Statistical Analysis Plan (SAP)

PATH-1 Study

Protocol Title: A Phase 1b Dose Range Finding Study of Phenserine Compared to Donepezil in Participants with Early or Mild Alzheimer's Disease

Short Title: PATH-1

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ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BPM	Beats Per Minute
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CSF	Cerebrospinal Fluid
DMC	Data Monitoring Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOS	End of Study
FLAME	Factors of Longitudinal Attention, Memory, and Executive Function
IMP	Investigational Medicinal Product
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
MoCA	Montreal Cognitive Assessment
NfL	Neurofilament Light Chain
PD	Pharmacodynamic
PK	Pharmacokinetic
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SOC	System Organ Class

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1 Introduction

1.1 Background and Rationale

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by synaptic dysfunction, neuronal loss, and accumulation of pathological protein aggregates. Current standard treatments, including acetylcholinesterase inhibitors (AChEIs) such as donepezil, provide modest symptomatic benefit but do not address underlying disease mechanisms or halt neurodegeneration.

Phenserine is a next-generation AChEI with additional non-cholinergic mechanisms that may confer disease-modifying properties. Preclinical and clinical evidence indicates that phenserine modulates pathways related to pre-programmed cell death, synaptic integrity, neuroinflammation, and amyloid precursor protein processing. Unlike currently approved AChEIs, phenserine has demonstrated effects on molecular markers associated with neuronal survival and apoptosis, including Bcl-2, caspase signaling, and neurotrophic factors.

Previous clinical development programs of phenserine were conducted primarily in patients with mild-to-moderate AD and were limited by suboptimal dosing strategies, lack of biomarker confirmation of AD pathology, and insufficient characterization of pharmacodynamic effects. These limitations support the need for a refined early-phase study focused on mechanistic biomarkers and individualized dose optimization

The full rationale for undertaking the trial and trial background is explained in detail in the protocol. This Statistical Analysis Plan (SAP) describes the statistical methodology and data handling procedures for the PATH-1 study, a Phase 1b randomized, open-label, parallel-group trial comparing phenserine with donepezil in participants with early or mild Alzheimer's disease (AD).

The SAP is finalized prior to database lock and unblinding of biomarker results and is written in accordance with ICH E9 and relevant EMA guidance for early-phase exploratory trials. Any deviations from this SAP will be documented in a separate SAP amendment prior to database lock.

2 Study Objectives and Endpoints

2.1 Primary Objective

To assess the effects of phenserine compared to donepezil on exosome biomarkers of pre-programmed cell death, synaptic integrity, neuroinflammation, and AD-related protein trafficking.

2.2 Secondary Objectives

- To evaluate safety and tolerability of phenserine versus donepezil
- To characterize PK–PD relationships
- To assess achievement and maintenance of ~45% AChE inhibition
- To assess treatment compliance

2.3 Exploratory Objectives

- Changes in CSF and plasma AD biomarkers
- Short-term cognitive effects using FLAME and MoCA

Endpoints are defined exactly as per Protocol Section 3 and Table 2

2.3.1 Hypothesis Framework

This trial is an exploratory Phase 1b dose range–finding study designed to characterize the pharmacodynamic, pharmacokinetic, and safety profile of phenserine compared with donepezil in participants with early or mild Alzheimer's disease. The study is not powered for formal confirmatory hypothesis testing.

The primary objective is mechanistic and descriptive in nature, focusing on changes in exosome-derived biomarkers associated with pre-programmed cell death, synaptic integrity, neuroinflammation, and AD-related protein trafficking.

Primary Hypothesis Framework

- Null hypothesis (H_0): There is no difference between phenserine and donepezil in changes from baseline in exosome biomarkers over the 8-week treatment period.
- Alternative hypothesis (H_1): Treatment with phenserine is associated with changes from baseline in exosome biomarkers that differ from those observed with donepezil over the 8-week treatment period.

These hypotheses will be **exploratorily** evaluated through estimation of treatment effects and their associated uncertainty (95% confidence intervals), rather than formal hypothesis testing with a pre-specified significance level. The focus is on estimating the magnitude and direction of treatment differences.

Secondary and Exploratory Analyses

There is a single identified primary analysis related to exosome biomarker changes. All secondary (safety, PK, AChE inhibition, compliance) and exploratory (CSF, plasma biomarkers, cognitive outcomes) analyses will be considered supportive or exploratory and will be interpreted descriptively.

