ALZHEIMER'S DISEASE COOPERATIVE STUDY (ADCS)

A Safety and PK/PD Study of Posiphen in Subjects with Early Alzheimer's Disease (DISCOVER)

NCT02925650

Statistical Analysis Plan (SAP)

February 7, 2022

Project Principal Investigators: Douglas R. Galasko, MD and Martin Farlow, M.D.

U-19 Principal Investigator: Howard Feldman, MD

Analysis Team: ADCS Biostatistics Core

Contributing Investigators (alphabetical):

Martin Farlow, M.D.
Howard Feldman, MD
Douglas R. Galasko, MD
Shelia Jin, MD MPH
Jeremiah Momper, PhD
Robert Rissman, PhD
Ron Thomas, PhD

Statistical Analysis Plan (SAP): Version 1

Protocol Title:	A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics (PK) and Pharmacodynamic (PD) Effects of Posiphen® in Subjects with Early Alzheimer's Disease (AD)
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Author:	Ronald G. Thomas, Ph.D., Professor Herbert Wertheim School of Public Health and Human Longevity Science, University of California, San Diego Signature: Date: 2/3/22

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 Signatories:

Douglas R. Galasko, MD
Signature:
Date: 9 Feb 2022

Martin Farlow, M.D.
Signature:
Date: 9 Feb 2022

ADCS Principal Investigator Signatory:

Howard Feldman, MD, Director, Alzheimer's Disease Cooperative Study
Signature:
Date: 9 Feb, 2022

Discover Statistical Analysis Plan

Prepared by: Dr. Ron Thomas, ADCS Biostatistics Core, February 7, 2022

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

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

Protocol Number: ADC-043-DISC (QR15001)

1 Study Eligibility

Clinical profile consistent with early AD including MCI-AD or mild AD according to the core clinical criteria outlined in the National Institute Aging (NIA) and Alzheimer's Association (NIA-AA) Guidelines Cerebrospinal fluid (CSF) AD biomarkers consist with AD (Beta Amyloid 42(Ab42)/Beta Amyloid 40(Ab40) ratio less than 0.131 as measured by mass spectrometry) Age 55-89 (inclusive), in good health with no frailty Mini-Mental State Examination (MMSE) score between 17 – 30 (inclusive) Clinical Dementia Rating (CDR) global score of 0.5 with a memory score of 0.5 or greater, or global score of 1.0

2 Study Aims

Aim 1. Safety and Tolerability

Aim 2. PK of Posiphen/metabolites in Plasma and CSF

Aim 3. PD on fractional synthesis rate (FSR) of Beta Amyloid 40 (Ab40) in CSF

Aim 4. Feasibility of SILK in a multicenter trial

Aim 5. PD on other biomarkers in CSF including Beta Amyloid 38 (Ab38), Ab40, Ab42, Soluble Amyloid Precursor Protein alpha (sAPPa), Soluble Amyloid Precursor Protein alpha (sAPPb), total Tau

Aim 6. Cognitive and/or neuropsychiatric effects of Posiphen

3 Randomization

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

4 Power and Sample Size Determination

Power estimates for SILK™ studies involving measurements of FSR of Ab40 were deter- mined by analysis of data provided by C2N, Inc., and modeled to evaluate a primary analytic goal of demonstrating a monotonic dose response relationship to Posiphen treatment. Based on a two-sided t-test for the difference between two independent means at a significance level (alpha) of 0.05 and a statistical power of 80% (1-beta), a sample size of 5 subjects in each treatment group (n active = 15) and 9 controls will provide at least 80% statistical power to detect a 27% change in the mean Ab40 FSR. The following table provides estimated power analysis to detect different effect size reductions of FSR Ab40 of 27% with 2-sided alpha and significance set at 0.05, assuming no dropouts during the SILK™ study:

4.1 Estimated Power Analysis

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

Table 1. Estimated Power Analysis

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

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

4.2 Study Populations

a. **Enrolled Population:** All participants who consented to screening
b. **Intention To Treat (ITT) Population:** list of all randomized participants
c. **Modified Intention-to-Treat (mITT) Population**: Randomized participants who took at least one dose of study medication confirmed by compliance assessment and had at least one post randomization study visit
d. **Per Protocol (completers)**: Randomized participants who were
    i. compliant through Confinement Visit,
    ii. compliant to with 20% of dose 1 week prior Pre-Confinement, and
    iii. completed the 36-hour sampling
e. **Safety Population**: Randomized participants who:
    i. took at least one dose of drug confirmed by compliance assessment

