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STATISTICAL ANALYSIS PLAN

A Multi-Center, Double-Masked Phase 3 Evaluation of the Long-Term Safety of LNZ101 in Presbyopic Subjects

Sponsor: LENZ Therapeutics, Inc.

[redacted]

Protocol Number: 22-150-0017

Author: [redacted]

Ora Inc.

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A Multi-Center, Double-Masked Phase 3 Evaluation of the Safety and Efficacy of LNZ101 for the Treatment of Presbyopia

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SAP Version: **1.0**

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Statistical Analysis Plan Approval

[redacted]

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[redacted]

List of Abbreviations

Abbreviation	Definition
ADaM	Analysis Data Model
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
BCDVA	Best-Corrected Distance Visual Acuity
CI	Confidence Interval
CS	Clinically Significant
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ETDRS	Early Treatment of Diabetic Retinopathy Study
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IOP	Intraocular Pressure
LL-BCDVA	Low-luminance Best-Corrected Distance Visual Acuity
logMAR	Logarithm of the Minimum Angle of Resolution
LS	Least Squares
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not Clinically Significant
QD	*Quaque die* (Once Daily)
PDF	Portable Document Format
PT	Preferred Term
RTF	Rich Text Format
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDC	Statistics & Data Corporation
SDTM	Study Data Tabulation Model
SE	Standard Error
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TE-SAE	Treatment-Emergent Serious Adverse Event
WHODrug	World Health Organization Drug Dictionary

[redacted]

1. Introduction

The purpose of this statistical analysis plan (SAP) is to describe in detail the planned analyses and reporting for protocol 22-150-0017, Amendment 4 Version 5.0 dated 17AUG2023. The statistical analysis methods presented in this document will supersede the statistical analysis methods described in the clinical protocol. If additional analyses are required to supplement the planned analyses described in this SAP, they may be completed and will be identified in the clinical study report.

This SAP is being written with due consideration of the recommendations outlined in the most recent International Conference on Harmonisation (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports.

2. Study Objectives

The primary objective of the study is to evaluate the long-term safety of LNZ101/LNZ100 compared with vehicle in presbyopic subjects.

3. Study Endpoints

3.1 Safety Endpoints

- Adverse events (AE) (reported, elicited, and observed)
- Vital signs (resting blood pressure and pulse)
- Physical exam (general health, head, eyes, ears, nose, throat, and other comments)
- Systemic labs (hematology, blood chemistry analysis, and urinalysis)
- Pregnancy test
- Monocular best-corrected distance visual acuity (BCDVA) at 4 m
- Slit lamp biomicroscopy
- Intraocular pressure (IOP)
- Dilated fundus exam
- Endothelial cell density assessments (assessed via specular microscopy)

3.2 Clinical Hypotheses

LNZ101 [redacted] and LNZ100 [redacted] have an acceptable safety profile when used in healthy adults with a history of presbyopia.

4. Study Design and Procedures

4.1 General Study Design

This is a [redacted] randomized, double-masked, multi-center, parallel-group study evaluating the safety of LNZ101 and LNZ100 compared to vehicle in approximately 350 subjects with presbyopia.

Enrolled subjects will be randomized to receive LNZ101, LNZ100, or vehicle.

Subjects will dose for approximately 26 weeks.

[redacted]

[redacted]

4.3 Study Treatments

The study treatments are as follows:

- LNZ101 [redacted]
- LNZ100 [redacted]
- Vehicle ophthalmic solution

The two active ingredients (Aceclidine and Brimonidine) have been formulated and will be provided in a sterile container.

4.3.1 Method of Assigning Subjects to Treatment Groups

Each subject who signs an informed consent form (ICF) will be assigned a subject number (a six-digit number starting with the 3-digit site number followed by a sequential three-digit number starting with 001). Once a subject meets all qualification criteria [redacted], he/she will be randomized [redacted] via an interactive response technology system to 1 of 3 treatment groups (1: LNZ101 or 2: LNZ100, or 3: vehicle). Randomization will not be stratified by site.

Randomization will be used to avoid bias in the assignment of subjects to treatment, to increase the likelihood that known and unknown subject attributes (e.g., iris color and baseline characteristics) are balanced across treatment groups, and to provide validity of statistical comparisons across treatment groups. Double-masked treatment will be used to reduce the potential of bias during data collection and the evaluation of clinical endpoints.

5. Sample Size

Approximately 350 subjects will be randomized into the study [redacted] of LNZ101: LNZ100: vehicle [redacted] completing dosing for approximately 26 weeks.

