STATISTICAL ANALYSIS PLAN
Protocol No. AL001-ALZ02

A Multiple-Dose, Steady-State, Double-Blind, Ascending Dose Safety, Tolerability, Pharmacokinetic Study Of AL001 In Patients With Mild To Moderate Alzheimer's Disease And Healthy Adult Subjects ("MAD Study")

AltaSciences Project No. ALZ-P9-319

Version 1.1 (Final)

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16.1.9 Documentation of Statistical Methods

Statistical Analysis Plan – Final Version 1.1

Statistical Analysis Plan – Final Version 1.0

STATISTICAL ANALYSIS PLAN

For:

Alzamend Neuro, Inc.

PROTOCOL No. AL001-ALZ02

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Altasciences Project No. ALZ-P9-319

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Version: *Final 1.1*

Date: 2023-05-05

STATISTICAL ANALYSIS PLAN APPROVAL

We have carefully read this statistical analysis plan and agree it contains the necessary information required to handle the statistical analysis of study data.

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VERSION CONTROL

Version	Date	Author	Description of Changes
1.0	*2023/02/22*	Riddhi Thakkar	Not applicable
1.1	*2023/04/26*	Riddhi Thakkar	To provide the safety data analysis by combined active and pooled placebo groups.

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ABBREVIATIONS

AD	Alzheimer's disease
AE	Adverse event
CI	Confidence interval
COVID-19	Coronavirus sars-cov2
CRO	Clinical research organization
CV	Coefficient of variation
DMP	Data management plan
ECG	Electrocardiogram
EDC	Electronic data capture
ET	Early termination
FDA	Food and drug administration
ICF	Informed consent form
ICH	International council for harmonisation
IEC	Independent ethics committee
IMP	Investigational medicinal product
IP	Investigational product
MAD	Multiple ascending dose
MMSE	Mini-Mental State Examination
MTD	Maximum tolerated dose
PK	Pharmacokinetic
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System organ class
SOP	Standard operating system
TEAE	Treatment-emergent adverse event
TID	3 times daily
WHO-DD	World Health Organization drug dictionary

1 INTRODUCTION

This statistical analysis plan (SAP) provides a detailed description of the statistical methods and procedures to be implemented for the analyses of data from protocol AL001-ALZ02.The analyses described in the SAP are based upon the final protocol version 8.0 (Amendment 06) dated 2022/11/28.

2 STUDY OBJECTIVES

The objectives of the study and corresponding study endpoints are detailed in Table 1.

Table 1: Objectives and Related Endpoints

Objective	Endpoint	Analysis
Primary		
To evaluate the safety and tolerability of AL001 under multiple-dose, steady-state conditions in Alzheimer's subjects and healthy adult subjects.	• Vital signs (blood pressure, heart rate, and oral body temperature) • 12-lead electrocardiogram • Physical examination • Safety laboratory tests (hematology, chemistry [including thyroid-stimulating hormone (TSH), vitamin B12, and Mg], HbA1c, and urinalysis • Reported and observed Adverse events (AEs)/serious AEs)/tolerability observations including signs and/or symptoms of lithium and/or salicylate toxicity	Refer to Section 8
Secondary		
To characterize the Maximum tolerated dose (MTD) of AL001 in patients with mild to moderate Alzheimer's disease (AD) and healthy adult subjects.	• Prevalence of peak salicylate concentrations above 30 mg/dL • Prevalence of trough plasma lithium concentrations above 1.2 mEq/L for healthy adult subjects or 1.0 mEq/L for healthy elderly and Alzheimer's subjects.	Refer to Section 7
Exploratory		
To explore the difference in PK profile between the non-elderly vs elderly subjects (healthy subjects only).	• Descriptive statistics for PK will be presented by treatment group and compared between non-elderly and elderly subjects.	Refer to Section 7
For AD subject cohorts (Cohorts 1, 2b, 3b, 4b, and 5b), determination of qualitative and quantitative evaluations of AD subject desirable characteristics for future Phase 2 and 3 clinical studies in order to: • facilitate recruitment into subsequent AL001 clinical trials • facilitate trial-adherence to completion of study requirements including treatment adherence	• Demographic characteristics of all screened and successfully enrolled AD subjects will be documented to determine what AD subject characteristics are needed and appropriate for successful recruitment and adherence to treatment and study requirements in future Phase 2 and 3 clinical studies. Baseline Mini-Mental State Examination scores, FDA classifications (protocol APPENDIX 3) and demographic characteristics, for example, will be reviewed for relationship to AD subjects' ability to provide informed consent and complete study requirements.	Refer to Section 6

3 STUDY DESIGN

3.1 General Description

This is a Phase 1/2a, multi-center, double-blind, placebo-controlled, randomized, MAD clinical study to determine the safety and MTD of AL001 in subjects with mild to moderate AD (FDA Classification of Stage 2 to 4 AD [protocol APPENDIX 3]) and healthy adult subjects. Cohorts 1, 2b, 3b, 4b, and 5b will include AD subjects (between the ages of 50 and 80 years, inclusive) and Cohorts 2a, 3a, 4a, and 5a will include healthy subjects (non-elderly adults between the ages of 18 to 64 years, inclusive, and healthy elderly adults between the ages of 65 to 80 years, inclusive). In each of the 9 cohorts, subjects will be confined to the study site from Day -1 until Day 15.

The study consists of 3 phases.

1. Screening Phase – up to 28 days (from Days -28 to -1)
2. Treatment Phase – 16 days:
    - Subjects will be admitted to the study site on Day -1. Subjects will receive 14 days of 3 times daily (TID) dosing with assigned study medication from Days 1 to 14 and will remain confined until Day 15.
    - Each of the 9 cohorts will be initiated sequentially starting with Cohort 1 and progressing through to Cohort 5 depending on acceptable review of the safety data by a 3-member expert adjudication panel. Each of the Cohorts 2 to 5 will be sub-divided into 2 cohorts: Cohorts 2a, 3a, 4a, and 5a will include healthy subjects and Cohorts 2b, 3b, 4b, and 5b will include AD subjects. The results from the healthy subject cohorts ‘a’ will be reviewed by the independent safety adjudication committee and if assessed as safe, the committee will permit progression to the corresponding AD subject cohort ‘b’ at the same dose level, as well as progression to the next dose level of healthy subject cohort. The healthy subject cohorts can proceed to the next higher dose level without having to wait for the corresponding AD subject cohort to be completed and reviewed by the safety adjudication panel.
    - The initiation of the next dose cohort may be delayed up to approximately 4 weeks following the last dose of the last subject in the preceding cohort. This is necessary to allow time for the safety and PK data to be compiled and tabulated for review by the safety committee (laboratory reports, AEs, tolerability, and PK data from all [up to 8] completed subjects in each cohort), to determine if it is safe to proceed with enrollment of the subsequent cohort.
3. Follow-up Phase - 1 month
    - Subjects will be followed-up after discharge from the study site on Day 15, with a post-treatment clinic visit on Day 23 and a post-treatment telephone call on Day 42.

Each patient will participate in the study for up to approximately 2.5 months.

