Clinical Trial Protocol 22-150-0015

Protocol Title:	A Multi-Center, Double-Masked Phase 3 Evaluation of the Safety and Efficacy of LNZ101 for the Treatment of Presbyopia
Protocol Number:	22-150-0015
Study Phase:	Phase 3
Investigational Product Name:	[redacted]
IND/IDE/PMA Number:	120,609
Indication:	Presbyopia
Investigators:	TBD
Sponsor:	LENZ Therapeutics, Inc. [redacted]
Contract Research Organization:	[redacted]
IRB/IEC:	[redacted]

	Date
Original Protocol:	Version 1.0 (17 Nov 2022)
Amendment 1	Version 2.0 (26 Jan 2023)
Amendment 2	Version 3.0 (01 May 2023)
Amendment 3	Version 4.0 (15 Jun 2023)
Amendment 4	Version 5.0 (31 Jul 2023)

Confidentiality Statement

SPONSOR PERSONNEL

Sponsor:	LENZ Therapeutics, Inc. [redacted]
Sponsor Representative:	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]

MEDICAL MONITOR

Medical Monitor:	[redacted]

1.0 SYNOPSIS

Protocol Title:	A Multi-Center, Double-Masked Phase 3 Evaluation of the Safety and Efficacy of LNZ101 for the Treatment of Presbyopia
Protocol Number:	22-150-0015
Investigational Products:	LNZ101 (Aceclidine 1.75%/Brimonidine 0.08%) ophthalmic solution LNZ100 (Aceclidine 1.75%) ophthalmic solution Brimonidine (0.08%) ophthalmic solution
Study Phase:	Phase 3
Primary Objective:	To evaluate the safety and efficacy of LNZ101 compared with LNZ100 and Brimonidine for the treatment of Presbyopia.
Secondary Objective:	Not applicable
Overall Study Design:	
Structure:	A multi-center, double-masked, randomized, active-controlled, safety and efficacy study
Duration:	Approximately 6 weeks
Controls:	**Screening Control:** Refresh® Plus Eye Drops **Randomized Control Treatment:** Brimonidine (0.08%) ophthalmic solution
Dosage/Dose Regimen/ Instillation/Application/Use:	Enrolled subjects will be randomized to receive LNZ101, LNZ100, or Brimonidine bilaterally once a day. Subjects will be instructed to dose 2 drops in both eyes (OU) (1 drop in each eye followed by 2 minutes later, another drop in each eye). Subjects will dose for 6 weeks.
Summary of Visit Schedule:	• Visit 1 (Day -60 to -4): Screening; • Visit 2 (Day 1): Baseline Assessments, Enrollment, Randomization, and Primary Efficacy Assessments; • Visit 3 (Day 15 ±2 days): Efficacy and Safety Assessments • Visit 4 (Day 28 ±2 days): Efficacy and Safety Assessments • Visit 5 (Day 42 ±2 days) End of Study Visit / Early Termination (ET) Visit: Safety Assessments

Measures Taken to Reduce Bias:	Randomization will be used to avoid bias in the assignment of subjects to treatment and to enhance the validity of statistical comparisons across treatment groups. Double-masked treatment will be used to reduce potential of bias during data collection and evaluation of clinical endpoints Randomization will be at a 1:1:1 ratio (LNZ101: LNZ100: Brimonidine).
Study Population Characteristics:	
Number of Subjects:	Approximately 435 subjects will be enrolled at Visit 2. Cohort 1 (Approximately 20% of population): Have baseline monocular (in study eye) BCDVA at 40 cm greater than or equal to 0.42 and less than 0.50 logMAR (approximately between $20/50^{-2}$ and 20/63 Snellen) at Visit 2. Cohort 2 (Approximately 80% of population): Have baseline monocular (in study eye) BCDVA at 40 cm greater than or equal to 0.50 logMAR (approximately 20/63 Snellen) at Visit 2.
Condition/Disease:	Healthy adult subjects ages 45 to 75 years who have presbyopia
Inclusion Criteria:	Subjects must: 1. Be able and willing to provide written informed consent and sign a Health Information Portability and Accountability Act (HIPAA) form prior to any study procedure being performed; 2. Be able and willing to follow all instructions and attend all study visits; 3. Be 45-75 years of age of either sex and any race or ethnicity at Visit 1; 4. Have +1.00 to -4.00 diopters (D) of sphere (calculated in minus cylinder) with a spherical equivalent (SE) that is no more myopic than -4.00 D MRSE in both eyes determined by manifest refraction documented at Visit 1; 5. Have ≤2.00 D of cylinder (minus cylinder) in both eyes determined by manifest refraction documented at Visit 1; 6. Be presbyopic with monocular BCDVA at 40 cm ≥ 0.42 logMAR (approximately $20/50^{-2}$ Snellen), assessed prior to placebo run-in at Visit 1 (in at least one eye), and at Visit 2 baseline (in the study eye);

	7. Have monocular BCDVA at 4 m of 0.1 logMAR (approximately 20/25 Snellen) or better assessed prior to placebo run-in at Visit 1 (in at least one eye), and at Visit 2 baseline (in the study eye); 8. For pseudophakic subjects, intraocular lens (IOL) must be confirmed monofocal and with no significant posterior capsular opacification (PCO); Note: Subjects must also qualify based on all other IE criteria, including having a pre-IOL refraction and/or prescription that meets inclusion criteria #4 (refractive parameters for the study); 9. Have a negative pregnancy test at Visit 1 and 2, if female of childbearing potential (those who have experienced menarche and who are not surgically sterilized [bilateral tubal ligation, hysterectomy, or bilateral oophorectomy] or post-menopausal [12 months after last menses]) and must use adequate birth control throughout the study period. Adequate birth control is defined as hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as condom or diaphragm; intrauterine device (IUD); or surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control; 10. Be able and willing to avoid all disallowed medications for the appropriate washout period and during the study without significant risk to the subject.
Exclusion Criteria:	1. Subject must not be a female of childbearing potential who is currently pregnant, nursing, or planning a pregnancy; 2. Subject must not have known contraindications or sensitivity to the use of any of the study medications or their components; 3. Neither eye can have an active ocular infection at Visit 1 or at Visit 2 (bacterial, viral, or fungal), positive history of an ocular herpetic infection, preauricular lymphadenopathy, or ongoing, active ocular inflammation (e.g., moderate to severe blepharitis, allergic conjunctivitis, peripheral ulcerative keratitis, scleritis, uveitis);

	4. Neither eye can have moderate or severe dry eye defined as corneal fluorescein staining superior, inferior, and central (combined) > 2 ([redacted] Calibra™ Scale) at Visit 1[i]; 5. Neither eye can have clinically significant abnormal lens findings (e.g., cataract) including early lens changes and/or any evidence of a media opacity during dilated slit-lamp biomicroscopy and fundus exam documented within 3 months of Visit 1 or at Visit 1; 6. Neither eye can have > +0.75D hyperopic shift in sphere from manifest refraction compared to cycloplegic refraction documented at Visit 1; 7. Subject must not have a > 0.2 logMAR (i.e., 10-letters) difference between eyes as determined by screening monocular BCDVA at 40 cm at Visit 1 or at Visit 2 baseline; 8. Subject must not have < 0.3 logMAR (i.e., 15 letters) improvement in monocular BCDVA at 40 cm with +1.50 D Sphere Add compared to monocular BCDVA at 40 cm at Visit 1 (in at least one eye) and at Visit 2 baseline (in the study eye); 9. Neither eye can have > 0.14 logMAR (i.e., 7 letters) improvement in placebo post-treatment monocular BCDVA at 40 cm compared to screening monocular BCDVA at 40 cm at Visit 1; 10. Neither eye can have average dark-adapted pupillometry measurements of < 4.2 mm at Visit 1; 11. Neither eye can have intraocular pressure (IOP) that is < 5 millimeters of mercury (mmHg) or > 22 mmHg documented at Visit 1, or have a prior diagnosis of ocular hypertension or glaucoma or currently being treated with any type of topical IOP lowering (glaucoma) medication at Visit 1; 12. Neither eye can have clinically significant abnormal findings on dilated fundus exam documented within 3 months of Visit 1 or at Visit 1, or a known history of retinal detachment or clinically significant retinal disease; 13. Neither eye can have a known history or past diagnosis of iritis, scleritis, or uveitis, whether active or inactive; 14. Subject must not have had surgical intervention (ocular or systemic) within 6 months prior to Visit 1, or planned surgical intervention during the study; 15. Subject must not have undergone prior LASIK or PRK surgery within 12 months of Visit 1:

	Subjects who have undergone LASIK or PRK more than 12 months prior to Visit 1 must qualify based on all other IE criteria, including having a pre-LASIK or pre-PRK prescription and/or refraction that meets Inclusion Criteria 4; 16. Subject must not use artificial tears or lubricant eye ointment on a daily basis; 17. Subject must not have planned use of artificial tears or lubricant eye ointment on the day of any study visits; 18. Subject must not have an inability or refuse to discontinue soft contact lens wear 7 days prior to study Visit 1 and rigid gas permeable (RGP) contact lens wear 14 days prior to Visit 1 and during the study; 19. Subject must not use any of the following disallowed medications during the 2 weeks (14 days) prior to Visit 1 and during the study: a. narcotic (opiate class) pain medication (e.g., codeine, OxyContin®, Vicodin®, Tramadol®) b. bladder medication (e.g., Urecholine®, bethanechol, oxybutynin, tolterodine) c. antipsychotics d. antidepressants e. attention–deficit/hyperactivity disorder (ADHD) medications f. alpha-blockers (e.g., tamsulosin, Flomax®, Jayln®, Uroxatral®, Rapaflo®) g. anticholinergics (e.g., atropine, belladonna, benztropine, dicyclomine, donepezil, hyoscyamine, propantheline, scopolamine, trihexyphenidyl) h. muscarinic receptor agonists or cholinergic agonists (e.g., Salagen®, Evoxac®) i. over-the-counter (OTC) or prescription antihistamines or decongestants j. any prescribed topical ophthalmic medications k. recreational drug use (e.g., marijuana, methadone, heroin, cocaine); l. any medications that are known to cause mydriasis or miosis, including, but not limited to: i. Buscopan®

[i] Punctal plugs are acceptable if they have been in place for a minimum of 90 days prior to visit 1.

	ii. Procyclidine iii. L-DOPA iv. amphetamine v. scopolamine vi. physostigmine vii. neostigmine viii. guanethidine ix. primidone x. Wegovy® , Ozempic® , Rybelsus® (or any other form of semaglutide) xi. Mounjaro® (tirzepatide) 20. Subject must not have been dosed with an investigational drug or device study within 1 month of Visit 1; 21. Neither eye can have undergone any prior corneal surgery that is not described and allowed in other IE criteria; 22. Neither eye can have undergone any prior retinal surgery, laser treatment or conventional surgery for retinal hole and retinal tears; 23. Subject must not have a diagnosis of diabetes mellitus or an elevated blood sugar greater than 150mg/dl, or HbA1c of greater than or equal to 6.5% as measured within the past 3 months[ii]; 24. Subject must not have a condition or a situation, which in the Investigator's opinion, may put the subject at increased risk, confound study data, or interfere significantly with the subject's study participation, including but not limited to unstable: cardiovascular, hepatic, renal, respiratory, gastrointestinal, endocrine, immunologic, dermatologic, hematologic, neurologic, or psychiatric disease; 25. Subject must not have been dosed with investigational drug in LENZ study 22-150-0017, 22-150-0018, or 22-100-0007.