No formal non-inferiority or superiority margins are prespecified, and no multiplicity adjustments will be applied.

2.3.2 Confidence Intervals and p-values

P-values will be reported for descriptive purposes only and will be interpreted cautiously in the context of the exploratory study design. All efficacy estimates will be presented with two-sided 95% confidence intervals, which should be interpreted as ranges of plausible treatment effects rather than formal inference tools. As there is only one primary null hypothesis to be tested in this trial, there will be no adjustments for multiplicity.

2.4 Timing of Outcome Assessments

The timing of all outcome assessments is predefined in the study protocol and summarized in the Schedule of Activities (SoA) (Protocol Section 1.3, Table 1). Outcome measures are collected at screening, baseline, and at scheduled on-treatment and follow-up visits during the 8-week treatment period and safety follow-up.

For all clinically planned outcome measures, visits should occur within the predefined visit windows. Assessments performed outside the allowable visit window will be considered protocol deviations, unless otherwise justified and documented. Only assessments obtained within the protocol-specified windows will be used for primary and secondary analyses.

The target days and visit windows relevant for efficacy, pharmacodynamic, pharmacokinetic, and safety analyses are defined as follows:

Visit Label	**Visit Window**
Screening	Within 28 days prior to baseline
V1: Baseline / Randomization	Day 0
V2: Week 2	Day 14 ± 3 days
V3: Week 4	Day 28 ± 3 days
V4: Week 6	Day 42 ± 3 days
V5: Week 8 / End of Treatment	Day 56 ± 3 days
Safety Follow-up / EOS	Within 28 days after last dose

Assessments collected outside the visit window will not be included in the primary analysis but may be listed and summarized descriptively.

3 Study Design Overview

- Design: Open-label, randomized (6:2), parallel-group Phase 1b study
- Treatment Arms:
  - Phenserine (dose-escalation up to 10 mg TDS)
  - Donepezil (5–10 mg OD)
- Treatment Duration: 8 weeks
- Sample Size: n = 16 (Phen: n=12; Donepezil: n=4)

Given the exploratory nature and small sample size, all analyses will be descriptive, and estimation focused.

3.1 Screening Data, Eligibility, and Recruitment

Participants are identified and screened according to the procedures described in the study protocol (Protocol Section 5). Screening data will be summarized to describe the representativeness of the study population and to document participant flow through the trial.

The total number of participants assessed for eligibility at screening will be summarized, together with the number and reasons for screen failures. Reasons for ineligibility will be categorized according to violations of inclusion and exclusion criteria as defined in the protocol.

Screening and eligibility data will be presented using descriptive summaries

3.2 Baseline Participant Characteristics

Baseline demographic and clinical characteristics will be summarized by randomized treatment arm and overall, for all randomized participants. Baseline characteristics will also be summarized for the primary analysis population to assess the potential impact of attrition on group balance.

The following baseline variables will be summarized, as available:

- Age (years)
- Sex
- Education level (if collected)
- Body weight, height, and body mass index (BMI)
- Alzheimer's disease stage (early vs mild AD)
- Baseline cognitive measures (MMSE, MoCA)
- Baseline exosome biomarker levels
- Baseline use of concomitant medications relevant to cognition or safety

Baseline characteristics will be summarized using descriptive statistics:

- Continuous variables: number of observations, mean, standard deviation, median, interquartile range (25th–75th percentile), minimum, and maximum
- Categorical variables: number and percentage of participants

No formal statistical comparisons between treatment groups will be performed. Any clinically relevant imbalances between treatment arms will be described qualitatively.

3.3 Withdrawal and Follow-up

Participant disposition and withdrawal status will be summarized by treatment group. Levels of withdrawal will be categorized as follows:

- Completed study intervention and all scheduled assessments
- Completed scheduled assessments but did not complete study intervention
- Withdrew from study intervention but continued follow-up
- Withdrew consent for further participation
- Lost to follow-up

The number and percentage of participants in each category will be tabulated by treatment arm and overall. Reasons for withdrawal or loss to follow-up will be summarized descriptively.