5 Statistical Analysis

5.1 Primary Aims

5.1.1 Aim 1

To determine the safety and tolerability of multiple ascending doses of Posiphen on subjects with early AD with up to 23-25 days of daily usage. Safety and tolerability will be summarized using the following tables, listing and figures:

- Vital signs
- Adverse events onset before randomization
- Adverse events onset after randomization
- AE overall
- AE by severity
- MedDRA coded AEs
- Percentage of AEs summarized by SOC
- Number of participants with at least one AE
- Number of AEs summarized by System Organ Class (SOC) and Preferred Terms (PT)
- Serious AEs (SAE) onset after randomization
- Death summary
- Hospitalization summary
- Safety Labs

5.1.2 Aim 2

To determine the PK of Posiphen and its metabolites in plasma and CSF of subjects with early AD treated with QD, BID and TID dosing regimens., samples will be analyzed at baseline time 0 pre first dose then at times 2H, 4H, 8H, 12 H, 16H. 20H and 24H post dose. Further doses are provided every 8 hours. Curves and area under the curve will be constructed for each of the three sequential dose cohorts of 60 mg daily, 60 mg BID, and 60 mg TID.

Standard PK parameters will be calculated for each subject, including AUC, C_{max}, t_{max}, $t_{½}$, $V_{ss.}$ Dose-proportionality will be assessed on C_{max} and AUC statistics. Plasma concentrations will be displayed with time series plots including individual and means by dose. PK parameters summarized descriptively (N,

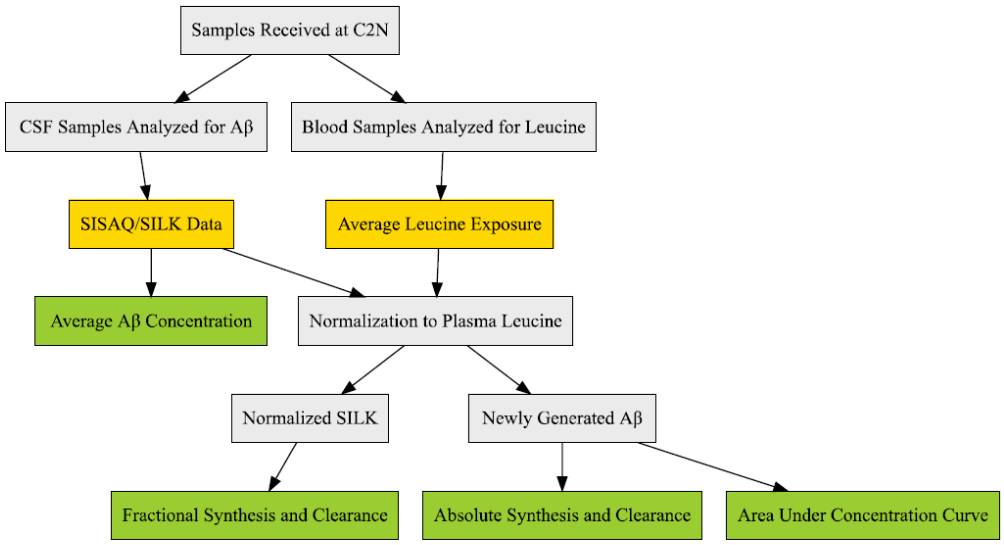
mean (SD) and 95% CI, median, min, max, and geometric mean (%CV) as appropriate) by each dose regimen. Accumulation ratio will be determined. CSF levels will be evaluated by dose levels and correlated where possible to blood levels.

The Charles River Lab in Montreal will analyze PK of Posiphen and itsmetabolites in Plasma and CSF. This will include Posiphen, N1-Desmethyl Posiphen (Phenserine) and N8-Desmethyl Posiphen (Phenserine). Phenserine is an enantiomer of Posiphen. The chromatographic assay used does not distinguish between the two enantiomers, so these metabolites represent the total amount of both.

5.1.3 Aim 3

To assess the PD effects on the FSR of Ab 40 using the SILK™ technique with multiple doses of Posiphen.

The schema for evaluating the Fractional Synthesis and Clearance Rate, the Absolute Synthesis and Clearance and Area under the concentration curve are presented in **Figure 1**.



The analytic techniques to calculation these values are undertaken by C2N.

The primary outcome measure of Fractional Synthesis Rate (FSR) of Abeta 40 will be compared across all Posiphen treated groups to placebo. A linear regression model will be used to evaluate treatment effects on the FSR Abeta 40, with age and gender as covariates. Quality control of samples for the FSR 40 will be ascertained to determine sufficiency of the sample values prior to this regression analysis.