[ii] Any blood sugar in an otherwise normal, non fasting blood chemistry panel that is greater than 150mg/dl would be considered abnormal and indicative of a diabetic or prediabetic state and is exclusionary.

Study Formulations:	LNZ101 (Aceclidine 1.75%/Brimonidine 0.08%) ophthalmic solution LNZ100 (Aceclidine 1.75%) ophthalmic solution Brimonidine (0.08%) ophthalmic solution
Evaluation Criteria:	
Efficacy Measures and Endpoints:	**Primary Efficacy Endpoint:** • Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) at 3 hours post-treatment in the study eye at Visit 2 (Day 1) for each LNZ treatment relative to Brimonidine. **Secondary Efficacy Endpoints** All secondary endpoints will be comparisons of each LNZ treatment relative to Brimonidine unless otherwise specified. • Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at: Onset of Action a) 30 minutes post-treatment Duration of Action a) 1 hour post-treatment b) 5 hours post-treatment c) 7 hours post-treatment d) 8 hours post-treatment e) 9 hours post-treatment f) 10 hours post-treatment • Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at: a) 30 minutes post-treatment b) 1 hour post-treatment c) 3 hours post-treatment

	• Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at: a) 30 minutes post-treatment b) 1 hour post-treatment c) 3 hours post-treatment d) 5 hours post-treatment e) 7 hours post-treatment f) 8 hours post-treatment g) 9 hours post-treatment h) 10 hours post-treatment • Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) between LNZ101 and LNZ100. **Exploratory Efficacy Endpoints:** • Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment • Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRSchart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment • Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment • Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment

	• Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 3 (Day 15) at 0.5, 1, and 3 hours post-treatment • Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment • Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment • Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1, 3 hours post-treatment • Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment • Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment • Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1, and 3 hours post-treatment • Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment • Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment

	• Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment • Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment • Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 1, 3, and 10 hours post-treatment • Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 1, 3, and 10 hours post-treatment • Mean and mean change in monocular BCDVA at 66 cm (ETDRS chart) in the study eye at Visit 2 (Day 1) at 1, 3, and 10 hours post-treatment • Mean and mean change in monocular BCDVA at 66 cm (ETDRS chart) in the study eye at Visit 4 (Day 28) at 1, 3, and 10 hours post-treatment • [redacted]

	[redacted]
Safety Measures:	• Adverse events (AE) (reported, elicited, and observed) • Pregnancy test (at Visit 1, Visit 2, and Visit 5) • Monocular and binocular BCDVA (normal and low-luminance) • Slit lamp biomicroscopy • IOP (at Visit 1 and Visit 5) • Dilated fundus exam (at Visit 1 and Visit 5) • Conjunctival redness

Other:	• Dark Adapted Pupillometry • Patient-reported outcome (PRO) questionnaire • Drop instillation assessment

General Statistical Methods and Types of Analyses

Analysis Sets:

Full Analysis Set (FAS) – The FAS will include all randomized subjects. [redacted] Subjects in the FAS will be analyzed as randomized.

Per Protocol (PP) Set – The PP set will include subjects in the FAS who do not have significant protocol deviations that affect the primary endpoint analysis. Protocol deviations will be assessed prior to database lock and unmasking. Subjects in the PP set will be analyzed as treated.

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.

Unit of Analysis:

The study eye will be used for all monocular efficacy analyses. The qualified fellow eye will be used in additional binocular analyses as specified. Both eyes will be displayed and analyzed for all ophthalmic safety variables.

The study eye will be defined at Visit 2 (Day 1) as the eye that meets all enrollment criteria. [redacted]

General Considerations:

In general, quantitative/continuous data will be summarized using descriptive statistics (number of subjects [n], mean, standard deviation [SD], median, minimum, and maximum). Qualitative/categorical data will be summarized using frequencies and percentages. Statistical testing, unless otherwise indicated, will be performed at a 2-sided 0.05 significance level.

For all variables, baseline is defined as the last measurement taken prior to the administration of first dose of study drug at Visit 2. Change from baseline will be calculated as follow-up measure minus baseline measure.

Missing Data:

The primary analysis will use the Full Analysis Set (FAS) with intercurrent events handled in the following manner:

1) Discontinuation of study drug and non-optimal dosing compliance (less than 80% or more than 125%) will be ignored [treatment policy strategy]. This will not cause any missing data and will be conservative to treatment effect.
2) Missing data due to withdrawal due to lack of efficacy will be imputed employing multiple imputations (MIs) using control treatment-based regression methods, regardless of randomized treatment, for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].
3) Missing data due to withdrawal due to adverse events will be imputed using worst observation carried forward method for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].

4) Missing data without withdrawal or with withdrawal due to reasons other than lack of efficacy or adverse events will be imputed employing MIs using randomized treatment-based regression methods for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].

Sensitivity analyses on the primary efficacy variable will be performed [redacted]

Hypotheses

The primary efficacy hypotheses are as follows:

H_{01}: The difference, between study eyes treated with LNZ101 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2 (Day 1), 3 hours = 0.

H_{11}: The difference, between study eyes treated with LNZ101 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from the baseline measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2, 3 hours ≠ 0.

H_{02}: The difference, between study eyes treated with LNZ100 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2 (Day 1), 3 hours = 0.

H_{12}: The difference, between study eyes treated with LNZ100 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from the baseline measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2, 3 hours ≠ 0.

[redacted]

The primary hypothesis for both treatments will be tested using Hochberg's step-up method with familywise error rate controlled at two-sided 0.05 significance level. The details of the method will be provided in the SAP. The study will be considered a success if either or both of the null hypotheses are rejected in favor of the corresponding alternative hypothesis.

If the primary endpoint for both treatments is positive, then the secondary endpoints within each treatment will be tested in a hierarchical order at 2-sided 0.05 significance level. If only one of the two treatments is positive, then the secondary endpoints will be tested in a hierarchical order in the respective treatment at 2-sided 0.025 significance level. If none of the treatments are positive, then secondary endpoints will not be tested. If at any point in the hierarchical order within a treatment (LNZ101 or LNZ100), statistical significance is not demonstrated, testing will stop within that treatment. LNZ101 vs LNZ100 overall comparison at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) in that order will be tested if either one of the treatments is positive for all other secondary endpoints.

Sample Size

A sample size of 435 subjects (145 per arm) yields >99% power to establish superiority of LNZ101/LNZ100 to Brimonidine in the proportion of study eyes demonstrating a ≥ 3-line (15-letter) improvement or greater from baseline in BCDVA at 40 cm and no loss in BDVA ≥ 1 line (5 letters) at 4 m at the 3-hour post-treatment time point on Day 1 using a Pearson chi-squared test with a 2-sided significance level of 0.05 assuming a response rate of 60% in LNZ101/LNZ100 and 6% in Brimonidine.

[redacted]

Primary Efficacy Analyses

The primary efficacy endpoint in this study is the percentage of study eyes with a ≥ 3-line (15-letter) improvement in BCDVA at 40 cm from baseline and no loss in best distance corrected visual acuity ≥ 1 line (5-letter) at 4 m at 3-hour post-treatment at Visit 2 (Day 1). The primary analysis will use the FAS with missing data imputations as detailed above within the Missing Data section.

Descriptive statistics will be presented by treatment group. Testing of the primary endpoint will be completed using logistic regression with treatment as the fixed effect and baseline BCDVA at 40 cm as a covariate. The adjusted odds ratios and marginal proportions and differences in proportions along with corresponding two-sided 95% confidence intervals (CIs) and p-values will be presented.

Treatment comparisons will also be made using Pearson's chi-squared test or Fisher's exact test if any of the expected cell counts are less than five as a sensitivity analysis to the primary model above.

Analyses will be repeated using the FAS with missing data imputed as failures and FAS and PP sets with observed data. Tipping point analysis may be performed.

Secondary Efficacy Analysis

Summaries and analyses for the secondary efficacy endpoints will be conducted using the same imputation strategy and statistical models as the primary efficacy endpoint. Analyses will be repeated using the FAS with observed data only.

[redacted]

Exploratory Efficacy Analysis

The categorical exploratory efficacy endpoints will be analyzed using the same model used for the primary endpoint.

[redacted]

Safety Analysis

All safety data will be analyzed using the Safety set. [redacted]

Verbatim descriptions of AEs will be mapped to Medical Dictionary for Regulatory Activities (MedDRA) terms and be presented in a data listing. Treatment emergent AEs (TEAEs), those that occur after the first dose of study drug, will be summarized by treatment group using frequency and percent for each system organ class (SOC) and preferred term (PT) within each SOC. Similar summaries will also be presented for expected and unexpected TEAEs, treatment emergent SAEs, TEAEs related to the study drug, and TEAEs by severity. When reporting the incidence of AEs, a subject will only be counted once if they ever experience an event within the SOC and ever experience the individual PT within each treatment group. Ocular and non-ocular events will be summarized separately.

Actual results and changes from baseline results in monocular and binocular BCDVA(normal and low-luminance), slit lamp biomicroscopy, IOP, dilated fundus examination, and conjunctival redness will be summarized descriptively at each visit by treatment group.

Full details of the safety analyses will be specified in the formal SAP.