Participants who discontinue study treatment but provide post-baseline outcome data will be included in the relevant analysis populations according to the definitions specified in Section 4. Early termination visits will be mapped to the end-of-treatment time point where applicable.

3.4 Adherence and Protocol Deviations

3.4.1 Adherence to Allocated Treatment

Adherence to the allocated study treatment will be assessed for all participants who receive at least one dose of investigational medicinal product (IMP). Adherence is defined as the extent to which participants take the study medication according to the prescribed dosing regimen.

Compliance will be assessed using capsule counts and subject diaries and will be calculated as:

$$\% \text{ Compliance} = \left(\frac{\text{Number of capsules taken}}{\text{Number of capsules prescribed}} \right) \times 100$$

The number of capsules prescribed will be calculated based on the duration of treatment (date of last dose − date of first dose + 1) multiplied by the number of capsules prescribed per day according to the assigned dose level.

Compliance will be summarized by treatment group and overall. Participants will be classified as compliant if their calculated compliance is within 80–120**%** of the prescribed dosing, in accordance with the protocol definition.

The following summaries will be presented:

- Mean, median, and range of percentage compliance
- Number and percentage of participants classified as compliant
- Number and percentage of participants with compliance <80% or >120%

Compliance summaries will be descriptive only and no formal statistical comparisons will be performed.

3.4.2 Protocol Deviations

A protocol deviation is defined as any departure from the approved protocol, including but not limited to deviations related to eligibility criteria, study treatment administration, study assessments, or visit scheduling.

Protocol deviations will be prospectively classified as major or minor prior to database lock and prior to unblinding of biomarker results.

Major Protocol Deviations

Major protocol deviations are defined as deviations that may affect participant safety, the scientific integrity of the study, or the interpretability of the primary endpoint. Predefined major protocol deviations include:

- Failure to meet inclusion or exclusion criteria with enrolment into the study
- Receipt of incorrect study treatment or dose not consistent with protocol-defined escalation rules
- Discontinuation of study treatment prior to completion of the dose-escalation phase without post-baseline biomarker data
- Use of prohibited concomitant medication with potential impact on safety or pharmacodynamic outcomes
- Missing all post-baseline exosome biomarker assessments

Participants with major protocol deviations may be excluded from specific analysis populations as defined in Section 4.

Minor Protocol Deviations

Minor protocol deviations include deviations that are not expected to materially affect safety or interpretation of study outcomes, such as:

- Study visits conducted outside the protocol-defined visit window
- Isolated missed or delayed assessments
- Administrative or documentation errors

Summary of Protocol Deviations

Protocol deviations will be summarized descriptively by treatment group and overall. The following summaries will be provided:

- Number and percentage of participants with at least one protocol deviation
- Number and percentage of participants with at least one major protocol deviation
- Number and percentage of participants with at least one minor protocol deviation

- Listing of protocol deviations by category and type

Percentages will be calculated using the randomized population as the denominator. No formal statistical testing will be undertaken.

4 Analysis Populations

4.1 Enrolled Population

All participants who sign informed consent.

4.2 Safety Analysis Set (SAF)

All participants who receive ≥1 dose of study medication.

4.3 Modified Intent-to-Treat (mITT) Population

All participants who:

- Receive ≥1 dose of study medication, and
- Have ≥1 post-baseline exosome biomarker assessment

This population is the **primary analysis population** for PD and biomarker endpoints.

4.4 Pharmacokinetic Population

All participants with sufficient PK samples to estimate at least one PK parameter.

4.5 Overview of Outcomes

An overview of the primary, secondary, and exploratory outcomes included in the PATH-1 study is provided in Table 4.4-1. Outcomes are categorized by level, timeframe, and data type to clarify their role in the statistical analyses.