3.2 Treatments

Cohort 1 will include 8 AD subjects (6 active and 2 placebo). Each of the Cohorts 2 to 5 will be sub-divided into 2 cohorts: Cohorts 2a, 3a, 4a, and 5a will include healthy subjects and Cohorts 2b, 3b, 4b, and 5b will include AD subjects. For each cohort, subjects will be randomized to 1 of 2 treatment groups in a 6:2 ratio of AL001 (active study medication) to placebo. Subjects in each of the cohorts will be dosed TID for 14 days at the dose level indicated in Table 2 below or placebo

Active Group: Ascending doses of AL001 are manufactured in 210 mg 0 size capsules; the number of capsules for each cohort dosing will vary based on the AL001 dosing equivalent (refer to Table 2). Dosing will be TID at approximately 8:00 AM, 2:00 PM, and 8:00 PM (within ±10 minutes). Patients will be fasted for each dose (dosed at least 1 hour before or 4 hours after meals).

Placebo Group: The placebo capsules will be manufactured to appear exactly as the capsule used for AL001; the number of capsules dosed, number of doses, dosing schedule, and fasting conditions will be the same as for the active group.

Table 2: Ascending Doses of AL001 for Each Cohort

Cohort	Daily Dosing Level	No. of AL001 210 mg Capsules	AL001 Daily Dose Equivalent
Cohort 1	60% of 450 mg lithium carbonate	3 capsules TID	1890 mg × 14 days
Cohort 2a/ Cohort 2b	100% of 450 mg lithium carbonate	5 capsules TID	3150 mg × 14 days
Cohort 3a/ Cohort 3b	140% of 450 mg lithium carbonate	7 capsules TID	4410 mg × 14 days
Cohort 4a/ Cohort 4b	160% of 450 mg lithium carbonate	8 capsules TID	5040 mg × 14 days
Cohort 5a/ Cohort 5b	200% of 450 mg lithium carbonate	10 capsules TID	6300 mg × 14 days[a]

Abbreviation: TID = 3 times daily.
a. Lithium dose equivalent to that used for bipolar/affective disorders.

3.3 Study Procedures

For complete details on the study assessments to be performed, refer to APPENDIX A.

3.4 Randomization and Blinding Procedures

Subjects will be randomized by computer in a ratio of 6:2 (active:placebo) in each of the 9 cohorts. The randomization will be performed separately for AD subject cohorts and healthy subject cohorts. For the AD subject cohorts (Cohorts 1, 2b, 3b, 4b, and 5b) subjects will be randomized in a 6:2 ratio (active:placebo) and for the healthy subject cohorts (Cohorts 2a, 3a, 4a, and 5a), the randomization will be performed in such a way that age will be used as a stratification factor for each cohort: 3 non-elderly and 3 elderly subjects will receive AL001, and 1 non-elderly and 1 elderly subject will receive placebo.

The subjects will be assigned a Screening Number (upon signing the informed consent at the Screening Visit) and a Subject Randomization Number (upon meeting eligibility criteria on Day 1) sequentially by the Investigator or authorized study staff. The Subject Randomization Numbers will start with 1001 for Cohort 1, 2001 for Cohort 2, etc. Prior to dosing on Day 1, the subject is assigned the next sequential randomization number for that cohort. Once a Subject Randomization Number is assigned to a subject, it will not be reused. The subject ID number will be the combination of the Screening Number and the Randomization Number.

A key to the randomization will be kept secured by the Sponsor's Medical Monitor or other Sponsor representative not involved in this study, in a sealed envelope under secure conditions at the study site for emergency use. In the occurrence of an SAE, or other safety issue which requires knowing the patient's treatment group, the Medical Monitor will unblind that patient.

3.5 Sample size

A maximum of 5 cohorts of 8 AD subjects each and 4 cohorts of 8 healthy subjects each will be enrolled in this clinical trial for a maximum total of 72 subjects. Each cohort will correspond to one of the 5 given dose level of AL001 and will be comprised of 6 subjects receiving AL001 and 2 subjects receiving placebo. This sample size is not based on any statistical considerations, but is judged adequate to meet the primary objective of this clinical trial.

4 ANALYSIS POPULATIONS

The following analysis sets will be defined:

- **Screened Analysis Set*:*** The Screened Analysis Set will include all the subjects who have signed the inform consent form.
- **Randomized Analysis Set:** The Randomized Analysis Set will include all subjects who are randomized within one of the cohorts.
- **Safety Analysis Set:** The Safety Analysis Set will include all subjects in the Randomized Analysis Set who receive any dose of study drug. This analysis set will be utilized for all safety analyses.
- **Pharmacokinetic Analysis Set***:* The PK Analysis Set will include all subjects in the Safety Analysis Set who receive AL001 and have either a Cmax measurement for salicylate or a trough measurement for lithium. This analysis set will be utilized for all PK analyses.

5 STUDY SUBJECTS

Disposition data, analysis population information, and protocol deviations will be listed and summarized as described in Table 3.

Table 3: Data Presentations for Study Subject Information

Data	Variables	Presentation
Disposition and analysis population	Subject, completion status (i.e., completed or withdrawn), reason for withdrawal, analysis set determination	Listings: • Randomization • Disposition • Analysis set Table: • Disposition
Protocol deviations	Protocol deviations	Listings: • Protocol deviations

5.1 Disposition

Subject disposition will be summarized for all subjects randomized. The following will be presented:

- Number of subjects (N) screened
- N of subjects randomized
- N and % of subjects who completed the study
- N and % of subjects who withdrew from the study by primary reason for withdrawal
- N and % of subjects included in each analysis set

The percentages will be calculated using the number of randomized subjects as the denominator. The summary will be presented by treatment (dose level of AL001, pooled placebo and overall) for AD cohorts and Healthy cohorts.

5.2 Protocol Deviations

Deviations identified at the site will be collected in the clinic deviation tracking system (DTS) and presented in a protocol deviation listing. Protocol deviations will be reviewed by the Sponsor, principal investigator, and lead statistician and finalized before unblinding the study. Protocol deviations will be listed for the safety population.

6 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Unless otherwise specified, all available data will be listed, summarized, for demographics and other baseline characteristics, and presented for all study population as detailed in Table 4. The screened analysis set will be used for exploratory analysis.

Table 4: Data Presentations for Demographic and Other Baseline Characteristics

Data	Variables	Presentation
Demographic and Other Baseline Characteristics	Sex, age, ethnicity, race, height, weight and body mass index (BMI), Mini-Mental State Examination (MMSE) scores, and AD stage FDA classification for AD subjects only	Listing Table – presented for all study populations (Screened, Randomized, Safety and PK).
Medical history	All medical history findings	Listing
Prior and Concomitant medications	All medications taken prior to study drug administration and concomitant medications	Listing

7 PHARMACOKINETIC ANALYSES

7.1 Pharmacokinetic Statistical Methodology

All PK tables, figures and listings (TFLs), when appropriate, will be stratified by treatment and cohort for each study part.

7.2 Summary Statistics

In order to characterize the MTD of AL001 in subjects with AD and healthy subjects, descriptive statistics will be presented by treatment group for each cohort and overall for the following:

- Proportion of healthy non-elderly subjects with trough lithium concentrations > 1.2 mEq/L
- Proportion of subjects with peak (Cmax) salicylate concentrations > 30 mg/dL
- Proportion of healthy elderly and, separately, Alzheimer's subjects with plasma trough measurements of lithium > 1.0 mEq/L

Summary statistics of the plasma concentration data will be calculated for the PK population, unless otherwise indicated. Summary statistics will be calculated for concentration at each individual time point.

Concentration data will be summarized by treatment group and compared between non-elderly and elderly subjects only for healthy cohorts using the following statistics: number of observations (N), arithmetic mean (mean), standard deviation (SD), geometric mean, minimum (min), median, maximum (max), and coefficient of variation (CV%).