Summary of Known and Potential Risks and Benefits to Human Subjects

Approved pharmacologic treatment for presbyopia is limited. Pilocarpine ophthalmic solution 1.25% is the only approved pharmacologic treatment for presbyopia in adults to date. There are currently no marketed Aceclidine-Brimonidine combination products. A 2% concentration of Aceclidine was approved in several European countries in 1969 for the treatment of glaucoma, and Aceclidine has been used in humans at concentrations of up to 4%. Possible AEs associated with Aceclidine include redness or brow ache (Romano 1970, Randazzo et al. 2005). Fewer adverse effects have been reported with Aceclidine (no angle closure, fewer subjects reporting pain on instillation, greater comfort with long-term use, and no tachyphylaxis) compared to other miotics, like pilocarpine (Francois and Goes 1977; Romano 1970).

Brimonidine was approved in the U.S. for human use in 1996 for the lowering of intraocular pressure in patients with open-angle glaucoma or ocular hypertension. It is commercially available in the U.S. at up to 0.2% concentration. Dosing at this concentration has been shown to be generally safe and effective. Adverse ocular effects of Brimonidine include possible discomfort, irritation, blurred vision, and light sensitivity.

In addition, vitreous floaters have also been reported.

The concentrations proposed for use in this study (Aceclidine 1.75% and Brimonidine 0.08%) are substantially lower than the concentrations in common clinical use for both of these active ingredients.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADHD	Attention-deficit/hyperactivity disorder
AE	Adverse event
BID	Twice daily
BCDVA	Best-corrected distance visual acuity
cd/m^2	Candela per square meter
CFR	Code of Federal Regulations
CI	Confidence interval
eCRF	Electronic case report form
D	Diopter
ETDRS	Early Treatment of Diabetic Retinopathy Study
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IND	IInvestigational new drug
IOL	ntraocular lens
IOP	Intraocular pressure
IP	Investigational product
IRB	Institutional review board
IUD	Intrauterine device
HIPAA	Health Information Portability and Accountability Act
LASEK	Laser-assisted epithelial keratomileusis
LASIK	Laser-assisted in-situ keratomileusis
logMAR	Logarithm of the minimum angle of resolution
MedDRA	Medical Dictionary for Regulatory Activities
mmHG	Millimeters of mercury
NSAID	Non-steroidal anti-inflammatory drug
OTC	Over-the-counter
PP	Per protocol
PRK	Photorefractive keratectomy
PT	Preferred term
RGP	Rigid gas permeable
SAE	Serious adverse event

Abbreviation	Definition
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
TEAE	Treatment-emergent adverse event
VA	Visual acuity

2.0 INTRODUCTION

Presbyopia is defined by a loss in the ability of the eye to adjust its focal length so that objects at different distances produce focused images on the retina. As the eye ages, the lens of the eye becomes less able to change shape. Hardening of the lens, along with weakening of the ciliary muscles, plays a major role in the pathogenesis of presbyopia and leads to symptoms such as blurred vision, eye strain and headache after reading or computer use (Truscott 2009; Helmholtz 1855; Ostrin and Glasser 2007; Scarcelli 2011).

Presbyopia affects most people over 45 years of age and continues to worsen until approximately 65 years of age. Based on data from 228 countries, it was estimated that in 2005, presbyopia affected more than 1 billion people worldwide. Presbyopia is projected to increase to 1.78 billion people by 2050. More than half of the people with this condition do not have adequate treatment options, which results in some level of disability when performing tasks that require near visual acuity (Holden 2008).

Current treatments available for presbyopia include the use of reading glasses, contact lenses, or refractive surgery (including laser) or intraocular lens (IOL) surgery. Pharmaceutical therapies have been explored but, thus far, continue to be limited in treating presbyopia without being associated with undesirable adverse effects (Gilmartin 1995). Pilocarpine ophthalmic solution 1.25% is the only approved pharmacologic treatment for presbyopia in adults to date.

Both Aceclidine and Brimonidine have been used extensively in humans for ophthalmic indications. Aceclidine is a parasympathomimetic miotic agent that is approved for lowering IOP in Europe. In 1969, a 2% solution (dosed four times a day) of Aceclidine was approved in several European countries for the treatment of glaucoma. Furthermore, Aceclidine has been used at concentrations of up to 4% in humans. Brimonidine is an alpha adrenergic drug that was approved for human use in the US in 1996. Brimonidine has a dual mechanism of action by reducing aqueous humor production and increasing uveoscleral outflow. Brimonidine at concentrations of up to 0.2% typically dosed twice daily has been shown to be generally safe and effective. Notably, the concentrations of Aceclidine and Brimonidine in LNZ-101 formulation in this study (Aceclidine 1.75% and Brimonidine 0.08%) are substantially lower than the concentrations of these agents that are in clinical use.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of the study is to evaluate the safety and efficacy of LNZ101 compared with LNZ100 and Brimonidine for the treatment of Presbyopia.

4.0 CLINICAL HYPOTHESES

The clinical hypothesis of this study is that LNZ101 is superior to LNZ100 (Aceclidine non-preserved product) and Brimonidine in improving near vision in subjects with presbyopia.

5.0 OVERALL STUDY DESIGN

This is a five-visit randomized, double-masked, multi-center, parallel-group study evaluating the safety and efficacy of LNZ101 compared to LNZ100 and Brimonidine in approximately 435 subjects with presbyopia.

Visit 1 (Screening, Day -60 to -4): Subjects will be screened through ophthalmic assessments using best corrected distance visual acuity (BCDVA) tests. Subjects who remain qualified will receive 2 drops of Refresh® Plus Eye Drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye, and BCDVA at 40 cm will be retested 30 minutes post-dosing.

Visit 2 (Randomization, Day 1): Visit 1 ophthalmic assessments will be repeated at Visit 2 per the Schedule of Visits and Measurements. Following the pre-treatment, baseline assessments, qualified subjects will be randomized at a 1:1:1 ratio to one of the following study arms:

- LNZ101 –Aceclidine 1.75% and Brimonidine 0.08% combination (non-preserved) ophthalmic solution
- LNZ100 – Aceclidine 1.75% (non-preserved) ophthalmic solution
- Brimonidine (0.08%) ophthalmic solution

An ophthalmic technician will administer dose in office. Primary efficacy and safety assessments will be performed.

All subjects will be instructed for home use to dose each eye once daily (QD, i.e. at 8 am ± 2 hours). For each dosing, subjects are instructed to administer 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye.

Visit 3 (Day 15 ± 2 days): Ophthalmic technician will administer dose in office. Primary efficacy and safety assessments will be performed.

Visit 4 (Day 28 ± 2 days): Ophthalmic technician will administer dose in office. Primary efficacy and safety assessments will be performed.

Visit 5 (Day 42 ± 2 days) End of Study Visit / Early Termination (ET) Visit: Study drugs will be collected. Safety assessments will be performed.

6.0 STUDY POPULATION

Approximately 435 healthy adult subjects from 45 to 75 years of age (inclusive) with presbyopia who do not have any conditions, in the investigator's opinion, that may put the subject at increased risk, confound study data, or interfere significantly with the subject's study participation.

6.1 Inclusion Criteria

Subjects must:

1. Be able and willing to provide written informed consent and sign a Health Information Portability and Accountability Act (HIPAA) form prior to any study procedure being performed;

2. Be able and willing to follow all instructions and attend all study visits;
3. Be 45-75 years of age of either sex and any race or ethnicity at Visit 1;
4. Have +1.00 to -4.00 diopters (D) of sphere (calculated in minus cylinder) with a spherical equivalent (SE) that is no more myopic than -4.00 D MRSE in both eyes determined by manifest refraction documented at Visit 1;
5. Have ≤2.00 D of cylinder (minus cylinder) in both eyes determined by manifest refraction documented at Visit 1;
6. Be presbyopic with monocular BCDVA at 40 cm ≥ 0.42 logMAR (approximately $20/50^{-2}$ Snellen), assessed prior to placebo run-in at Visit 1 (in at least one eye), and at Visit 2 baseline (in the study eye);
7. Have monocular BCDVA at 4 m of 0.1 logMAR (approximately 20/25 Snellen) or better assessed prior to placebo run-in at Visit 1 (in at least one eye), and at Visit 2 baseline (in the study eye);
8. For pseudophakic subjects, intraocular lens (IOL) must be confirmed monofocal and with no significant posterior capsular opacification (PCO);

   Note: Subjects must also qualify based on all other IE criteria, including having a pre-IOL refraction and/or prescription that meets inclusion criteria #4 (refractive parameters for the study);
9. Have a negative pregnancy test at Visit 1 and 2, if female of childbearing potential (those who have experienced menarche and who are not surgically sterilized [bilateral tubal ligation, hysterectomy, or bilateral oophorectomy] or post-menopausal [12 months after last menses]) and must use adequate birth control throughout the study period. Adequate birth control is defined as hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as condom or diaphragm; intrauterine device (IUD); or surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control;
10. Be able and willing to avoid all disallowed medications for the appropriate washout period and during the study without significant risk to the subject.

6.2 Exclusion Criteria

1. Subject must not be a female of childbearing potential who is currently pregnant, nursing, or planning a pregnancy;
2. Subject must not have known contraindications or sensitivity to the use of any of the study medications or their components;
3. Neither eye can have an active ocular infection at Visit 1 or at Visit 2 (bacterial, viral, or fungal), positive history of an ocular herpetic infection, preauricular lymphadenopathy, or ongoing, active ocular inflammation (e.g., moderate to severe blepharitis, allergic conjunctivitis, peripheral ulcerative keratitis, scleritis, uveitis);

4. Neither eye can have moderate or severe dry eye defined as corneal fluorescein staining superior, inferior and central (combined) > 2 ([redacted] Calibra™ Scale) at Visit 1[i];
5. Neither eye can have clinically significant abnormal lens findings (e.g., cataract) including early lens changes and/or any evidence of a media opacity during dilated slit-lamp biomicroscopy and fundus exam documented within 3 months of Visit 1 or at Visit 1;
6. Neither eye can have > +0.75 D hyperopic shift in sphere from manifest refraction compared to cycloplegic refraction documented at Visit 1;
7. Subject must not have a > 0.2 logMAR (i.e., 10-letters) difference between eyes as determined by screening monocular BCDVA at 40 cm at Visit 1 or at Visit 2 baseline;
8. Subject must not have < 0.3 logMAR (i.e., 15 letters) improvement in monocular BCDVA at 40 cm with +1.50 D Sphere Add compared to monocular BCDVA at 40 cm at Visit 1 (in at least one eye) and at Visit 2 baseline (in the study eye);
9. Neither eye can have > 0.14 logMAR (i.e., 7 letters) improvement in placebo post-treatment monocular BCDVA at 40 cm compared to screening monocular BCDVA at 40 cm at Visit 1;
10. Neither eye can have average dark-adapted pupillometry measurements of < 4.2 mm at Visit 1;
11. Neither eye can have intraocular pressure (IOP) that is < 5 millimeters of mercury (mmHg) or > 22 mmHg documented at Visit 1, or have a prior diagnosis of ocular hypertension or glaucoma or currently being treated with any type of topical IOP lowering (glaucoma) medication at Visit 1;
12. Neither eye can have clinically significant abnormal findings on dilated fundus exam documented within 3 months of Visit 1 or at Visit 1, or a known history of retinal detachment or clinically significant retinal disease;
13. Neither eye can have a known history or past diagnosis of iritis, scleritis, or uveitis, whether active or inactive;
14. Subject must not have had surgical intervention (ocular or systemic) within 6 months prior to Visit 1, or planned surgical intervention during the study;
15. Subject must not have undergone prior LASIK or PRK surgery within 12 months of Visit 1:

    Subjects who have undergone LASIK or PRK more than 12 months prior to Visit 1 must qualify based on all other IE criteria, including having a pre-LASIK or pre-PRK prescription and/or refraction that meets Inclusion Criteria 4;
16. Subject must not use artificial tears or lubricant eye ointment on a daily basis;

[i] Punctal plugs are acceptable if they have been in place for a minimunm of 30 days prior to visit 1.