A sample size of 100 subjects treated with LNZ101/LNZ100 will have at least 95% confidence of detecting AEs that occur at a rate of 3% or greater.

6. Data Preparation

6.1 Input Data

Study data will primarily be recorded on the electronic Case Report Forms (eCRFs) supplied by Ora Inc. (Ora) using iMednet Electronic Data Capture (EDC) system. Additional details about the EDC system can be found in the Data Management Plan. In addition, the following study data which is not captured directly within the EDC system but is obtained from external vendors will also be included for analysis. These data sources are described in detail in data transfer agreements developed between data management and the respective external laboratory or reading center:

- Central laboratory data
- Ocular imaging assessments

When all prerequisites for database lock have been met, the database will be locked. Following database lock, approval will be obtained from the Sponsor to unmask the study. Any changes to the database after data have been locked can only be made with the approval of the Sponsor in consultation with Ora.

Final analysis will be carried out after the following have occurred:

- Database lock has occurred, with written authorization provided by appropriate Ora and Sponsor personnel.
- Protocol deviations have been identified and status defined (major/minor).
- Randomized treatment codes have been unmasked.

6.2 Output Data

Data from EDC will be transferred to Ora Biostatistics and incorporated into standard formats following the Study Data Tabulation Model (SDTM). Data will then be mapped to analysis datasets using the Analysis Data Model (ADaM). Both SDTM- and ADaM-formatted data will be used to create the subject listings, while all tables and figures will be based on the ADaM-formatted data.

The SDTM and ADaM versions, implementation guide versions, and Pinnacle 21 version will be documented in the respective reviewer's guides in the final CDISC package.

7. Analysis Sets

7.1 Safety Set

The Safety set will include all subjects who have received at least one dose of the study drug. Subjects in the Safety set will be analyzed as treated.

8. General Statistical Considerations

8.1 Unit of Analysis

Both eyes will be displayed and analyzed for all ophthalmic safety variables. All AEs and medical history will be presented at the subject level.

8.2 Missing or Inconclusive Data Handling

In general, there will be no imputation of missing data other than for partial or missing dates where complete dates are required to flag data as treatment-emergent or concomitant with treatment. Partial/missing start and end dates for AEs and concomitant medications will be imputed as follows:

Partial/missing start date:

- Dates with missing day only will be imputed as the 1st of the month unless the month and year are same as the month and year of first dose of study medication, in which case missing day will be imputed as the first dose day of study medication.
- Dates with both day and month missing will be imputed as 1 Jan unless the year is same as the year of first dose of study medication, in which case missing day and month will be imputed as the first dose day and month of study medication.
- Completely missing dates will be imputed as the first dose date of study medication unless the end date is on or before the first dose date of study medication, in which case missing date will be imputed as 1 Jan of the same year as the end date.

Partial/missing end date:

- Dates with missing day only will be imputed as the last day of the month unless the month and year are the same as the month and year of the last dose of study medication, in which case missing day will be imputed as the last dose day of study medication.
- Dates with both day and month missing will be imputed as 31 Dec unless the year is same as the year of the last dose of study medication, in which case missing day and month will be imputed as the last dose day and month of study medication.
- If the ongoing flag is missing or “Yes” then the date will not be imputed unless death date is available, in which case the missing date will be imputed as the death date. If ongoing is “No” then the missing end date will be imputed as the last dose date.
- If the imputed date is after the date of death, then the end date will be set equal to the date of death.

The original dates will be displayed in data listings and the imputed dates will be used in derivations only (study day, treatment-emergence status, etc.).

8.3 Definition of Baseline

For all variables, baseline is defined as the last measurement taken prior to the administration of first dose of study drug [redacted] Change from baseline will be calculated as Follow-up Measure minus Baseline Measure.

8.4 Data Analysis Conventions

All data analysis will be performed by Ora. Statistical programming and analyses will be performed using SAS® Version 9.4 or higher. Output will be provided in rich text format (RTF) for tables and portable document format (PDF) for tables, listings, and figures using landscape orientation.

Summaries for continuous and ordinal variables will include the number of observations (n), arithmetic mean, standard deviation (SD), median, minimum, and maximum. Minima and maxima will be reported with the same precision as the raw values; means and medians will be presented to one additional decimal place than reported in the raw values. Standard deviations will be presented to two additional decimal places than reported in the raw values. All percentages will be rounded to one decimal place (i.e., XX.X%). Differences between active treatment group and vehicle will be calculated as, Active minus Vehicle.