7.3 Statistical Analysis

In order to assess whether PK steady state has been approximately achieved, descriptive statistics for trough (Cmin) measurements of plasma lithium and Cmax (peak) measurements of plasma salicylate will be presented for all available days by cohort and overall. In addition, these measurements for plasma lithium and salicylate on Days 13 and 14 for each cohort will be $\log^e$-transformed and a 90% CI for the difference in the measurements between Days 13 and 14 for lithium and salicylate will be calculated on the $\log^e$ scale and then transformed back to the original scale for each cohort. If the 90% CI on the original scale is contained within the interval 85.0% to 117.6%, then an approximation of steady state will be concluded for the respective analyte and cohort. Alternative approaches to estimate achievement of steady-state conditions are to be used, as appropriate, such as when at least 3 trough concentrations for plasma lithium and/or at least 3 Cmax concentrations for plasma salicylate can be assessed using linear regression. All cohorts except Cohort 1 are expected to complete at least 3 lithium trough and 3 salicylate peak concentration measurements and should be candidates for this statistical approach.

8 SAFETY ANALYSIS

Unless otherwise specified, all available data will be listed and summary tables for safety assessments will be presented by treatment (dose level of AL001 and pooled placebo) per cohort for the safety population as detailed in Table 5 and all applicable safety data will be presented by combined active and pooled placebo groups, such as, disposition, demographic, adverse events, labs, vital signs, and 12-ECG data.

Continuous variables will be summarized (absolute values and change from baseline) using descriptive statistics (n, mean, SD, minimum, median and maximum).

Table 5: Data Presentations for Safety Assessments

Data	Variables	Presentation
Adverse events	Adverse event (AE) description, date and time (start and end), intensity, relationship to study drug, action taken, study days and outcome	Listing Table
Extent of exposure	Study drug administration dose, units, date, time, cohort	Listing
Clinical laboratory evaluations	Laboratory results (refer to section 8.3 for parameters)	Listing Table
Vital signs	Blood pressure, pulse, and body temperature	Listing Table
Physical examinations	Physical examinations findings	Listing
12-Lead ECGs	ECG interpretations and findings	Listing Table

8.1 Adverse Events

Treatment-emergent adverse events (TEAEs) are AEs that are not present prior to the exposure to study treatment. All AEs reported following exposure to study treatment are considered TEAEs. All TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 25.0. The listing and summaries will include coded System Organ Class (SOC) and Preferred Term (PT).

An overall summary by treatment (dose level of AL001 and pooled placebo and overall) will present the number and percentage of subjects with:

- At least one TEAE
- At least one drug-related TEAE
- Severity intensity

  - Mild
  - Moderate
  - Severe
- Life-threatening
- Death
- at least one SAE
- at least one drug-related SAE
- death

For the study, the following AE tables will also be presented:

- TEAEs by SOC and PT
- SAEs by SOC and PT
- Drug-related TEAEs by SOC and PT
- TEAEs by SOC, PT, and maximum intensity
- TEAEs that lead to premature discontinuation

Events will be listed by treatment, subject and AE onset date. Treatment-emergent AE duration (stop date/time - start date/time) will be included in the listing.

8.2 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESI) for this study is upper gastrointestinal upset (lower gastrointestinal; diarrhea). The number and percentage of subjects with an AESI, as well as the total number of AESIs, will be summarized for each cohort and overall by SOC and PT.

8.3 Clinical Laboratory Evaluations

Laboratory data will be presented using units as reported by the clinical laboratory. Specific hematology, chemistry, and urinalysis parameters are listed in Table 6.

Table 6: Clinical Laboratory Evaluations

Hematology	Serum chemistry
Complete blood count including: Red blood cells (RBCs), hemoglobin, hematocrit, reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count White blood cell (WBC) count with differential including (absolute and percentage): neutrophils, lymphocytes, monocytes, eosinophils, basophils, band neutrophils HbA1c[a]	Albumin Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Alkaline phosphatase (ALP) Blood Urea Nitrogen (BUN) or Urea Bicarbonate Creatinine Electrolytes (Na, K, Cl, Ca, P) Glucose (fasting) Lactate dehydrogenase (LDH) Total bilirubin Direct bilirubin Total cholesterol, High-density lipoprotein (HDL)[c] Low-density lipoprotein (LDL)[c] Triglycerides[c] TSH[a] Mg

	Vitamin B12[a]
Urinalysis	
Urobilinogen Glucose Ketones Bilirubin Protein Nitrate Leukocytes Blood pH Specific gravity	

Abbreviations: TSH = thyroid-stimulating hormone.

a. Performed at Screening and Day 23 or early termination only.
b. Urobilinogen may be measured via a urine dipstick test at the site if their local laboratory does not include it in their urinalysis panel.
c. Lipid panel (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides) measured at the Screening Visit does not need to be repeated on Day -1.

Absolute values for continuous parameters (hematology, general biochemistry, and urinalysis) will be summarized using descriptive statistics by each cohort (dose level of AL001 and pooled placebo; overall) and change from baseline will be presented.

Number (Percentage) of Subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values will be presented for each laboratory category by each cohort (dose level of AL001 and pooled placebo, overall) and visit.

Serology, alcohol/drug screen, pregnancy test results will be presented in separate listings.

Data listings will include out-of-range flags (Low, High) and whether the abnormal values are clinically significant or not.

8.4 Vital Signs

Vital signs will include heart rate, blood pressure, and body temperature.

Absolute values in vital signs measurements will be summarized descriptively by each cohort (dose level of AL001 and pooled placebo; overall), time point and change from baseline will be presented.

Number (%) of subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values of vital signs will be presented by each cohort (dose level of AL001 and pooled placebo, overall), visit and time point.

Data listing will identify if values are “abnormal, clinically significant” or “abnormal, not clinically significant”. Vital sign results will be presented.

8.5 Physical Examination Findings

The physical examination will include a general review of the following body systems (at minimum): general appearance, head and neck, cardiovascular, respiratory, abdomen, and brief neurological examination.

Physical examination results will be listed as normal or abnormal. Abnormal findings of clinical significance will be included in the listing.

8.6 12-lead Electrocardiogram

12- Lead safety ECG results will be summarized descriptively by each cohort ((dose level of AL001 and pooled placebo, overall), time point and change from baseline will be presented.

Number (%) of subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values of ECG will be presented by each cohort (dose level of AL001 and pooled placebo, overall), visit and time point.

Data listing will identify if values are “abnormal, clinically significant” or “abnormal, not clinically significant”.

9 DATA HANDLING AND PRESENTATION

All safety and statistical outputs will be generated using SAS software, version 9.4.
All programs used to generate statistical analyses will be validated according to Altasciences's standard operating procedure (SOPs).
Tables, figures and listings will be provided in RTF formats.

9.1 Safety Analysis Presentation

Adverse events and medical history will be classified using the MedDRA terminology as defined in the study data management plan (DMP).

Prior and concomitant medications will be coded with the World Health Organization drug dictionary (WHO-DD) (March 2022 B3) as defined in the study DMP.

Generally, summaries will be presented by cohort (dose level of AL001 and pooled placebo, overall). Some summaries will also be presented to include overall (i.e., subject disposition and demographics) Also, applicable safety data will be presented by combined active and pooled placebo groups, such as, disposition, demographic, adverse events, labs, vital signs, and 12-ECG data.