17. Subject must not have planned use of artificial tears or lubricant eye ointment on the day of any study visits;
18. Subject must not have an inability or refuse to discontinue soft contact lens wear 7 days prior to study Visit 1 and rigid gas permeable (RGP) contact lens wear 14 days prior to Visit 1 and during the study;
19. Subject must not use any of the following disallowed medications during the 2 weeks (14 days) prior to Visit 1 and during the study:
    a. narcotic (opiate class) pain medication (e.g., codeine, OxyContin®, Vicodin®, Tramadol®)
    b. bladder medication (e.g. Urecholine®, bethanechol, oxybutynin, tolterodine)
    c. antipsychotics
    d. antidepressants
    e. attention–deficit/hyperactivity disorder (ADHD) medications
    f. alpha-blockers (e.g., tamsulosin, Flomax®, Jayln®, Uroxatral®, Rapaflo®)
    g. anticholinergics (e.g., atropine, belladonna, benztropine, dicyclomine, donepezil, hyoscyamine, propantheline, scopolamine, trihexyphenidyl)
    h. muscarinic receptor agonists or cholinergic agonists (e.g., Salagen®, Evoxac®)
    i. over-the-counter (OTC) or prescription antihistamines or decongestants
    j. any prescribed topical ophthalmic medications
    k. recreational drug use (e.g., marijuana, methadone, heroin, cocaine);
    l. any medications that are known to cause mydriasis or miosis, including, but not limited to:
        i. Buscopan®
        ii. Procyclidine
        iii. L-DOPA
        iv. amphetamine
        v. scopolamine
        vi. physostigmine
        vii. neostigmine
        viii. guanethidine
        ix. primidone
        x. Wegovy®, Ozempic®, Rybelsus® (or any other form of semaglutide)
        xi. Mounjaro® (tirzepatide)

20. Subject must not have been dosed in an investigational drug or device study within 1 month of Visit 1;
21. Neither eye can have undergone any prior corneal surgery that is not described and allowed in other IE criteria;
22. Neither eye can have undergone any prior retinal surgery, laser treatment or conventional surgery for retinal hole and retinal tears;
23. Subject must not have a diagnosis of diabetes mellitus or an elevated blood sugar greater than 150mg/dl, or HbA1c of greater than or equal to 6.5% as measured within the past 3 months[ii];
24. Subject must not have a condition or a situation, which in the Investigator's opinion, may put the subject at increased risk, confound study data, or interfere significantly with the subject's study participation, including but not limited to unstable: cardiovascular, hepatic, renal, respiratory, gastrointestinal, endocrine, immunologic, dermatologic, hematologic, neurologic, or psychiatric disease;
25. Subject must not have been dosed with investigational drug in LENZ study 22-150-0017, 22-150-0018, or 22-100-0007.

6.3 Cohort Assignment

At Visit 2, approximately 435 subjects will be enrolled based on study eye to one of the following cohorts:

Cohort 1 (Approximately 20% of population): Have baseline monocular (in study eye) BCDVA at 40 cm greater than or equal to 0.42 and less than 0.50 logMAR (approximately between $20/50^{-2}$ and 20/63 Snellen) at Visit 2 .

Cohort 2 (Approximately 80% of population): Have baseline monocular (in study eye) BCDVA at 40 cm greater than or equal to 0.50 logMAR (approximately 20/63 Snellen) at Visit 2 .

If a subject has one eye that meets the criteria for Cohort 1 and the other eye meets the criteria for Cohort 2, selection of the study eye is at the discretion of the Investigator.

6.4 Withdrawal Criteria

Subjects will be withdrawn from the study if any of the following criteria are met:

- Be a female of childbearing potential who is currently pregnant, nursing, or planning a pregnancy; tests positive to a pregnancy test; or refuses to use an adequate method of contraception for the duration of the study;
- Have an active ocular infection (bacterial, viral, or fungal), active ocular inflammation (e.g. moderate to severe blepharitis, allergic conjunctivitis, peripheral ulcerative keratitis, scleritis, uveitis) in either eye.

[ii] Any blood sugar in an otherwise normal, non fasting blood chemistry panel that is greater than 150mg/dl would be considered abnormal and indicative of a diabetic or prediabetic state and is exclusionary.

- Subjects may also be withdrawn from the study for the following reasons:
  - Adverse event (AE)
  - Lost to follow-up
  - Withdrawal of consent by subject
  - Investigator's discretion
  - Death
  - Subject not adequately following required study procedures
  - Study terminated by the Sponsor
  - Other

Subject withdrawals will be documented on the subject's source document.

7.0 STUDY PARAMETERS

7.1 Efficacy Endpoints

Primary Efficacy Endpoint:

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) at 3 hours post-treatment in the study eye at Visit 2 (Day 1) for each LNZ treatment relative to brimonidine.

Secondary Efficacy Endpoints

All secondary endpoints will be comparisons of each LNZ treatment relative to brimonidine unless otherwise specified.

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at:

  Onset of Action

  a) 30 minutes post-treatment

  Duration of Action

  b) 1 hour post-treatment
  c) 5 hours post-treatment
  d) 7 hours post-treatment
  e) 8 hours post-treatment
  f) 9 hours post-treatment
  g) 10 hours post-treatment

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at:
    a) 30 minutes post-treatment
    b) 1 hour post-treatment
    c) 3 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at:
    a) 30 minutes post-treatment
    b) 1 hour post-treatment
    c) 3 hours post-treatment
    d) 5 hours post-treatment
    e) 7 hours post-treatment
    f) 8 hours post-treatment
    g) 9 hours post-treatment
    h) 10 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) between LNZ101 and LNZ100.

Exploratory Efficacy Endpoints:

- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment

- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 1, 3, and 10 hours post-treatment

- Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 1, 3, and 10 hours post-treatment
- [redacted]
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment at Visit 2 (Day 1)
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, and 3 hours post-treatment at Visit 3 (Day 15)
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment at Visit 4 (Day 28)
- [redacted]

Note that the baseline value for all assessments performed at Visits 3 and 4 is the pre-treatment value at Visit 2.

Criteria for Efficacy

Changes in BCDVA at 40 cm distance will be calculated as the difference, in logMAR units, between the post-treatment monocular (study eye) minus the pre-treatment baseline monocular (study eye) at Visit 2. A 3-line improvement or greater in BCDVA at 40 cm is considered clinically meaningful.

7.2 Safety Endpoints

- Adverse events (AE) (reported, elicited, and observed)
- Pregnancy test (at Visit 1, Visit 2, and Visit 5)
- Monocular and binocular BCDVA (normal and low-luminance)
- Slit lamp biomicroscopy
- IOP
- Dilated fundus exam (at Visit 1 and Visit 5)
- Conjunctival redness

8.0 STUDY MATERIALS

8.1 Study Treatments

8.1.1 Investigational Product

The study treatments are as follows:

- LNZ101 –Aceclidine 1.75% and Brimonidine 0.08% combination (non-preserved) ophthalmic solution
- LNZ100 – Aceclidine 1.75% (non-preserved) ophthalmic solution
- Brimonidine (0.08%) ophthalmic solution

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

Brimonidine ophthalmic solution will be provided in a sterile container.

8.1.2 Instructions for Use and Administration

In order to maintain masking to the study drug administrator and study subject, the LNZ-101, LNZ100, and Brimonidine treatments will be prepared in sterile containers having an identical appearance. The sterile containers will be identical in size and color and have identical clinical labels (except for the subject number and Visit number).

The treatment will be administered by a trained study technician via instillation of 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye. If the drop is not properly instilled in each eye (e.g., subject blinks during instillation), the technician will administer another drop to ensure each drop is properly instilled. The same treatment should be administered to both eyes.

A new clinical kit will be assigned to each subject at Visits 2, 3, and 4.

Both the technician who administers the treatment and the subject will be masked to the treatment identity.

8.2 Subject Instructions

After randomization at Visit 2, subjects will receive IP and a dosing diary. [redacted]

Subjects should not dose the morning of any study visits. At all study visits post-randomization, study site personnel will administer the dose at the visit after the required pre-treatment assessments are completed. Efficacy and safety measures will follow administration of IP. Subjects will exit the study after all assessments are complete at Visit 5.

9.0 STUDY METHODS AND PROCEDURES

9.1 Subject Entry Procedures

9.1.1 Overview

Subjects as defined by the criteria in Section 6.1 and Section 6.2 will be considered for entry into this study.

A subject may be re-screened to allow for adequate washout of disallowed medications. Re-screening for any other reason requires advanced discussion and agreement by the Sponsor. Re-screened subjects should be assigned a new screening number.

9.1.2 Informed Consent

Prior to a subject's participation in the trial (e.g. changes in a subject's medical treatment and/or study related procedures), the study will be discussed with each subject, and subjects wishing to participate must give written informed consent (and/or assent) using an informed consent form (ICF). The ICF must be the most recent version that has received approval/favorable review by a properly constituted IRB.

9.1.3 Washout Intervals

- [redacted]

9.1.4 Procedures for Final Study Entry

Subjects must satisfy all inclusion and none of the exclusion criteria in order to be entered into the study.