Unless otherwise specified, summaries will be presented by treatment group and, where appropriate, visit, time point, and parameter. Listings will be presented by subject number, randomized treatment, visit, time point, and parameter as applicable.

8.5 Adjustments for Multiplicity

As there are no statistical hypotheses tested in this study, multiplicity is not applicable for this study.

9. Disposition of Subjects

Subject disposition will be presented in terms of the numbers of subjects who were screened and screen failed, and the number and percentage of subjects who were randomized into the study, who are included in the Safety set, who completed the study, and who discontinued from the study. Subjects who are not discontinued from the study will be considered study completers. Disposition will be summarized by treatment group and overall. Percentages will be based on the number of randomized subjects unless otherwise noted.

The number and percentage of subjects prematurely discontinued from the study and the reasons for study discontinuation will be summarized by treatment group and overall. The reasons for study discontinuation will be based on the total number of discontinuations for that treatment group and overall, and the reasons that will be summarized include: AE, lost to follow-up, investigator decision, protocol violation, study terminated by sponsor, withdrawal by subject (with subcategories of: due to an AE, due to lack of efficacy per subject, due to unknown reasons, and other), and other. The number and percentage of subjects whose reason for discontinuation was related to COVID-19 will be presented by treatment group and overall. The subject disposition summary will also include the number and percentage of subjects with any protocol

deviations, any minor deviations, or any major deviations by treatment group and overall. A subject listing will be provided that includes the informed consent date, the date of study completion or discontinuation, and reason for premature study discontinuation.

The number and percentage of subjects with any major protocol deviations (major status decided by the study team at the conclusion of the study prior to database lock and unmasking) will be summarized by treatment group and overall. The protocol deviation categories (codes) that will be summarized include: informed consent, inclusion/exclusion and randomization, test article/study drug instillation, improper protocol procedures at site (missed, repeated, not per protocol), site's failure to report serious adverse event (SAE)/AE, visit out of window (missed, early, late), subject's non-compliance with test article, subject's failure to follow instructions, and other. A subject listing will be provided that includes the start date of the deviation, the deviation code, whether the deviation was COVID-19 related, deviation description, action taken, whether the deviation was required to be reported to the Investigational Review Board (IRB) (and if yes, the date reported to the IRB), and the classification of whether the deviation was judged to be major or minor.

Listings will also be provided for randomization schedule, screen failures, and inclusion/exclusion criteria.

10. Demographics and Baseline Characteristics

10.1 Demographics

The demographic variables collected in this study include date of birth, age, sex at birth, childbearing potential for female subjects, race, ethnicity, and iris color of the right and left eyes. Subjects who record more than one race will be grouped into a single category denoted as Multi-racial. Demographic variables will be summarized for the Safety set.

Age (years) will be summarized, by treatment group and overall, using continuous descriptive statistics. Age will also be categorized as follows: < 65 years and ≥ 65 years. The number and percentage of subjects will be summarized for age category, sex at birth, childbearing potential, race, ethnicity, and iris color.

A subject listing that includes all demographic variables will be provided.

11. Medical History and Concomitant Medications

Listings of medical history, prior and concomitant medications, and concomitant procedures will be generated separately. Medical history and prior and concomitant medications will also be listed separately for ocular and non-ocular data.

11.1 Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 25.1.

Non-ocular medical history will be summarized using discrete summary statistics and presented by treatment group at the subject level by System Organ Class (SOC) and Preferred Term (PT) using the Safety set. Ocular medical history will be similarly summarized at the subject level. If a subject reports the same PT multiple times within the same SOC, that PT will only be reported once within that SOC. As with the PT, if a subject reports multiple conditions within the same SOC, that SOC will only be reported once.

In the summaries, SOCs and PTs within an SOC will be ordered by descending frequency based on all subjects.

11.2 Concomitant Medications

Concomitant medications will be coded using World Health Organization Drug Dictionary (WHODrug) Global, B3, September 2022 and summarized to the therapeutic drug class (Anatomical Therapeutic Chemical [ATC] 4 classification) and preferred name. If the ATC 4 classification is not provided, then the next highest classification that is provided in the coding dictionary will be used. The preferred name will be defined as the active ingredient; if the active ingredient is not provided or includes more than two ingredients (e.g., multivitamins), then the drug name will be summarized as the preferred name.