Summaries for data collected at scheduled times specified in the protocol will be presented by cohort (dose level of AL001 and pooled placebo, overall), visit, and, where applicable, time point. Continuous variables will be summarized using the number of observations, mean, standard deviation, minimum, median, and maximum. Categorical variables will be summarized using the number of observations and the corresponding percentage.

In general, the data listings will include data from all subjects who receive at least one dose of either AL001 or placebo.

Study days will be included in adverse event and concomitant medication listings in addition to dates. Study days will be calculated relative to the first day of double-blind treatment (Day 1) derived as (event date - first day of AL001 or placebo dosing) +1 for events after the first day of dosing and (event date - first day of AL001 or placebo dosing) for events before the first day of dosing.
The following general comments also apply to all statistical analyses and data presentations:

- Duration variables will be calculated using the general formula: (end date - start date) +1.
- If the reported value of a clinical laboratory parameter cannot be used in a statistical summary table (e.g., a character string is reported for a parameter of the numerical type), a coded value must be appropriately determined and used in the statistical analyses. In general, a value or lower and upper limit of normal range such as '<10' or '≤5' will be treated as '10' or '5' respectively, and a value such as '>100' will be treated as '100'. However, the actual values as reported in the database will be presented in data listings.
- When assessments are repeated for a given time point or performed at unscheduled times, only the result which is the closest to the dosing time will be included in summary tables.

In general, summary statistics for raw variables (i.e., variables measured at the study site or central laboratory) will be displayed as follows:

- Minima and maxima will be displayed to the same number of decimal places as the raw data.
- Means, medians, and quartiles will be displayed to 1 additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.
- Percentages will be displayed to 1 decimal place. Percentages between 0 and 0.1 (exclusive) will be displayed as '<0.1'.
- P-values will be displayed to 3 decimal places. P-values that are less than 0.001 will be displayed as '<0.001' if applicable.

The number of decimal places for summary statistics of derived variables (i.e., variables that are not measured by the study site but are calculated for analysis based on other measured variables) will be determined on a case by case basis. In general:

- Minima and maxima will be displayed to the commonly used unit of precision for the parameter.
- Means, medians, quartiles, and confidence limits will be displayed to 1 additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.

9.2 Baseline

The baseline is defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the first dose of study drug in each cohort.

10 INTERIM ANALYSES AND DATA SAFETY MONITORING

There is no interim analysis plan for this study.

11 GENERAL INFORMATION RELATED TO DATA PRESENTATIONS

The formats and layouts of TFLs are provided in a separate document and are common displays. Their numbering and general content follow the International Conference on Harmonisation (ICH) E3 guidelines. Actual formats and layouts may be altered slightly from those presented as necessary to accommodate actual data or statistics.

Minor format changes will not require updates to the SAP; rather they may be documented in a Note to SAP.

APPENDIX A STUDY SCHEDULE(S)

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Informed Consent	X							
Admission[1]		X						
Clinic Confinement			X	X				
Discharge					X			
Medical and Surgical History	X							
Physical Examination	X				X	X		X
Demographic information	X							
Body Height/Body Weight	X							
Vital Signs[2]	X	X	X	X	X	X		X
12-lead ECG[3]	X		X	X	X	X		X

[1] Subjects will be admitted to the clinical research unit at least 16 hours prior to the first study drug administration.
[2] Vital signs will include blood pressure, heart rate and oral body temperature. On Days 1 to 14, vital signs will be measured within 1 hour prior todosing using the same arm, if possible, after 5 minutes in seated position.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Safety Laboratory Tests[4]	X	X		X	X	X		X
Serology (HIV/HBV/HCV)	X							
Serum Pregnancy Test (females of childbearing potential only)	X							
Urine Pregnancy Test (females of childbearing potential only)		X						
COVID-19 Test[5]	X	X						
Urine Drug Screen and Alcohol Breath or Urine Test	X	X						
Inclusion/Exclusion Criteria	X	X						
Randomization			X					
Fasted Study Drug			X	X				

[3] On Day 1, ECGs will be conducted pre-dose and 1 hour (±10 minutes) after the third dose of Day 1. On Days 7, 13, and 14, ECGs will be performed prior to first daily dose.

[4] Safety laboratory tests (chemistry, hematology, and urinalysis) will be performed on Day -1, Day 7 (pre-dose), Day 15, and Day 23. For all subjects, total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides will not be included in the safety laboratory tests performed on Day -1. For subjects who arescreened within 5 days of check-in (on Day -6 to Day -2), the safety laboratory tests will not be performed on Day -1.

[5] The COVID-19 test should be repeated upon check-in on Day -1 using a COVID rapid antigen test kit or PCR. Additionally,, study site and local health authoritypolicies and recommendations can be applied for compliance. If the initial result is positive at the Screening Visit, the test may be repeated after 14 days or on Day -1 whichever comes first.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Administration (all doses)								
Baseline and Trough plasma lithium and peak plasma salicylate concentration measurements[6]			X	X				X
MMSE Score (AD subjects only)	X							
Hachinski Ischemic Scale Score[7] (AD subjects only)	X							
Meals[8]		X	X	X	X			

[6] Blood samples for baseline plasma lithium and plasma salicylate concentration measurements will be collected before first dose on Day 1. Blood samples for trough plasma lithium and peak plasma salicylate concentration measurements will be collected on Days 3, 5, 7, 10, 13, and 14, and at the discretion of the Investigator (the latter are 'Safety Samples' for Therapeutic Drug Monitoring), particularly to assess the relationship to possible lithium or salicylate related AEs. These safety samples could be sent for expedited analysis to either a local laboratory with an aliquot saved for the bioanalytical laboratory, or to the bioanalytical laboratory directly (Sannova or Altasciences). In addition, for AD subject Cohorts 2b, 3b, 4b, and 5b, the trough lithium sample on Day 5 will be treated as a 'Safety Sample' for Therapeutic Drug Monitoring to assess any lithium-related PK exposure safety concerns at an early treatment time point which may require dosing to be discontinued for an individual subject. This sample will be sent to Sannova for expedited analysis. Similarity between Days 13 and 14 trough lithium plasma levels and peak salicylate plasma levels will be used to determine the status of lithium and salicylate steady-state PK conditions, and/or by another appropriate method, such as linear regression. Blood collection for trough lithium plasma concentrations will occur just prior to the first daily dose, while blood collection for peak plasma salicylate concentrations will occur at 1.25 hours post third daily dose (the estimated time to a maximum blood concentration C_{max}). For early termination, blood samples for lithium and salicylate plasma concentrations will be collected. A ±10-minute window will be allowed for collection of PK samples for both lithium and salicylate.

[7] Hachinski Ischemic Scale Score is presented in protocol APPENDIX 5.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Adverse Event Reporting/Safety-Tolerability Assessment	X	X	X	X	X	X	X	X
Prior Medications	X	X						
Concomitant Medications	X	X	X	X	X	X	X	X
Health Status Questionnaire (SF-36)						X	X	X

Abbreviations: AD = Alzheimer's disease; COVID-19 = Coronavirus Disease 2019; ECG = electrocardiogram; ET = Early Termination; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; MMSE = Mini-Mental State Exam; PK = pharmacokinetic(s); TSH = thyroid-stimulating hormone

[8] Meals during confinement should include breakfast (at least 1 hour after first dose), lunch (at least 1 hour after second dose), dinner (at least 1 hour after third dose), and a snack (if time permits and 10-hour fast is respected). On Day 15, breakfast will be provided prior to subject/caregiver being discharged from the study site.

STATISTICAL ANALYSIS PLAN

For:

Alzamend Neuro, Inc.