9.1.5 Pregnancy

Females must have a negative pregnancy test at Visit 1 and Visit 2, if female of childbearing potential (those who have experienced menarche and who are not surgically sterilized [bilateral tubal ligation, hysterectomy or bilateral oophorectomy] or post-menopausal [12 months after last menses]) and must use adequate birth control throughout the study period. Adequate birth control is defined as hormonal – oral, implantable, injectable, or transdermal contraceptives; mechanical – spermicide in conjunction with a barrier such as condom or diaphragm; intrauterine device (IUD); or surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control.

In the event a female has a positive pregnancy test, the subject will be withdrawn from the study and the Investigator will notify [redacted] the sponsor within 24 hours of knowledge of the event.

9.1.6 Methods for Assignment to Treatment Groups

Each subject who signs an 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 at Visit 2, he/she will be randomized in a 1:1:1 ratio via an interactive response technology system to 1 of 3 treatment groups (1: LNZ101 or 2: LNZ100 or 3: Brimonidine). [redacted]

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 evenly balanced across treatment groups, and to enhance the 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.

9.2 Concurrent Therapies and Medical History

The use of any concurrent medication, prescription, or over-the counter taken within 30 days of Visit 1, is to be recorded on the subject's source document and corresponding electronic case report form (eCRF) along with the reason the medication was taken.

All significant current and prior ocular medical and surgical history is to be recorded on the subject's source document and corresponding eCRF. All current and prior significant general medical and surgical history is to be recorded on the subject's source document and corresponding eCRF.

Concurrent enrollment in another investigational drug or medical device study is not permitted.

9.2.1 Prohibited Medications/Treatments

Washout intervals as described in Section 9.1.3 should be followed for all prohibited medications. Soft contact lenses must be removed at least 7 days prior to study Visit 1 and during the study, and rigid gas permeable (RGP) contact lenses must be removed at least 14 days prior to study Visit 1 and during the study.

9.2.2 Escape Medications

Not applicable.

9.2.3 Special Diet or Activities

Not applicable.

9.3 Examination Procedures

9.3.1 Procedures to be Performed at the Study Visit with Regard to Study Objective

The following procedures should be conducted at each study visit in the following order.

9.3.1.1 Visit 1 (Day -60 to -4): Screening Visit

1. Informed consent / HIPAA
2. Demographic data
3. Medical and medication history
4. Inclusion and exclusion criteria review
5. Urine pregnancy test (for females of child-bearing potential)
6. Dark adapted pupillometry
7. Manifest refraction and BCDVA at 4 meters: Visual acuity to be assessed with best distance-correction determined by the manifest refraction within 3 months of Visit 1 or at Visit 1
8. Pre-treatment monocular near BCDVA at 40 cm (ETDRS chart)
9. Pre-treatment monocular near BCDVA at 40 cm with +1.50 D add (ETDRS chart)
10. Pre-treatment monocular BCDVA at 4 m (ETDRS)
11. Pre-treatment slit lamp biomicroscopy
12. Pre-treatment conjunctival redness assessment
13. Instillation of Refresh® Plus Eye Drops-All subjects will receive 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye.
14. Drop instillation assessment
15. Post-treatment monocular BCDVA at 40 cm (ETDRS chart) (30 minutes post-placebo)
16. Post-treatment binocular BCDVA at 40 cm (ETDRS chart) (30 minutes post-placebo)

17. Fluorescein staining
18. IOP
19. Cycloplegic Refraction
20. Dilated indirect fundoscopy
21. End of visit slit lamp biomicroscopy
22. End of visit AE query

9.3.1.2 Visit 2 (Day 1) Randomization

1. Medical and medication history update
2. AE query
3. Urine pregnancy test (for females of child-bearing potential)
4. Inclusion and exclusion criteria review
5. Subjects will listen to instructions read aloud about what will be completed at each visit prior to beginning baseline/pre-treatment assessments.
6. Pre-treatment light adapted pupillometry under near VA at 40 cm testing conditions
7. Pre-treatment monocular near BCDVA at 40 cm (ETDRS chart)
8. Pre-treatment binocular near BCDVA at 40 cm (ETDRS chart)
9. Pre-treatment monocular near BCDVA at 40 cm with +1.50 D add (ETDRS chart)
10. Pre-treatment monocular near BCDVA at 66 cm (ETDRS chart)
11. Pre-treatment monocular BCDVA at 4 m (ETDRS chart)
12. Pre-treatment binocular BCDVA at 4 m (ETDRS chart)
13. Pre-treatment monocular LL-BCDVA at 4 m (ETDRS chart)
14. Pre-treatment binocular LL-BCDVA at 4 m (ETDRS chart)
15. Pre-treatment slit lamp biomicroscopy
16. Pre-treatment conjunctival redness assessment
17. Selection of study eye and Cohort Assignment
18. Randomization
19. Dispense IP
20. Instill IP

    Instillation of LNZ101 – 2 drops OU, LNZ100 – 2 drops OU, or Brimonidine – 2 drops OU

    - Subjects will receive 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye.

- [redacted].

21. Study drug drop instillation assessment
22. Post-treatment light adapted pupillometry under near VA at 40 cm testing conditions (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
23. Post-treatment monocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
24. Post-treatment binocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, 3, 7, 8, 9, and 10h post-treatment)
25. Post-treatment monocular BCDVA at 66 cm (ETDRS chart) (1, 3, and 10h post-treatment
26. Post-treatment monocular BCDVA at 4 m (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
27. Post-treatment binocular BCDVA at 4 m (0.5, 1, 3, 7, 8, 9, and 10h post-treatment)
28. Post-treatment monocular LL-BCDVA at 4 m (1, 3, and 10h post-treatment)
29. Post-treatment binocular LL-BCDVA at 4 m (1, 3, and 10h post-treatment)
30. AE query (3h post-treatment)
31. PRO Questionnaire (5h post-treatment)
32. Post-treatment conjunctival redness assessment (3 and 10h post-treatment)
33. End of visit slit lamp biomicroscopy
34. IOP
35. End of visit AE query
36. Provide Dosing Diary and dispensed IP kit

9.3.1.3 Visit 3 (Day 15 ±2 days)

1. Collect IP kit/Dosing Diary from previous visit
2. Medical and medication history update
3. AE query
4. Subjects will listen to instructions read aloud about what will be completed at each visit prior to beginning baseline/pre-treatment assessments.
5. Pre-treatment light adapted pupillometry under near VA at 40 cm testing conditions
6. Pre-treatment monocular near BCDVA at 40 cm (ETDRS chart)
7. Pre-treatment binocular near BCDVA at 40 cm (ETDRS chart)
8. Pre-treatment monocular BCDVA at 4 m (ETDRS chart)

9. Pre-treatment binocular BCDVA at 4 m (ETDRS chart)
10. Pre-treatment slit lamp biomicroscopy
11. Pre-treatment conjunctival redness assessment
12. Dispense IP
13. Instill IP

    Instillation of LNZ101 – 2 drops OU, LNZ100 – 2 drops OU, or Brimonidine – 2 drops OU

    - Subjects will receive 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye
    - [redacted]
14. Study drug drop instillation assessment
15. Post-treatment light adapted pupillometry under near VA at 40 cm testing conditions (0.5, 1 and 3 h post-treatment)
16. Post-treatment monocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, and 3h post-treatment)
17. Post-treatment binocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, and 3h post-treatment)
18. Post-treatment monocular BCDVA at 4 m (0.5, 1 and 3h post-treatment)
19. Post-treatment binocular BCDVA at 4 m (0.5, 1 and 3h post-treatment)
20. Post-treatment conjunctival redness assessment (3h post-treatment)
21. End of visit slit lamp biomicroscopy
22. IOP
23. End of visit AE query
24. Provide Dosing Diary and dispensed IP kit

9.3.1.4 Visit 4 (Day 28 ±2 days)

1. Collect IP kit/Dosing Diary from previous visit
2. Medical and medication history update
3. AE query
4. Subjects will listen to instructions read aloud about what will be completed at each visit prior to beginning baseline/pre-treatment assessments.
5. Pre-treatment light adapted pupillometry under near VA at 40 cm testing conditions
6. Pre-treatment monocular near BCDVA at 40 cm (ETDRS chart)
7. Pre-treatment binocular near BCDVA at 40 cm (ETDRS chart)

8. Pre-treatment monocular near BCDVA at 66 cm (ETDRS chart)
9. Pre-treatment monocular BCDVA at 4 m (ETDRS chart)
10. Pre-treatment binocular BCDVA at 4 m (ETDRS chart)
11. Pre-treatment monocular LL-BCDVA at 4 m (ETDRS chart)
12. Pre-treatment binocular LL-BCDVA at 4 m (ETDRS chart)
13. Pre-treatment slit-lamp biomicroscopy
14. Pre-treatment conjunctival redness assessment
15. Dispense IP
16. Instill IP

    Instillation of LNZ101 – 2 drops OU, LNZ100 – 2 drops OU, or Brimonidine – 2 drops OU

    - Subjects will receive 2 drops bilaterally, 1 drop in each eye followed by 2 minutes later, another drop in each eye
    - [redacted]
17. Study drug drop instillation assessment
18. Post-treatment light adapted pupillometry under near VA at 40 cm testing conditions (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
19. Post-treatment monocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
20. Post-treatment binocular BCDVA at 40 cm (ETDRS chart) (0.5, 1, 3, 7, 8, 9, and 10h post-treatment)
21. Post-treatment monocular BCDVA at 66 cm (ETDRS chart) (1, 3, and 10h post-treatment)
22. Post-treatment monocular BCDVA at 4 m (0.5, 1, 3, 5, 7, 8, 9, and 10h post-treatment)
23. Post-treatment binocular BCDVA at 4 m (0.5, 1, 3, 7, 8, 9, and 10h post-treatment)
24. Post-treatment monocular LL-BCDVA at 4 m (1, 3, and 10h post-treatment)
25. Post-treatment binocular LL-BCDVA at 4 m (1, 3, and 10h post-treatment)
26. AE query (3h post-treatment)
27. PRO Questionnaire (5-h post-treatment)
28. Post-treatment conjunctival redness assessment (3 and 10h post-treatment)
29. End of visit slit-lamp biomicroscopy
30. IOP

31. End of visit AE query
32. Provide Dosing Diary and dispensed IP kit

9.3.1.5 Visit 5 (Day 42 ±2 days) End of Study Visit / Early Termination (ET) Visit

1. Collect IP kit/Dosing Diary from previous visit
2. Medical and medication history update
3. AE query
4. Urine pregnancy test (for females of child-bearing potential)
5. Dark adapted pupillometry
6. Light adapted pupillometry under near VA at 40 cm testing conditions
7. Monocular near BCDVA at 40 cm (ETDRS chart)
8. Binocular near BCDVA at 40 cm (ETDRS chart)
9. Monocular BCDVA at 4 m (ETDRS chart)
10. Conjunctival redness assessment
11. Slit lamp biomicroscopy
12. IOP
13. Dilated indirect fundoscopy
14. End of visit Slit lamp biomicroscopy
15. End of visit AE query
16. Study exit

Participants who prematurely discontinue from the study after administration of the study drug will be encouraged to complete an early termination (ET) visit.