Concomitant medications are defined as those medications listed as having been taken (1) prior to initiation of study drug administration and continuing for any period of time following the first administration of study drug or (2) at any time following the first administration of study drug.

Concomitant medications will be summarized using the Safety set. Medications will be tabulated for each treatment group and overall using number and percentage of subjects. Subjects may have more than one medication per ATC class. At each level of subject summarization, a subject will be counted once if they report one or more medications. Percentages will be based on the number of subjects in each treatment group. In the summaries, ATC classes and preferred names within an ATC class will be ordered by descending frequency based on all subjects.

[redacted]

11.3 Concomitant Procedures

Concomitant procedures will be coded using MedDRA Version 25.1. Concomitant procedures will be listed but not summarized.

12. Dosing Compliance

Subjects will be provided with a dosing diary to document once daily (QD) dosing. Dosing compliance (% compliance) will be assessed by calculating the number of actual doses received and comparing that to the number of expected doses as follows:

$$\text{Compliance (\%)} = \frac{\text{Number of Actual Doses Received}}{\text{Number of Expected Doses}} \times 100\%$$

The number of actual doses received will be calculated by counting the number of used ampoules in the Study Drug Accountability eCRF along with the number of doses instilled in-office at Visit 2 (Day 1) on the Study Drug Instillation eCRF across all visits. [redacted]

Dosing compliance (%) will be summarized with continuous descriptive statistics for each treatment group using the Safety set. The compliance category defined above will be summarized with discrete summary statistics.

Subject listings will be provided for study drug assignment, dispensation, and instillation, as well as dosing diary dispensation and collection.

13. Safety Analyses

All safety analyses will be conducted using the Safety set.

13.1 Adverse Events

An AE is defined as any untoward medical occurrence associated with the use of an investigational product (IP) in humans, whether or not considered IP-related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IP, without any judgment about causality. An AE can arise from any use of the IP (e.g. off-label use, use in combination with another drug or medical device) and from any route of administration, formulation, or dose, including an overdose. All AEs will be collected from the time a subject signs the ICF through the subject's study exit visit. Study drug includes the investigational drug under evaluation and vehicle given during the study. All AEs will be coded using the MedDRA Version 25.1.

Treatment-emergent adverse events (TEAE) are defined as an AE with a start date on or after the first dose of study drug or that worsened following administration of study drug. Adverse events recorded in the eCRF which began prior to treatment will not be included in the summary tables but will be included in the AE data listings.

Severity of an AE is defined as a qualitative assessment of the degree of intensity of an AE as determined by the investigator or reported to them by the subject. The assessment of severity is made irrespective of relationship to study drug or seriousness of the event and should be evaluated according to the following scale:

- *Mild:* Event is noticeable to the subject, but is easily tolerated and does not interfere with the subject's daily activities.
- *Moderate:* Event is bothersome, possibly requiring additional therapy, and may interfere with the subject's daily activities.
- *Severe*: Event is intolerable, necessitates additional therapy or alteration of therapy, and interferes with the subject's daily activities.

The relationship of each AE to the study drug should be determined by the investigator using these explanations:

- *Suspected*: A reasonable possibility exists that the IP caused the AE.
- *Not Suspected*: A reasonable possibility does not exist that the IP caused the AE.

An overall summary will be presented that includes the number of events and the number and percentage of subjects who experienced at least one TEAE by treatment group. This summary will also include breakdowns of TEAEs further categorized as ocular and non-ocular TEAEs, treatment-emergent serious adverse events (TE-SAEs), treatment-related TEAEs, treatment-related TE-SAEs, TEAEs leading to early treatment discontinuation, TEAEs leading to death, and ocular and non-ocular TEAEs by maximum severity.

Additional summaries of TEAEs will be provided showing the number and percentage of subjects who experienced at least one TEAE by treatment group. Ocular and non-ocular TEAEs will be summarized separately by treatment group at the subject level by SOC and PT. [redacted] In the summary, SOCs and PTs within a SOC will be ordered in descending frequency of LNZ101 subjects.

Separate summaries will be provided for the following categories of AEs:

- Ocular TEAEs
- Non-ocular TEAEs
- Treatment-related ocular TEAEs

- Treatment-related non-ocular TEAEs
- Expected TEAEs
- Unexpected TEAEs
- Ocular TE-SAEs
- Non-ocular TE-SAEs
- Ocular TEAEs by Maximum Severity
- Non-Ocular TEAEs by Maximum Severity

All AEs will be presented in a subject listing. The AEs leading to study drug discontinuation will be listed separately. In addition, all SAEs will be presented in a separate listing.