PROTOCOL No. AL001-ALZ02

A Multiple-Dose, Steady-State, Double-Blind, Ascending Dose Safety, Tolerability, Pharmacokinetic Study Of AL001 In Patients With Mild To Moderate Alzheimer's Disease And Healthy Adult Subjects ("MAD Study")

Altasciences Project No. ALZ-P9-319

Prepared by:

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Version: *Final 1.0*

Date: 2023-02-22

STATISTICAL ANALYSIS PLAN APPROVAL

We have carefully read this statistical analysis plan and agree it contains the necessary information required to handle the statistical analysis of study data.

Riddhi Thakkar

Digitally signed by Riddhi Thakkar
Reason: I am the author of this document
Date: 2023.02.22 10:19:22 -05'00'

Riddhi Thakkar — Date

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On behalf of the Sponsor:

Eve del Rio

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Eve del Rio, MD, PhD — Date

Senior Clinical & Regulatory Consultant

VERSION CONTROL

Version	Date	Author	Description of Changes
1.0	*2023/02/22*	Riddhi Thakkar	Not applicable

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ABBREVIATIONS

AD	Alzheimer's disease
AE	Adverse event
CI	Confidence interval
COVID-19	Coronavirus sars-cov2
CRO	Clinical research organization
CV	Coefficient of variation
DMP	Data management plan
ECG	Electrocardiogram
EDC	Electronic data capture
ET	Early termination
FDA	Food and drug administration
ICF	Informed consent form
ICH	International council for harmonisation
IEC	Independent ethics committee
IMP	Investigational medicinal product
IP	Investigational product
MAD	Multiple ascending dose
MMSE	Mini-Mental State Examination
MTD	Maximum tolerated dose
PK	Pharmacokinetic
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System organ class
SOP	Standard operating system
TEAE	Treatment-emergent adverse event
TID	3 times daily
WHO-DD	World Health Organization drug dictionary

1 INTRODUCTION

This statistical analysis plan (SAP) provides a detailed description of the statistical methods and procedures to be implemented for the analyses of data from protocol AL001-ALZ02.The analyses described in the SAP are based upon the final protocol version 8.0 (Amendment 06) dated 2022/11/28.

2 STUDY OBJECTIVES

The objectives of the study and corresponding study endpoints are detailed in Table 1.

Table 1: Objectives and Related Endpoints

Objective	Endpoint	Analysis
Primary		
To evaluate the safety and tolerability of AL001 under multiple-dose, steady-state conditions in Alzheimer's subjects and healthy adult subjects.	• Vital signs (blood pressure, heart rate, and oral body temperature) • 12-lead electrocardiogram • Physical examination • Safety laboratory tests (hematology, chemistry [including thyroid-stimulating hormone (TSH), vitamin B12, and Mg], HbA1c, and urinalysis • Reported and observed Adverse events (AEs)/serious AEs)/tolerability observations including signs and/or symptoms of lithium and/or salicylate toxicity	Refer to Section 8
Secondary		
To characterize the Maximum tolerated dose (MTD) of AL001 in patients with mild to moderate Alzheimer's disease (AD) and healthy adult subjects.	• Prevalence of peak salicylate concentrations above 30 mg/dL • Prevalence of trough plasma lithium concentrations above 1.2 mEq/L for healthy adult subjects or 1.0 mEq/L for healthy elderly and Alzheimer's subjects.	Refer to Section 7
Exploratory		
To explore the difference in PK profile between the non-elderly vs elderly subjects (healthy subjects only).	• Descriptive statistics for PK will be presented by treatment group and compared between non-elderly and elderly subjects.	Refer to Section 7
For AD subject cohorts (Cohorts 1, 2b, 3b, 4b, and 5b), determination of qualitative and quantitative evaluations of AD subject desirable characteristics for future Phase 2 and 3 clinical studies in order to: • facilitate recruitment into subsequent AL001 clinical trials • facilitate trial-adherence to completion of study requirements including treatment adherence	• Demographic characteristics of all screened and successfully enrolled AD subjects will be documented to determine what AD subject characteristics are needed and appropriate for successful recruitment and adherence to treatment and study requirements in future Phase 2 and 3 clinical studies. Baseline Mini-Mental State Examination scores, FDA classifications (protocol APPENDIX 3) and demographic characteristics, for example, will be reviewed for relationship to AD subjects' ability to provide informed consent and complete study requirements.	Refer to Section 6

3 STUDY DESIGN

3.1 General Description

This is a Phase 1/2a, multi-center, double-blind, placebo-controlled, randomized, MAD clinical study to determine the safety and MTD of AL001 in subjects with mild to moderate AD (FDA Classification of Stage 2 to 4 AD [protocol APPENDIX 3]) and healthy adult subjects. Cohorts 1, 2b, 3b, 4b, and 5b will include AD subjects (between the ages of 50 and 80 years, inclusive) and Cohorts 2a, 3a, 4a, and 5a will include healthy subjects (non-elderly adults between the ages of 18 to 64 years, inclusive, and healthy elderly adults between the ages of 65 to 80 years, inclusive). In each of the 9 cohorts, subjects will be confined to the study site from Day -1 until Day 15.

The study consists of 3 phases.

1. Screening Phase – up to 28 days (from Days -28 to -1)
2. Treatment Phase – 16 days:
    - Subjects will be admitted to the study site on Day -1. Subjects will receive 14 days of 3 times daily (TID) dosing with assigned study medication from Days 1 to 14 and will remain confined until Day 15.
    - Each of the 9 cohorts will be initiated sequentially starting with Cohort 1 and progressing through to Cohort 5 depending on acceptable review of the safety data by a 3-member expert adjudication panel. Each of the Cohorts 2 to 5 will be sub-divided into 2 cohorts: Cohorts 2a, 3a, 4a, and 5a will include healthy subjects and Cohorts 2b, 3b, 4b, and 5b will include AD subjects. The results from the healthy subject cohorts ‘a’ will be reviewed by the independent safety adjudication committee and if assessed as safe, the committee will permit progression to the corresponding AD subject cohort ‘b’ at the same dose level, as well as progression to the next dose level of healthy subject cohort. The healthy subject cohorts can proceed to the next higher dose level without having to wait for the corresponding AD subject cohort to be completed and reviewed by the safety adjudication panel.
    - The initiation of the next dose cohort may be delayed up to approximately 4 weeks following the last dose of the last subject in the preceding cohort. This is necessary to allow time for the safety and PK data to be compiled and tabulated for review by the safety committee (laboratory reports, AEs, tolerability, and PK data from all [up to 8] completed subjects in each cohort), to determine if it is safe to proceed with enrollment of the subsequent cohort.
3. Follow-up Phase - 1 month
    - Subjects will be followed-up after discharge from the study site on Day 15, with a post-treatment clinic visit on Day 23 and a post-treatment telephone call on Day 42.

Each patient will participate in the study for up to approximately 2.5 months.

3.2 Treatments

Cohort 1 will include 8 AD subjects (6 active and 2 placebo). Each of the Cohorts 2 to 5 will be sub-divided into 2 cohorts: Cohorts 2a, 3a, 4a, and 5a will include healthy subjects and Cohorts 2b, 3b, 4b, and 5b will include AD subjects. For each cohort, subjects will be randomized to 1 of 2 treatment groups in a 6:2 ratio of AL001 (active study medication) to placebo. Subjects in each of the cohorts will be dosed TID for 14 days at the dose level indicated in Table 2 below or placebo

Active Group: Ascending doses of AL001 are manufactured in 210 mg 0 size capsules; the number of capsules for each cohort dosing will vary based on the AL001 dosing equivalent (refer to Table 2). Dosing will be TID at approximately 8:00 AM, 2:00 PM, and 8:00 PM (within ±10 minutes). Patients will be fasted for each dose (dosed at least 1 hour before or 4 hours after meals).

