the randomization scheme, which has been administered through the study EDC. Randomization has incorporated blocking to maintain temporal balance between arms with stratification to ensure balance across likely correlates of the study outcome. Specifically, randomization has been stratified by site, diagnostic category (MCI due to AD or mild AD 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.

5.13 Software

Statistical software R (version 4.1.2) will be used *http://www.r-project.org*.

6 REFERENCES

1. White H. A Heteroskedasticity-Consistent Covariance Matrix Estimator and a Direct Test for Heteroskedasticity. *JSTOR.* 1980;48:817-838.
2. Nilsson ME, Suryawanshi S, Gassmann-Mayer C, Dubrava S, McSorley P, Jiang K. Columbia–Suicide Severity Rating Scale Scoring and Data Analysis Guide. https://cssrs.columbia.edu/2013.