9.4 Schedule Of Visits, Measurements and Dosing

9.4.1 Scheduled Visit

Refer to Appendix 1: Schedule of Visits and Measurements for a schedule of measurements at each visit.

9.4.2 Unscheduled Visits

In the case of an AE, an Unscheduled Visit may be performed by the Investigator. Unscheduled Visit assessments can include any of the following:

- Medical/medication history
- Pregnancy test
- VA assessments
- Slit lamp biomicroscopy
- Conjunctival redness

- IOP
- Fluorescein staining
- AE query

The Investigator may perform additional assessments, if needed. All additional assessments will be documented in the subject's source document.

9.4.3 Early Termination

Participants who prematurely discontinue from the study after administration of the study drug will be encouraged to complete an early termination (ET) visit.

9.5 Compliance with Protocol

This study will be conducted in compliance with the protocol, the ethical principles that have their origin in the Declaration of Helsinki, the International Conference on Harmonization (ICH) consolidated Guideline E6 for Good Clinical Practice (GCP) (CPMP/ICH/135/95), and applicable regulatory requirement(s), such as Food and Drug Administration (FDA) GCP Regulations and Code of Federal Regulations (CFR) Title 21, parts 11, 50, 54, 56 and 312, as appropriate.

9.6 Subject Disposition

9.6.1 Screened Subjects

Subject who has completed the Informed Consent process and if eligible to proceed with additional assessments to ensure candidacy at Visit 1 (Screening Visit).

9.6.2 Screen failed Subjects

Screened subject who does not meet the inclusion/exclusion criteria at any time prior to randomization at Visit 2.

9.6.3 Randomized Subjects

Subject who has signed the ICF, been issued a unique 6-digit subject number and has been randomized to receive investigational product at Visit 2. Once randomized, the subject is enrolled in the study.

9.6.4 Discontinued Subjects

Randomized subject who discontinues participation in the trial prior to the last study procedure at Visit 5.

Prior to discontinuing a subject, every effort should be made to obtain as much follow-up data as possible, and to retrieve all study materials. Adverse events (AEs) will be followed as described in Section 10.

9.6.5 Completed Subjects

A completed subject is one who has not been discontinued from the study.

9.6.6 Withdrawn Subjects

A subject may be withdrawn for meeting any of the withdrawal criteria as described in Section 6.3.

Prior to discontinuing a subject, every effort should be made to obtain as much follow-up data as possible, and to retrieve all study materials. Adverse events (AEs) will be followed as described in Section 10.0.

9.7 Study Termination

The study may be terminated at any time by the investigator, the sponsor, and/or [redacted] with appropriate notification.

9.8 Study Duration

This study is comprised of 5 visits over a total duration of approximately 9 weeks.

9.9 Monitoring and Quality Assurance

During the course of the study [redacted], or designee, will make routine site visits to review protocol compliance, assess IP accountability, and ensure the study is being conducted according to the pertinent regulatory requirements. The review of the subjects' medical records will be performed in a manner that adequately maintains subject confidentiality. Further details of the study monitoring will be outlined in a monitoring plan.

Regulatory authorities of domestic and foreign agencies, [redacted] quality assurance and/or its designees, and the study sponsor may carry out on-site inspections and/or audits that may include source data checks. Therefore, direct access to the original source data will be required for inspections and/or audits. All inspections and audits will be carried out giving consideration to data protection as well as subject confidentiality to the extent that local, state, and federal laws apply.

10.0 ADVERSE EVENTS

10.1 Adverse Event

Adverse events will be monitored throughout the study. At each visit, the Investigator, or designee, will query for adverse events by asking subjects general, non-directed questions such as:

[redacted]

Directed questioning and examinations will be done as appropriate.

An AE is defined as any untoward medical occurrence associated with the use of an 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 spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the subject's source document and eCRF. Any clinically relevant deterioration in clinical finding is considered an AE and must be recorded. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

Documentation regarding the AE should be made as to the nature, date of onset, end date, severity, relationship to IP, expectedness, action(s) taken, seriousness, and outcome of any sign or symptom observed by the physician or reported by the patient upon indirect questioning.

All AEs will be collected from the time a subject signs the ICF through the subject's study exit visit.

10.2 Severity

The 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 him/her by the patient/subject. The assessment of severity is made irrespective of relationship to IP 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.

10.3 Relationship to Investigational Product

The relationship of each AE to the IP 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.

"Suspected adverse reaction" means any AE for which there is a reasonable possibility that the IP caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the IP and the AE. Types of evidence that would suggest a causal relationship between the IP and the AE include: a single occurrence of an event that is uncommon and known to be strongly associated with IP exposure (e.g. angioedema, hepatic injury, Stevens-Johnson Syndrome); one or more occurrences of an event that is not commonly associated with IP exposure, but is otherwise uncommon in the population exposed to the IP (e.g. tendon rupture); an aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the IP-treatment group than in a concurrent or historical control group.

10.4 Expectedness

The expectedness of an AE should be determined based upon existing safety information about the IP using these explanations:

- *Unexpected*: an AE that is not listed in the Investigator's Brochure (IB) or is not listed at the specificity or severity that has been observed.
- *Expected*: an AE that is listed in the IB at the specificity and severity that has been observed.
- *Not applicable:* an AE unrelated to the IP.

AEs that are mentioned in the IB as occurring with a class of products or as anticipated from the pharmacological (or other) properties of the product but are not specifically mentioned as occurring with the particular product under investigation are to be considered unexpected.

The investigator should initially classify the expectedness of an AE. The medical monitor will review and determine the expectedness of any serious adverse event (SAE) following the investigator's assessment. The final classification of an AE is subject to the sponsor's determination.

10.5 Serious Adverse Events

An AE is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening AE;

  Note: An AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization;

  Note: The term "inpatient hospitalization" refers to any inpatient admission (even if < 24 hours). For chronic or long-term inpatients, inpatient admission includes transfer within the hospital to an acute/intensive care inpatient unit. Inpatient hospitalization does not include: emergency room visits; outpatient/same-day/ambulatory procedures; observation/short stay units; rehabilitation facilities; hospice facilities; nursing homes; or clinical research/Phase 1 units.

  Note: The term "prolongation of existing hospitalization" refers to any extension of an inpatient hospitalization beyond the stay anticipated or required for the reason for the initial admission as determined by the investigator or treating physician.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;

Note: An SAE specifically related to visual threat would be interpreted as any potential impairment or damage to the subject's eyes (e.g. intra-ocular hemorrhage, retinal detachment, central corneal ulcer, or damage to the optic nerve).

- A congenital anomaly/birth defect.

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.6 Procedures for Reporting Adverse Events

All AEs and their outcomes must be reported [redacted] the study sponsor, and the IRB as required by the IRB, federal, state, or local regulations and governing health authorities and recorded on the appropriate subject source document and eCRF.

10.7 Reporting a Suspected Unexpected Adverse Reaction

All AEs that are 'suspected' and 'unexpected' are to be reported to [redacted] the study sponsor, and the IRB as required by the IRB, federal, state, or local regulations and governing health authorities.

10.8 Reporting a Serious Adverse Event

To ensure subject safety, all SAEs, regardless of relationship to the IP, must be immediately reported by the investigator [redacted] the sponsor within 24 hours of becoming aware of the event. All information relevant to the SAE must be recorded on the appropriate source document, SAE Report Form, and eCRF. The investigator is obligated to pursue and obtain information requested by [redacted] and/or the sponsor in addition to the information reported on the source document, SAE Report Form, and eCRF. All subjects experiencing a SAE must be followed-up with and the outcome reported.

In the event of a SAE, the investigator must notify [redacted] the sponsor immediately; obtain and maintain in his/her files all pertinent medical records, information, and medical judgments from colleagues who assisted in the treatment and follow-up of the subject; provide [redacted] the study sponsor with a complete case history, which includes a statement as to whether the event was or was not suspected to be related to the use of the IP; and inform the IRB of the AE within their guidelines for reporting SAEs.

Contact information for reporting SAEs:

Medical Monitor:	[redacted]
Project Manager:	[redacted]

10.9 Procedures for Unmasking (if applicable)

When medically necessary, the investigator may need to determine what treatment has been assigned to a subject. When possible (i.e. in non-emergent situations), [redacted] the study sponsor should be notified before unmasking the IP.

10.10 Type and Duration of the Follow-up of Subjects after Adverse Events

The investigator will follow unresolved AEs to resolution, until the subject is lost to follow-up or until the AE is otherwise explained. Resolution means the subject has returned to baseline state of health or the investigator does not expect any further improvement or worsening of the AE. If the subject is lost to follow-up, the investigator should make 3 reasonable attempts to contact the subject via telephone, post, or certified mail. All follow-up will be documented in the subject's source document. Non-serious AEs identified on the last scheduled contact must be recorded on the source document with the status noted.

If the investigator becomes aware of any new information regarding a SAE (i.e. resolution, change in condition, or new treatment), a new SAE Report Form must be completed and faxed/emailed to [redacted] the study sponsor within 24 hours. The original SAE Report Form is not to be altered. The SAE Report Form should describe whether the event has resolved or continues and how the event was treated.

11.0 STATISTICAL HYPOTHESES AND METHODS OF ANALYSES

11.1 General Considerations

In general, quantitative/continuous data will be summarized using descriptive statistics (number of subjects [n], mean, standard deviation [SD], median, minimum, and maximum). Qualitative/categorical data will be summarized using frequencies and percentages. Statistical testing, unless otherwise indicated, will be performed at a 2-sided 0.05 significance level.

For all variables, baseline is defined as the last measurement taken prior to the administration of first dose of study drug at Visit 2. Change from baseline will be calculated as follow-up measure minus baseline measure.

11.2 Hypotheses

The primary efficacy hypotheses are as follows:

> H_{01}: The difference, between study eyes treated with LNZ101 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2 (Day 1), 3 hours = 0.
>
> H_{11}: The difference, between study eyes treated with LNZ101 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from the baseline measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2, 3 hours ≠ 0.