Placebo Group: The placebo capsules will be manufactured to appear exactly as the capsule used for AL001; the number of capsules dosed, number of doses, dosing schedule, and fasting conditions will be the same as for the active group.

Table 2: Ascending Doses of AL001 for Each Cohort

Cohort	Daily Dosing Level	No. of AL001 210 mg Capsules	AL001 Daily Dose Equivalent
Cohort 1	60% of 450 mg lithium carbonate	3 capsules TID	1890 mg × 14 days
Cohort 2a/ Cohort 2b	100% of 450 mg lithium carbonate	5 capsules TID	3150 mg × 14 days
Cohort 3a/ Cohort 3b	140% of 450 mg lithium carbonate	7 capsules TID	4410 mg × 14 days
Cohort 4a/ Cohort 4b	160% of 450 mg lithium carbonate	8 capsules TID	5040 mg × 14 days
Cohort 5a/ Cohort 5b	200% of 450 mg lithium carbonate	10 capsules TID	6300 mg × 14 days[a]

Abbreviation: TID = 3 times daily.
a. Lithium dose equivalent to that used for bipolar/affective disorders.

3.3 Study Procedures

For complete details on the study assessments to be performed, refer to APPENDIX A.

3.4 Randomization and Blinding Procedures

Subjects will be randomized by computer in a ratio of 6:2 (active:placebo) in each of the 9 cohorts. The randomization will be performed separately for AD subject cohorts and healthy subject cohorts. For the AD subject cohorts (Cohorts 1, 2b, 3b, 4b, and 5b) subjects will be randomized in a 6:2 ratio (active:placebo) and for the healthy subject cohorts (Cohorts 2a, 3a, 4a, and 5a), the randomization will be performed in such a way that age will be used as a stratification factor for each cohort: 3 non-elderly and 3 elderly subjects will receive AL001, and 1 non-elderly and 1 elderly subject will receive placebo.

The subjects will be assigned a Screening Number (upon signing the informed consent at the Screening Visit) and a Subject Randomization Number (upon meeting eligibility criteria on Day 1) sequentially by the Investigator or authorized study staff. The Subject Randomization Numbers will start with 1001 for Cohort 1, 2001 for Cohort 2, etc. Prior to dosing on Day 1, the subject is assigned the next sequential randomization number for that cohort. Once a Subject Randomization Number is assigned to a subject, it will not be reused. The subject ID number will be the combination of the Screening Number and the Randomization Number.

A key to the randomization will be kept secured by the Sponsor's Medical Monitor or other Sponsor representative not involved in this study, in a sealed envelope under secure conditions at the study site for emergency use. In the occurrence of an SAE, or other safety issue which requires knowing the patient's treatment group, the Medical Monitor will unblind that patient.

3.5 Sample size

A maximum of 5 cohorts of 8 AD subjects each and 4 cohorts of 8 healthy subjects each will be enrolled in this clinical trial for a maximum total of 72 subjects. Each cohort will correspond to one of the 5 given dose level of AL001 and will be comprised of 6 subjects receiving AL001 and 2 subjects receiving placebo. This sample size is not based on any statistical considerations, but is judged adequate to meet the primary objective of this clinical trial.

4 ANALYSIS POPULATIONS

The following analysis sets will be defined:

- **Screened Analysis Set*:*** The Screened Analysis Set will include all the subjects who have signed the inform consent form.
- **Randomized Analysis Set:** The Randomized Analysis Set will include all subjects who are randomized within one of the cohorts.
- **Safety Analysis Set:** The Safety Analysis Set will include all subjects in the Randomized Analysis Set who receive any dose of study drug. This analysis set will be utilized for all safety analyses.
- **Pharmacokinetic Analysis Set***:* The PK Analysis Set will include all subjects in the Safety Analysis Set who receive AL001 and have either a Cmax measurement for salicylate or a trough measurement for lithium. This analysis set will be utilized for all PK analyses.

5 STUDY SUBJECTS

Disposition data, analysis population information, and protocol deviations will be listed and summarized as described in Table 3.

Table 3: Data Presentations for Study Subject Information

Data	Variables	Presentation
Disposition and analysis population	Subject, completion status (i.e., completed or withdrawn), reason for withdrawal, analysis set determination	Listings: • Randomization • Disposition • Analysis set Table: • Disposition
Protocol deviations	Protocol deviations	Listings: • Protocol deviations

5.1 Disposition

Subject disposition will be summarized for all subjects randomized. The following will be presented:

- Number of subjects (N) screened
- N of subjects randomized
- N and % of subjects who completed the study
- N and % of subjects who withdrew from the study by primary reason for withdrawal
- N and % of subjects included in each analysis set

The percentages will be calculated using the number of randomized subjects as the denominator. The summary will be presented by treatment (dose level of AL001, pooled placebo and overall) for AD cohorts and Healthy cohorts.

5.2 Protocol Deviations

Deviations identified at the site will be collected in the clinic deviation tracking system (DTS) and presented in a protocol deviation listing. Protocol deviations will be reviewed by the Sponsor, principal investigator, and lead statistician and finalized before unblinding the study. Protocol deviations will be listed for the safety population.

6 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Unless otherwise specified, all available data will be listed, summarized, for demographics and other baseline characteristics, and presented for all study population as detailed in Table 4. The screened analysis set will be used for exploratory analysis.

Table 4: Data Presentations for Demographic and Other Baseline Characteristics

Data	Variables	Presentation
Demographic and Other Baseline Characteristics	Sex, age, ethnicity, race, height, weight and body mass index (BMI), Mini-Mental State Examination (MMSE) scores, and AD stage FDA classification for AD subjects only	Listing Table – presented for all study populations (Screened, Randomized, Safety and PK).
Medical history	All medical history findings	Listing
Prior and Concomitant medications	All medications taken prior to study drug administration and concomitant medications	Listing

7 PHARMACOKINETIC ANALYSES

7.1 Pharmacokinetic Statistical Methodology

All PK tables, figures and listings (TFLs), when appropriate, will be stratified by treatment and cohort for each study part.

7.2 Summary Statistics

In order to characterize the MTD of AL001 in subjects with AD and healthy subjects, descriptive statistics will be presented by treatment group for each cohort and overall for the following:

- Proportion of healthy non-elderly subjects with trough lithium concentrations > 1.2 mEq/L
- Proportion of subjects with peak (Cmax) salicylate concentrations > 30 mg/dL
- Proportion of healthy elderly and, separately, Alzheimer's subjects with plasma trough measurements of lithium > 1.0 mEq/L

Summary statistics of the plasma concentration data will be calculated for the PK population, unless otherwise indicated. Summary statistics will be calculated for concentration at each individual time point.

Concentration data will be summarized by treatment group and compared between non-elderly and elderly subjects only for healthy cohorts using the following statistics: number of observations (N), arithmetic mean (mean), standard deviation (SD), geometric mean, minimum (min), median, maximum (max), and coefficient of variation (CV%).