Overview of Study Outcomes

Level	Outcome	Timeframe	Outcome Type
Primary	Change from baseline in exosome biomarkers of pre-programmed cell death, synaptic integrity, neuroinflammation, and AD-related protein trafficking	Baseline to Week 8	Continuous
Secondary (Key)	Safety and tolerability (AEs, SAEs, labs, ECGs, vital signs)	Throughout study	Categorical / Continuous
Secondary	Steady-state pharmacokinetic parameters (e.g. plasma concentrations)	Weeks 2–8	Continuous
	Proportion achieving target AChE inhibition (~45%)	During treatment	Dichotomous
	Time to achievement of target AChE inhibition	During treatment	Time-to-event
	Duration of maintenance of target AChE inhibition	During treatment	Continuous

	Treatment compliance (capsule count, diary)	During treatment	Continuous / Categorical
Exploratory	Change in CSF biomarkers (Aβ1-40, Aβ1-42, Tau, p-tau)	Baseline to Week 8	Continuous
	Change in plasma biomarkers (p-tau217, NfL)	Baseline to Week 8	Continuous
	Change in FLAME cognitive domain scores	Baseline to Week 8	Continuous
	Change in MoCA total score	Baseline to Week 8	Continuous

5 General Statistical Principles

- All analyses will be conducted using R (version ≥4.2) or SAS (version ≥9.4)
- Continuous variables: mean, SD, median, IQR, min, max
- Categorical variables: counts and percentages
- No formal hypothesis testing or multiplicity correction
- Emphasis on effect size estimation, confidence intervals, and visualizations
- Outliers and extreme values in biomarker measurements will be retained in primary analyses unless determined to result from assay or data collection errors. Sensitivity analyses may exclude extreme values (e.g., >10 MAD from median) to assess their influence on results. If performed, such analyses will be justified and documented.
- No formal subgroup analyses are pre-planned. Exploratory descriptive summaries may examine biomarker responses by baseline disease severity (early vs. mild AD) or by achievement of target AChE inhibition, but these will be clearly labeled as descriptive and post-hoc.

6 Handling of Missing Data

- No imputation will be performed for missing biomarker or cognitive data
- Analyses will use observed data only in a complete-case manner
- Missingness patterns will be summarized descriptively
- Early termination participants will be included where data are available

Given the short duration and intensive follow-up, missingness is expected to be limited.

7 Primary Endpoint Analysis

Exosome Biomarkers

7.1 Data Structure

For each biomarker panel:

- Pre-programmed cell death
- Synaptic integrity

- Neuroinflammation
- AD-related trafficking

Measurements will be summarized at:

- Baseline
- Week 2
- Week 4
- Week 6
- Week 8

7.2 Statistical Methods

- Change from baseline will be calculated for each time point
- Within-arm trajectories will be visualized using spaghetti plots
- Between-arm comparisons will use:
    - Mean change differences with 95% CI
    - Mixed-effects models will be fitted exploratorily to estimate treatment effects over time.
        - Model specification:
            - Response: Change from baseline in biomarker level;
            - Fixed effects: Treatment (phenserine vs donepezil), Visit (categorical: Weeks 2, 4, 6, 8), Treatment × Visit interaction.
            - Random effects: Random intercept per participant.
            - Covariance structure: Unstructured (if convergence permits, otherwise compound symmetry).
            - Given the small sample size, models may have convergence issues or unstable variance estimates. If models fail to converge, results will be presented descriptively.

8 Secondary Analyses

8.1 Safety and Tolerability

Summarized by treatment arm:

- Incidence of AEs and SAEs
- GI AEs of special interest
- Laboratory abnormalities

- ECG changes
- Dose reductions and discontinuations

AEs will be coded using MedDRA.

8.2 Pharmacokinetics

- Summary of plasma concentrations at steady state
- PK parameters (Cmax, Tmax, AUC if derivable)
- Graphical exposure–response plots versus:
    - AChE inhibition
    - Key exosome markers

8.3 AChE Inhibition

- Proportion achieving ≥45% inhibition
- Time to reach target inhibition
- Duration above target threshold

Summarized descriptively and graphically.

8.4 Compliance

- Percentage of prescribed doses taken
- Proportion within 80–120% compliance window

9 Exploratory Analyses

9.1 CSF and Plasma Biomarkers

- Change from baseline at Week 8
- Descriptive summaries only
- Correlation analyses with exosome biomarkers (Spearman)

9.2 Cognitive Outcomes

- FLAME domain scores: change from baseline
- MoCA: baseline vs Week 8
- Individual-level change plots

No adjustment for practice effects due to short follow-up.

10 Interim Analyses

No formal interim efficacy analyses are planned. Safety data will be reviewed on an ongoing basis by the medical monitor and DSMB according to protocol.

11 Changes from Protocol

Any deviations from protocol-specified analyses will be documented and justified in the Clinical Study Report (CSR).