H_{02}: The difference, between study eyes treated with LNZ100 and study eyes treated with Brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2 (Day 1), 3 hours = 0.

H_{12}: The difference, between study eyes treated with LNZ100 and study eyes treated with brimonidine, in the percentage of study eyes with a ≥ 3-line (15-letter) improvement or greater from the baseline measurement of monocular BCDVA at 40 cm and no loss in BCDVA ≥ 1 line (5 letters) at 4 m at Visit 2, 3 hours ≠ 0.

Secondary hypotheses will be similarly constructed for all time points (excluding primary time point) at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) for each LNZ treatment.

The primary hypothesis for both treatments will be tested using Hochberg's step-up method with familywise error rate controlled at two-sided 0.05 significance level. The details of the method will be provided in the SAP. The study will be considered a success if either or both of the null hypotheses are rejected in favor of the corresponding alternative hypothesis.

If the primary endpoint for both treatments is positive, then the secondary endpoints within each treatment will be tested in a hierarchical order at 2-sided 0.05 significance level. If only one of the two treatment groups is positive, then the secondary endpoints will be tested in a hierarchical order in the respective treatment at 2-sided 0.025 significance level. If none of the treatments are positive, then secondary endpoints will not be tested. If at any point in the hierarchical order within a treatment group (LNZ101 or LNZ100), statistical significance is not demonstrated, testing will stop within that treatment group.

11.3 Analysis Sets

Full Analysis Set (FAS) – The FAS will include all randomized subjects. No subjects will be excluded from the FAS due to protocol violations/deviations. Subjects in the FAS will be analyzed as randomized.

Per Protocol (PP) Set – The PP set will include subjects in the FAS who do not have significant protocol deviations that affect the primary endpoint analysis. Protocol deviations will be assessed prior to database lock and unmasking. Subjects in the PP set will be analyzed as treated.

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.

11.4 Unit of Analysis

The study eye will be used for all monocular efficacy analyses. The qualified fellow eye will be used in additional binocular analyses as specified. Both eyes will be displayed and analyzed for all ophthalmic safety variables.

The study eye will be defined at Visit 2 (Day 1) as the eye that meets all enrollment criteria. [redacted]

11.5 Efficacy Endpoints

11.5.1 Primary Efficacy Endpoint:

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm and no loss in BCDVA $\geq$ 5 letters (ETDRS chart at 4 m) at 3 hours post-treatment in the study eye at Visit 2 (Day 1) for each LNZ treatment relative to Brimonidine.

11.5.2 Secondary Efficacy Endpoints

All secondary endpoints will be comparisons of each LNZ treatment relative to brimonidine unless otherwise specified.

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRSchart) and no loss in BCDVA $\geq$ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at:

Onset of Action

a) 30 minutes post-treatment

Duration of Action

a) 1 hour post-treatment
b) 5 hours post-treatment
c) 7 hours post-treatment
d) 8 hours post-treatment
e) 9 hours post-treatment
f) 10 hours post-treatment

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA $\geq$ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at:

a) 30 minutes post-treatment
b) 1 hour post-treatment
c) 3 hours post-treatment

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at:

a) 30 minutes post-treatment

b) 1 hour post-treatment

c) 3 hours post-treatment

d) 5 hours post-treatment

e) 7 hours post-treatment

f) 8 hours post-treatment

g) 9 hours post-treatment

h) 10 hours post-treatment

- Percentage of subjects who achieve a 3-line (15-letter) or greater improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) between LNZ101 and LNZ100

11.5.3 Exploratory Efficacy Endpoints:

- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of monocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Mean and mean change in monocular BCDVA at 40 cm (ETDRS chart) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment

- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Mean and mean change in monocular BCDVA (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 3-line (15-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 3 (Day 15) at 0.5, 1 and 3 hours post-treatment
- Percentage of subjects who achieve a 2-line (10-letter) improvement from baseline in the measurement of binocular BCDVA at 40 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 0.5, 1, 3, 7, 8, 9, and 10 hours post-treatment
- Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 2 (Day 1) at 1, 3, and 10 hours post-treatment
- Percentage of subjects who achieved no improvement, a 1-line (5-letter) or greater, 2-line (10-letter) or greater, and 3-line (15-letter) or greater improvement from pre-treatment by 1-letter increments in the measurement of monocular BCDVA at 66 cm (ETDRS chart) and no loss in BCDVA ≥ 5 letters (ETDRS chart at 4 m) in the study eye at Visit 4 (Day 28) at 1, 3, and 10 hours post-treatment

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment at Visit 2 (Day 1)
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, and 3 hours post-treatment at Visit 3 (Day 15)
- Mean and mean change in pupil diameter (mm) as measured by light adapted pupillometry using near VA testing conditions at 0.5, 1, 3, 5, 7, 8, 9, and 10 hours post-treatment at Visit 4 (Day 28)
- [redacted]

Note that the baseline value for all assessments performed at Visits 3 and 4 is the pre-treatment value at Visit 2.

11.6 Safety Endpoints

The safety endpoints are:

- Adverse events (AE) (reported, elicited, and observed)
- Pregnancy test (at Visit 1, Visit 2, and Visit 5)
- Monocular and binocular BCDVA (normal and low-luminance)
- Slit lamp biomicroscopy

[redacted]

- IOP
- Dilated fundus exam (at Visit 1 and Visit 5)
- Conjunctival redness

11.7 Sample Size

A sample size of 435 subjects (145 per arm) yields >99% power to establish superiority of LNZ101/LNZ100 to brimonidine in the proportion of study eyes demonstrating a ≥ 3-line (15-letter) improvement or greater from baseline in BCDVA at 40 cm and no loss in BDVA ≥ 1 line (5 letters) at 4 m at the 3-hour post-treatment time point on Day 1 using a Pearson chi-squared test with a 2-sided significance level of 0.05 assuming a response rate of 60% in LNZ-101/LNZ100 and 6% in Brimonidine.

[redacted]

11.8 Demographic and Baseline Characteristics

Subject demographics including age, gender, race, ethnicity, and iris color will be presented using continuous/categorical summary statistics as appropriate.

11.9 Efficacy Analysis

11.9.1 Primary Efficacy Analyses

The primary efficacy endpoint in this study is the percentage of study eyes with a ≥ 3-line (15-letter) improvement in BCDVA at 40 cm from baseline and no loss in best distance corrected visual acuity ≥ 1 line (5-letter) at 4 m at 3-hour post-treatment at Visit 2 (Day 1). The primary analysis will use the FAS with missing data imputations as detailed above within the Missing Data section.

Descriptive statistics will be presented by treatment group. Testing of the primary endpoint will be completed using logistic regression with treatment as the fixed effect and baseline BCDVA at 40 cm as a covariate. The adjusted odds ratios and marginal proportions and differences in proportions along with corresponding two-sided 95% confidence intervals (Cis) and p-values will be presented.

Treatment comparisons will also be made using Pearson's chi-squared test or Fisher's exact test if any of the expected cell counts are less than five as a sensitivity analysis to the primary model above.

Analyses will be repeated using the FAS with missing data imputed as failures and FAS and PP sets with observed data. Tipping point analysis may be performed.

11.9.2 Secondary Efficacy Analysis

Summaries and analyses for the secondary efficacy endpoints will be conducted using the same imputation strategy and statistical models as the primary efficacy endpoint.

[redacted]

[redacted]

[redacted]

[redacted]

11.9.3 Exploratory Efficacy Analysis

The categorical exploratory efficacy endpoints will be analyzed using the same model used for the primary endpoint.

[redacted]

Exploratory efficacy endpoints will be analyzed on the FAS with observed data only.

[redacted]

11.10 Safety Analysis

All safety data will be analyzed using the Safety set. Safety of [redacted] LNZ-100 will be assessed by the review of all safety parameters.

Verbatim descriptions of Aes will be mapped to Medical Dictionary for Regulatory Activities (MedDRA) terms and be presented in a data listing. Treatment emergent Aes (TEAEs), those that occur after the first dose of study drug, will be summarized by treatment group using frequency and percent for each system organ class (SOC) and preferred term (PT) within each SOC. Similar summaries will also be presented for expected and unexpected TEAEs, treatment emergent SAEs, TEAEs related to the study drug, and TEAEs by severity. When reporting the incidence of Aes, a subject will only be counted once if they ever experience an event within the SOC and ever experience the individual PT within each treatment group. Ocular and non-ocular events will be summarized separately.

Actual results and changes from baseline results in monocular and binocular BCDVA (normal and low-luminance), slit lamp biomicroscopy, IOP, dilated fundus examination, and conjunctival redness, will be summarized descriptively at each visit by treatment group.

Full details of the safety analyses will be specified in the formal SAP.

11.11 Interim Analysis

No interim analysis is planned for this study.

11.12 Missing Data

The primary analysis will use the Full Analysis Set (FAS) with intercurrent events handled in the following manner:

1) Discontinuation of study drug and non-optimal dosing compliance (less than 80% or more than 125%) will be ignored [treatment policy strategy]. This will not cause any missing data and will be conservative to treatment effect.
2) Missing data due to withdrawal due to lack of efficacy will be imputed employing multiple imputations (Mis) using control treatment-based regression methods, regardless of randomized treatment, for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].
3) Missing data due to withdrawal due to adverse events will be imputed using worst observation carried forward method for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].
4) Missing data without withdrawal or with withdrawal due to reasons other than lack of efficacy or adverse events will be imputed employing Mis using randomized treatment-based regression methods for both BCDVA at 40 cm and BCDVA at 4 m [hypothetical strategy].

Sensitivity analyses on the primary efficacy variable will be performed using the FAS with missing data imputed as failures, the FAS with observed data, and PP set with observed data. Additional sensitivity analyses such as tipping point may be performed and will be specified in the statistical analysis plan (SAP).

11.13 Adjustment for Multiplicity

There is no adjustment necessary for the primary endpoint because only one time point is being considered for the primary analysis.

The primary hypothesis for both treatments will be tested using Hochberg's step-up method with familywise error rate controlled at two-sided 0.05 significance level. The details of the method will be provided in the SAP. The study will be considered a success if either or both of the null hypotheses are rejected in favor of the corresponding alternative hypothesis.