13.2 Vital Signs

Each subject will have vital signs assessments (resting blood pressure, pulse rate, and whether the pulse was regular or irregular)

The observed and change from baseline resting blood pressure and pulse rate will be summarized using continuous descriptive statistics for each treatment group, respectively. A subject listing of both resting blood pressure and pulse will also be produced, respectively.

13.3 Physical Examination

Each subject will have a physical examination

A table will summarize the results using counts and percentages for each treatment group. Percentages will be based on the number of subjects with non-missing values for that parameter at that visit in each treatment group with responses.

A shift table for the physical examination parameters will also be provided comparing the follow-up visit to baseline. On the shift table, percentages are based on the number of the respective subjects with non-missing assessment at baseline and at the respective post-baseline visit for the population being analyzed.

The physical examination results will be presented in a listing.

13.4 Systemic Labs

Each subject will have systemic labs

The quantitative variables will be summarized by treatment group with continuous descriptive statistics. The qualitative variables (counts and percentages) will be summarized by treatment group at each visit. Change from baseline will also be summarized by treatment group. A shift table for the systemic labs will also be provided comparing the follow-up visit to baseline. On the shift table, percentages are based on the number of the respective subjects with non-missing assessment at baseline and at the respective post-baseline visit for the population being analyzed. All quantitative values within range will be defined normal and out of range as abnormal-high or abnormal-low.

The systemic lab results will be presented in a listing.

13.5 Monocular Best-Corrected Distance Visual Acuity

Monocular best-corrected distance visual acuity (BCDVA) using an Early Treatment of Diabetic Retinopathy Study (ETDRS) chart calibrated for testing at 4 m is conducted at each visit. Results will be recorded in logarithm of the minimum angle of resolution (logMAR) units. If a subject is unable to read any letters, the subject will be asked to count fingers (with distance recorded), recognize hand movement, or perceive light perception.

[redacted]

The above analysis will be performed with respect to baseline and all post-baseline BCDVA assessments at 4 m.

13.6 Slit Lamp Biomicroscopy Examination

A slit lamp biomicroscopy examination will be conducted [redacted]. The results will be graded as normal, abnormal (not clinically significant [NCS]), or abnormal (clinically significant [CS]).

A table will summarize the results using counts and percentages for each treatment group at each region for each eye. Percentages will be based on the number of subjects in each treatment group with responses. Shift tables and data listing will also be provided.

13.7 Intraocular Pressure

Intraocular pressure is measured [redacted]. The data for IOP examinations will be presented in a listing.

13.8 Dilated Fundus Exam

Dilated fundus exam will be conducted [redacted].

A table will summarize results using counts and percentages for each treatment group at each location for each eye. Percentages will be based on the number of subjects in each treatment with responses.

A shift table for the dilated fundus exam parameters will also be provided comparing the follow-up visit to baseline. On the shift table, percentages are based on the number of the respective eyes with a non-missing assessment at baseline and at the respective post-baseline visit for the population being analyzed.

The dilated fundus exam results will be presented in a listing.

13.9 Undilated Fundus Exam

[redacted] an undilated fundus exam will be conducted [redacted]. The results will be graded as normal, abnormal (NCS), or abnormal (CS).

The undilated fundus exam results will be presented in a listing.

13.10 Urine Pregnancy Test

Female subjects of childbearing potential will have a urine pregnancy test performed [redacted]. A subject-level listing by visit will be produced.

13.11 Specular Microscopy

Konan specular microscopy will be performed [redacted]

The observed and change from baseline [redacted] will be summarized [redacted] for each treatment group. The change from baseline will be compared between the treatment groups for each eye using a two sample t-test. A subject listing for specular microscopy will also be produced.

14. Interim Analyses

There are no interim analyses planned for this study.

15. Changes from Protocol-Stated Analyses

There are no changes to the protocol-stated analyses.

[redacted]

16. References

1. *ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. 05 February 1998.
2. *ICH Harmonised Tripartite Guideline: Addendum on Estimands and Sensitivity Analysis in Clinical Trials to the Guideline on Statistical Principles for Clinical Trials E9(R1)*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. 20 November 2019.
3. *ICH Harmonised Tripartite Guideline: Structure and Content of Clinical Study Reports E3*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. 30 November 1995.

17. Revision History

Documentation of revision to the SAP will commence after approval of the final version 1.0.

[redacted]