A secondary analysis will test for possible dose group by treatment interaction.

Exploratory analyses will include PK AUC correlation to Abeta 40 FSR and other SILK measured analytes (Abeta 38, 42, ratio 42/40, total Abeta peptides) including absolute synthesis and clearance.

C2N will analyze Beta Amyloid 40 (Ab40).

5.2 Secondary Aims

5.2.1 Aim 1

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

Enrollment comparisons of research subjects at each of the 5 sites. Screen failure rates will be evaluated by cause, by number and by site. Demographics will be described across sites. There will be comparison of protocol deviations and sampling results between the sites. Adequacy of sample collections to permit analyses of PK and PD effects of Posiphen during the SILK will be tabulated. Description of the adequacy of SILK sampling, Pk and PD during the confinement visits will be undertaken with comparisons across sites.

Research Satisfaction Questionnaires: Descriptive ratings of participants in response to the research study will be undertaken across visits including baseline and post-confinement when this questionnaire was administered. The responses on the survey will be grouped and compared to address the experience across the trial with characterization of the most frequent terms. Cross tabulations and shift tables will be used to address the response to the SILK procedure through its categorical variables and its influence on participant satisfaction. We will explore whether there is any indication of an effect of treatment on these participant profiles.

5.2.2 Aim 2

To assess the PD effects of treatment with Posiphen versus placebo on the levels of the following biomarkers in CSF of subjects with early AD: Ab 38 and 40, sAPPa, sAPPb, total tau protein.

Mean changes in these biomarkers will be compared across treatment arms between baseline and day 21 time 0 prior to the leucine infusion pooling all participants treated with Posiphen vs placebo across cohorts. Linear regression modelling will allow the comparison of mean change scores in the analytes between the active and placebo groups while adjusting for covariates including age, gender, MMSE at baseline, and Apo-e status as well as armcode and dose cohort and cohort-by-armcode interaction.

To evaluate the PK PD relationships, trough levels of Posiphen at day 21 (time 0) will be examined as a predictor of change in the CSF levels of each of these biomarkers between baseline and day 21. A linear regression model will be used to predict the change in CSF analyte with the covariates of trough PK, age, and gender. Other PK measures will be similarly evaluated including AUC, and Cmax.

In a secondary analysis to evaluate the effect of dose, we will use a similar regression model with terms for placebo and each dose group as predictors, including age and gender as covariates.

5.2.3 Aim 3

To assess whether there are short-term cognitive and/or neuropsychiatric effects of Posiphen in subjects with early AD, either positive or negative on ADAS-Cog12, MMSE, Neuropsychiatric Inventory (NPI).

These cognitive and neuropsychiatric measures will be compared between Posiphen and placebo using multiple linear regression. This methodology will allow the comparison of mean change scores between the active and placebo groups while initially adjusting for covariates; age, gender, treatment arm and baseline MMSE. Dose effects will be assessed as part of this analysis to investigate trends. The effects of ApoE genotype on these clinical outcome measures will also be explored as a tertiary analysis.

C2N will analyze Beta Amyloid 38 (Ab38), Ab40, and Ab42. The ADCS Biomarker Core will analyze Soluble Amyloid Precursor Protein alpha (sAPPa), Soluble Amyloid Precursor Protein alpha (sAPPb), and total Tau protein.

5.3 Tertiary Analyses

The data of the normalized SILK curve sampling will provide for exploratory analyses of fractional synthesized rates of Abeta 38, 42, and total Abeta as well as the ratio curve of Abeta 42/40. The fractional clearance rate will be explored as well using the normalized SILK curve data for these same analytes also including Abeta 40. To compare all Posiphen treated groups to placebo a linear regression model will be used to evaluate treatment effects on the fractional and absolute synthesis rates, with age and gender as covariates. Quality control of samples for each of these analytes will be ascertained to determine sufficiency of the sample values prior to this regression analysis.

The absolute synthesis and clearance rates of newly generated Abeta 38, 40, 42, total Abeta and ratio of Abeta 42/40 will also be analyzed compared across all Posiphen treated groups to placebo. A linear regression model will be used to evaluate treatment effects on the both the fractional and absolute synthesis rates, with age and gender as covariates. Quality control of samples for each of these analytes will be ascertained to determine sufficiency of the sample values prior to this regression analysis. The area under the curve will be calculated for newly generated Abeta.

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

Concluding Comments
This phase 1B trial of Posiphen in the treatment of early AD, investigates its pharmacokinetics, pharmacodynamics and target engagement, as well as safety and tolerability. The SAP addresses each of the study aims with plans for preplanned analyses