7.3 Statistical Analysis

In order to assess whether PK steady state has been approximately achieved, descriptive statistics for trough (Cmin) measurements of plasma lithium and Cmax (peak) measurements of plasma salicylate will be presented for all available days by cohort and overall. In addition, these measurements for plasma lithium and salicylate on Days 13 and 14 for each cohort will be $\log^e$-transformed and a 90% CI for the difference in the measurements between Days 13 and 14 for lithium and salicylate will be calculated on the $\log^e$ scale and then transformed back to the original scale for each cohort. If the 90% CI on the original scale is contained within the interval 85.0% to 117.6%, then an approximation of steady state will be concluded for the respective analyte and cohort. Alternative approaches to estimate achievement of steady-state conditions are to be used, as appropriate, such as when at least 3 trough concentrations for plasma lithium and/or at least 3 Cmax concentrations for plasma salicylate can be assessed using linear regression. All cohorts except Cohort 1 are expected to complete at least 3 lithium trough and 3 salicylate peak concentration measurements and should be candidates for this statistical approach.

8 SAFETY ANALYSIS

Unless otherwise specified, all available data will be listed and summary tables for safety assessments will be presented by treatment (dose level of AL001 and pooled placebo) per cohort for the safety population as detailed in Table 5.

Continuous variables will be summarized (absolute values and change from baseline) using descriptive statistics (n, mean, SD, minimum, median and maximum).

Table 5: Data Presentations for Safety Assessments

Data	Variables	Presentation
Adverse events	Adverse event (AE) description, date and time (start and end), intensity, relationship to study drug, action taken, study days and outcome	Listing Table
Extent of exposure	Study drug administration dose, units, date, time, cohort	Listing
Clinical laboratory evaluations	Laboratory results (refer to section 8.3 for parameters)	Listing Table
Vital signs	Blood pressure, pulse, and body temperature	Listing Table
Physical examinations	Physical examinations findings	Listing
12-Lead ECGs	ECG interpretations and findings	Listing Table

8.1 Adverse Events

Treatment-emergent adverse events (TEAEs) are AEs that are not present prior to the exposure to study treatment. All AEs reported following exposure to study treatment are considered TEAEs. All TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 25.0. The listing and summaries will include coded System Organ Class (SOC) and Preferred Term (PT).
An overall summary by treatment (dose level of AL001 and pooled placebo and overall) will present the number and percentage of subjects with:

- At least one TEAE
- At least one drug-related TEAE
- Severity intensity
  - Mild
  - Moderate

- Severe
- Life-threatening
- Death

- at least one SAE
- at least one drug-related SAE
- death

For the study, the following AE tables will also be presented:

- TEAEs by SOC and PT
- SAEs by SOC and PT
- Drug-related TEAEs by SOC and PT
- TEAEs by SOC, PT, and maximum intensity
- TEAEs that lead to premature discontinuation

Events will be listed by treatment, subject and AE onset date. Treatment-emergent AE duration (stop date/time - start date/time) will be included in the listing.

8.2 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESI) for this study is upper gastrointestinal upset (lower gastrointestinal; diarrhea). The number and percentage of subjects with an AESI, as well as the total number of AESIs, will be summarized for each cohort and overall by SOC and PT.

8.3 Clinical Laboratory Evaluations

Laboratory data will be presented using units as reported by the clinical laboratory. Specific hematology, chemistry, and urinalysis parameters are listed in Table 6.

Table 6: Clinical Laboratory Evaluations

Hematology	**Serum chemistry**
Complete blood count including: Red blood cells (RBCs), hemoglobin, hematocrit, reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count White blood cell (WBC) count with differential including (absolute and percentage): neutrophils, lymphocytes, monocytes, eosinophils, basophils, band neutrophils HbA1c[a]	Albumin Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Alkaline phosphatase (ALP) Blood Urea Nitrogen (BUN) or Urea Bicarbonate Creatinine Electrolytes (Na, K, Cl, Ca, P) Glucose (fasting) Lactate dehydrogenase (LDH) Total bilirubin Direct bilirubin Total cholesterol, High-density lipoprotein (HDL)[c] Low-density lipoprotein (LDL)[c] Triglycerides[c] TSH[a] Mg Vitamin B12[a]
Urinalysis	

Urobilinogen
Glucose
Ketones
Bilirubin
Protein
Nitrate
Leukocytes
Blood
pH
Specific gravity

Abbreviations: TSH = thyroid-stimulating hormone.

a. Performed at Screening and Day 23 or early termination only.
b. Urobilinogen may be measured via a urine dipstick test at the site if their local laboratory does not include it in their urinalysis panel.
c. Lipid panel (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides) measured at the Screening Visit does not need to be repeated on Day -1.

Absolute values for continuous parameters (hematology, general biochemistry, and urinalysis) will be summarized using descriptive statistics by each cohort (dose level of AL001 and pooled placebo; overall) and change from baseline will be presented.

Number (Percentage) of Subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values will be presented for each laboratory category by each cohort (dose level of AL001 and pooled placebo, overall) and visit.

Serology, alcohol/drug screen, pregnancy test results will be presented in separate listings.

Data listings will include out-of-range flags (Low, High) and whether the abnormal values are clinically significant or not.

8.4 Vital Signs

Vital signs will include heart rate, blood pressure, and body temperature.

Absolute values in vital signs measurements will be summarized descriptively by each cohort (dose level of AL001 and pooled placebo; overall), time point and change from baseline will be presented.

Number (%) of subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values of vital signs will be presented by each cohort (dose level of AL001 and pooled placebo, overall), visit and time point.

Data listing will identify if values are “abnormal, clinically significant” or “abnormal, not clinically significant”. Vital sign results will be presented.

8.5 Physical Examination Findings

The physical examination will include a general review of the following body systems (at minimum): general appearance, head and neck, cardiovascular, respiratory, abdomen, and brief neurological examination.

Physical examination results will be listed as normal or abnormal. Abnormal findings of clinical significance will be included in the listing.

8.6 12-lead Electrocardiogram

12- Lead safety ECG results will be summarized descriptively by each cohort ((dose level of AL001 and pooled placebo, overall), time point and change from baseline will be presented.

Number (%) of subjects with Normal, Abnormal Clinically Significant (CS) and Abnormal Non-Clinically Significant (NCS) values of ECG will be presented by each cohort (dose level of AL001 and pooled placebo, overall), visit and time point.

Data listing will identify if values are “abnormal, clinically significant” or “abnormal, not clinically significant”.

9 DATA HANDLING AND PRESENTATION

All safety and statistical outputs will be generated using SAS software, version 9.4.
All programs used to generate statistical analyses will be validated according to Altasciences's standard operating procedure (SOPs).
Tables, figures and listings will be provided in RTF formats.

9.1 Safety Analysis Presentation

Adverse events and medical history will be classified using the MedDRA terminology as defined in the study data management plan (DMP).

Prior and concomitant medications will be coded with the World Health Organization drug dictionary (WHO-DD) (March 2022 B3) as defined in the study DMP.

Generally, summaries will be presented by cohort (dose level of AL001 and pooled placebo, overall). Some summaries will also be presented to include overall (i.e., subject disposition and demographics).

Summaries for data collected at scheduled times specified in the protocol will be presented by cohort (dose level of AL001 and pooled placebo, overall), visit, and, where applicable, time point. Continuous variables will be summarized using the number of observations, mean, standard deviation, minimum, median, and maximum. Categorical variables will be summarized using the number of observations and the corresponding percentage.

In general, the data listings will include data from all subjects who receive at least one dose of either AL001 or placebo.

Study days will be included in adverse event and concomitant medication listings in addition to dates. Study days will be calculated relative to the first day of double-blind treatment (Day 1) derived as (event date - first day of AL001 or placebo dosing) +1 for events after the first day of dosing and (event date - first day of AL001 or placebo dosing) for events before the first day of dosing.
The following general comments also apply to all statistical analyses and data presentations:

- Duration variables will be calculated using the general formula: (end date - start date) +1.
- If the reported value of a clinical laboratory parameter cannot be used in a statistical summary table (e.g., a character string is reported for a parameter of the numerical type), a coded value must be appropriately determined and used in the statistical analyses. In general, a value or lower and upper limit of normal range such as '<10' or '≤5' will be treated as '10' or '5' respectively, and a value such as '>100' will be treated as '100'. However, the actual values as reported in the database will be presented in data listings.
- When assessments are repeated for a given time point or performed at unscheduled times, only the result which is the closest to the dosing time will be included in summary tables.

In general, summary statistics for raw variables (i.e., variables measured at the study site or central laboratory) will be displayed as follows:

- Minima and maxima will be displayed to the same number of decimal places as the raw data.
- Means, medians, and quartiles will be displayed to 1 additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.
- Percentages will be displayed to 1 decimal place. Percentages between 0 and 0.1 (exclusive) will be displayed as '<0.1'.
- P-values will be displayed to 3 decimal places. P-values that are less than 0.001 will be displayed as '<0.001' if applicable.

The number of decimal places for summary statistics of derived variables (i.e., variables that are not measured by the study site but are calculated for analysis based on other measured variables) will be determined on a case by case basis. In general:

- Minima and maxima will be displayed to the commonly used unit of precision for the parameter.
- Means, medians, quartiles, and confidence limits will be displayed to 1 additional decimal place.
- Standard deviations will be displayed to 2 additional decimal places.

9.2 Baseline

The baseline is defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the first dose of study drug in each cohort.

10 INTERIM ANALYSES AND DATA SAFETY MONITORING

There is no interim analysis plan for this study.

11 GENERAL INFORMATION RELATED TO DATA PRESENTATIONS

The formats and layouts of TFLs are provided in a separate document and are common displays. Their numbering and general content follow the International Conference on Harmonisation (ICH) E3 guidelines. Actual formats and layouts may be altered slightly from those presented as necessary to accommodate actual data or statistics.

Minor format changes will not require updates to the SAP; rather they may be documented in a Note to SAP.

APPENDIX A STUDY SCHEDULE(S)

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Informed Consent	X							
Admission[1]		X						
Clinic Confinement			X	X				
Discharge					X			
Medical and Surgical History	X							
Physical Examination	X				X	X		X
Demographic information	X							
Body Height/Body Weight	X							
Vital Signs[2]	X	X	X	X	X	X		X
12-lead ECG[3]	X		X	X	X	X		X

[1] Subjects will be admitted to the clinical research unit at least 16 hours prior to the first study drug administration.
[2] Vital signs will include blood pressure, heart rate and oral body temperature. On Days 1 to 14, vital signs will be measured within 1 hour prior todosing using the same arm, if possible, after 5 minutes in seated position.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Safety Laboratory Tests[4]	X	X		X	X	X		X
Serology (HIV/HBV/HCV)	X							
Serum Pregnancy Test (females of childbearing potential only)	X							
Urine Pregnancy Test (females of childbearing potential only)		X						
COVID-19 Test[5]	X	X						
Urine Drug Screen and Alcohol Breath or Urine Test	X	X						
Inclusion/Exclusion Criteria	X	X						
Randomization			X					
Fasted Study Drug			X	X				

[3] On Day 1, ECGs will be conducted pre-dose and 1 hour (±10 minutes) after the third dose of Day 1. On Days 7, 13, and 14, ECGs will be performed prior to first daily dose.

[4] Safety laboratory tests (chemistry, hematology, and urinalysis) will be performed on Day -1, Day 7 (pre-dose), Day 15, and Day 23. For all subjects, total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides will not be included in the safety laboratory tests performed on Day -1. For subjects who arescreened within 5 days of check-in (on Day -6 to Day -2), the safety laboratory tests will not be performed on Day -1.

[5] The COVID-19 test should be repeated upon check-in on Day -1 using a COVID rapid antigen test kit or PCR. Additionally,, study site and local health authoritypolicies and recommendations can be applied for compliance. If the initial result is positive at the Screening Visit, the test may be repeated after 14 days or on Day -1 whichever comes first.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Administration (all doses)								
Baseline and Trough plasma lithium and peak plasma salicylate concentration measurements[6]			X	X				X
MMSE Score (AD subjects only)	X							
Hachinski Ischemic Scale Score[7] (AD subjects only)	X							
Meals[8]		X	X	X	X			

[6] Blood samples for baseline plasma lithium and plasma salicylate concentration measurements will be collected before first dose on Day 1. Blood samples for trough plasma lithium and peak plasma salicylate concentration measurements will be collected on Days 3, 5, 7, 10, 13, and 14, and at the discretion of the Investigator (the latter are 'Safety Samples' for Therapeutic Drug Monitoring), particularly to assess the relationship to possible lithium or salicylate related AEs. These safety samples could be sent for expedited analysis to either a local laboratory with an aliquot saved for the bioanalytical laboratory, or to the bioanalytical laboratory directly (Sannova or Altasciences). In addition, for AD subject Cohorts 2b, 3b, 4b, and 5b, the trough lithium sample on Day 5 will be treated as a 'Safety Sample' for Therapeutic Drug Monitoring to assess any lithium-related PK exposure safety concerns at an early treatment time point which may require dosing to be discontinued for an individual subject. This sample will be sent to Sannova for expedited analysis. Similarity between Days 13 and 14 trough lithium plasma levels and peak salicylate plasma levels will be used to determine the status of lithium and salicylate steady-state PK conditions, and/or by another appropriate method, such as linear regression. Blood collection for trough lithium plasma concentrations will occur just prior to the first daily dose, while blood collection for peak plasma salicylate concentrations will occur at 1.25 hours post third daily dose (the estimated time to a maximum blood concentration C_{max}). For early termination, blood samples for lithium and salicylate plasma concentrations will be collected. A ±10-minute window will be allowed for collection of PK samples for both lithium and salicylate.

[7] Hachinski Ischemic Scale Score is presented in protocol APPENDIX 5.

Study Procedures	Screening (28 days prior to randomization)	In-patient admission	Treatment Period		Clinic Discharge	Follow-up Period		Early Termination (ET)
Study Day	Days -28 to -1	Day -1	Day 1	Days 2 to 14	Day 15	Day 23 ±1 day Clinic Visit	Day 42 ±2 days Telephone call	
Adverse Event Reporting/Safety-Tolerability Assessment	X	X	X	X	X	X	X	X
Prior Medications	X	X						
Concomitant Medications	X	X	X	X	X	X	X	X
Health Status Questionnaire (SF-36)						X	X	X

Abbreviations: AD = Alzheimer's disease; COVID-19 = Coronavirus Disease 2019; ECG = electrocardiogram; ET = Early Termination; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; MMSE = Mini-Mental State Exam; PK = pharmacokinetic(s); TSH = thyroid-stimulating hormone

[8] Meals during confinement should include breakfast (at least 1 hour after first dose), lunch (at least 1 hour after second dose), dinner (at least 1 hour after third dose), and a snack (if time permits and 10-hour fast is respected). On Day 15, breakfast will be provided prior to subject/caregiver being discharged from the study site.