If the primary endpoint for both treatments is positive, then the secondary endpoints within each treatment will be tested in a hierarchical order at 2-sided 0.05 significance level. If only one of the two treatments is positive, then the secondary endpoints will be tested in a hierarchical order in the respective treatment at 2-sided 0.025 significance level. If none of the treatments are positive, then secondary endpoints will not be tested. If at any point in the hierarchical order within a treatment (LNZ101 or LNZ100), statistical significance is not demonstrated, testing will stop within that treatment. LNZ101 vs LNZ100 overall comparison at Visit 2 (Day 1), Visit 3 (Day 15), and Visit 4 (Day 28) in that order will be tested if either one of the treatments is positive for all other secondary endpoints.

12.0 COMPLIANCE WITH GOOD CLINICAL PRACTICES, ETHICAL CONSIDERATIONS, AND ADMINISTRATIVE ISSUES

This study will be conducted in compliance with the protocol, current GCPs, including the ICH Guidelines, and will, in general, be consistent with the Declaration of Helsinki. In addition, all applicable local, state, and federal requirements relevant to the use of Ips in the countries involved will be adhered to.

12.1 Protection of Human Subjects

12.1.1 Subject Informed Consent

Informed consent must take place before any study-specific procedures are initiated. Signed and dated written informed consent must be obtained from each subject and/or from the subject's parent or legal guardian prior to enrollment into the study.

All ICFs must be approved for use by the sponsor and receive approval/favorable opinion from an IRB prior to their use. If the ICF requires revision (e.g. due to a protocol amendment or significant new safety information), it is the investigator's responsibility to ensure that the amended informed consent is reviewed and approved by [redacted] prior to submission to the governing IRB and that it is read, signed, and dated by all subjects subsequently enrolled in the study as well as those currently enrolled in the study.

If informed consent is taken under special circumstances (oral informed consent), then the procedures to be followed must be determined by [redacted] the sponsor and provided in writing by [redacted] the sponsor prior to the consent process.

12.1.2 IRB Approval

This study is to be conducted in accordance with IRB regulations (U.S. 21 CFR Part 56.103). The investigator must obtain appropriate IRB approval before initiating the study and re-approval at least annually.

Only an IRB-approved version of the ICF will be used.

12.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that originated with the Declaration of Helsinki.

12.3 Subject Confidentiality

All personal study subject data collected and processed for the purposes of this study should be maintained by the investigator and his/her staff with adequate precautions as to ensure that the confidentiality of the data is in accordance with local, state, and federal laws and regulations.

Monitors, auditors and other authorized representatives of [redacted] the sponsor, the IRB approving this study, the FDA, the Department of Health and Human Services, other domestic government agencies, and other foreign regulatory agencies will be granted direct access to the study subject's original medical and study records for verification of the data and/or clinical trial procedures. Access to this information will be permitted to the aforementioned individuals to the extent permitted by law.

A report of the results of this study may be published or sent to the appropriate health authorities in any country in which the IP may ultimately be marketed in, but the subject's identity will not be disclosed in these documents.

12.4 Documentation

Source documents may include a subject's medical records, hospital charts, clinic charts, the investigator's subject files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms. The investigator's copy of the eCRFs serves as the investigator's record of a subject's study-related data.

12.4.1 Retention of Documentation

All study-related correspondence, patient records, consent forms, records of the distribution and use of all Ips, and copies of case report forms should be maintained on file for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region; or until at least two years have elapsed since the formal discontinuation of clinical development of the IP. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

If the responsible investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping study records, custody must be transferred to a person who will accept the responsibility. The sponsor must be notified in writing of the name and address of the new custodian.

12.5 Labeling, Packaging, Storage, Accountability, and Return or Disposal of Investigational Product

12.5.1 Labeling/Packaging

Investigational drug will be packaged and labeled into clinical kits, following the randomization list generated prior to the start of the study. Each clinical kit will be uniquely identified by a 5-digit kit number .

The primary packaging of the LNZ101, LNZ100, and Brimonidine Ophthalmic Solution will be blow-fill-seal ampoules. The secondary packaging is a foil pouch that contains five ampoules in each pouch. Four pouches of five ampoules will be packaged in a 2-week clinical kit. Each subject will receive 3 kits total, one at Visit 2, one at Visit 3, and one at Visit 4.

Clinical label texts for the primary packaging and secondary packaging (clinical kits) meet applicable regulatory requirements and include the statement "Caution: New Drug-Limited by Federal Law to Investigational Use.

12.5.2 Storage of Investigational Product

Investigational drug must be stored in a secure area of the investigative site, accessible only to Investigator or designees, at temperature 2-8ºC. The investigational product will be administered only to subjects entered into the clinical study, in accordance with the conditions specified in this protocol. All investigational drugs will be returned to inventory after use.

12.5.3 Accountability of Investigational Product

The investigational product is to only be administered by a trained study technician delegated by the principal Investigator,) and is to only be used in accordance with this protocol. The investigational product must only be distributed to subjects properly qualified under this protocol to receive investigational product.

The Investigator must keep an accurate accounting of the investigational product received from the supplier. This includes the amount of investigational product administered to subjects and the amount returned or disposed upon the completion of the study. A detailed inventory must be completed for the investigational product.

12.5.4 Return or Disposal of Investigational Product

All Ips will be returned to the sponsor or their designee or destroyed. The return or disposal of IP will be specified in writing. Any remaining IP will be collected from subjects before study exit.

12.6 Recording of Data on Source Documents and Electronic Case Reports Forms (eCRFs)

The investigator is responsible for ensuring that study data is completely and accurately recorded on each subject's source document, eCRF, and all study-related material. All study data should also be attributable, legible, contemporaneous, and original. Recorded datum should only be corrected in a manner that does not obliterate, destroy, or render illegible the previous entry (e.g. by drawing a single line through the incorrect entry and writing the revision next to the corrected data). An individual who has corrected a data entry should make clear who made the correction and when by adding to the correction his/her initials as well as the date of the correction.

12.7 Handling of Biological Specimens

Not applicable

12.8 Publications

Authorship and manuscript composition will reflect cooperation among all parties involved in the study. Authorship will be established before writing the manuscript. [redacted] and the study sponsor will have the final decision regarding the manuscript and publication.

13.0 REFERENCES

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14.0 APPENDICES

14.1 Appendix 1: Schedule of Visits and Measurements

For the suggested order of assesments, please refer to Section 9.3.

Study Parameter	Visit 1 (Day -60 to -4)	Visit 2 (Day 1)	Visit 3 (Day 15 ± 2)	Visit 4 (Day 28 ± 2)	Visit 5 / ET Visit[d] (Day 42 ± 2)
Informed consent / HIPAA	X				
Demographic data	X				
Medical and medication history	X				
Medical and medication history update		X	X	X	X
Urine pregnancy test (for females of child-bearing potential)	X	X			X
Inclusion and exclusion criteria review	X	X			
Manifest refraction	X				
Dark-adapted pupillometry	X				X
Pre-treatment light adapted pupillometry under near visual acuity at 40 cm testing conditions		X	X	X	X[c]
Pre-treatment monocular BCDVA 40 cm (ETDRS chart)	X	X	X	X	X[c]
Pre-treatment binocular BCDVA 40 cm (ETDRS chart)		X	X	X	X[c]
Pre-treatment monocular BCDVA 40 cm with +1.50 D Add (ETDRS chart)	X	X			
Pre-treatment monocular BCDVA 66cm (ETDRS chart)		X		X	
Pre-treatment monocular BCDVA at 4 m (ETDRS)	X	X	X	X	X[c]
Pre-treatment binocular BCDVA at 4 m (ETDRS)		X	X	X	
Pre-treatment monocular low-luminance BCDVA at 4 m (ETDRS)		X		X	
Pre-treatment binocular low-luminance BCDVA at 4 m (ETDRS)		X		X	
Pre-treatment slit lamp biomicroscopy	X	X	X	X	X
Pre-treatment conjunctival redness assessment	X	X	X	X	
Instillation of Refresh® Plus Eye Drops	X				
Adverse event query[d]	X	X	X	X	X
Selection of study eye		X			
Randomization		X			
Instillation of Investigational Product		X	X	X	
Drop instillation assessment	X	X	X	X	
Post-treatment light adapted pupillometry under near visual acuity at 40 cm testing conditions (0.5, 1, 3h)			X		
Post-treatment monocular BCDVA 40 cm (ETDRS chart) (0.5, 1, 3h)	X[a]		X[a]		
Post-treatment binocular BCDVA 40 cm (ETDRS chart) (0.5, 1, 3h)	X[a]		X[a]		

Study Parameter	Visit 1 (Day -60 to -4)	Visit 2 (Day 1)	Visit 3 (Day 15 ± 2)	Visit 4 (Day 28 ± 2)	Visit 5 / ET Visit[d] (Day 42 ± 2)
Post-treatment monocular BCDVA at 4 m (ETDRS) (0.5, 1, 3h)			X		
Post-treatment binocular BCDVA at 4 m (ETDRS) (0.5, 1, 3h)			X		
PRO Questionnaire (5h)		X		X	
Post-treatment light adapted pupillometry under near visual acuity at 40 cm testing conditions (0.5, 1, 3, 5, 7 8, 9, 10h)		X		X	
Post-treatment monocular BCDVA 40 cm (ETDRS chart) (0.5, 1, 3, 5, 7, 8, 9, 10h)		X		X	
Post-treatment binocular BCDVA 40 cm (ETDRS chart) (0.5, 1, 3, 7, 8, 9, 10h)		X		X	
Post-treatment monocular BCDVA 66cm (ETDRS chart) (1, 3, 10h)		X		X	
Post-treatment monocular BCDVA at 4 m (ETDRS) (0.5, 1, 3, 5, 7, 8, 9, 10h)		X		X	
Post-treatment binocular BCDVA at 4 m (ETDRS) (0.5, 1, 3, 7, 8, 9, 10h)		X		X	
Post-treatment monocular low-luminance BCDVA at 4 m (ETDRS) (1, 3, and 10h)		X		X	
Post-treatment binocular low-luminance BCDVA at 4 m (ETDRS) (1, 3, and 10h)		X		X	
Conjunctival redness		X[b]	X[b]	X[b]	X
Fluorescein staining	X				
IOP	X	X	X	X	X
Cycloplegic Refraction	X				
Dilated indirect fundoscopy	X				X
End of visit slit-lamp biomicroscopy	X	X	X	X	X
Dispense study drug/dosing diary[e]		X	X	X	
Collect study drug/dosing diary			X	X	X
Study exit					X

Abbreviations: AE = adverse event; BCDVA = best corrected distance visual acuity; ETDRS = Early Treatment of Diabetic Retinopathy Study; IOP = intraocular pressure

[a]At Visit 1, post-treatment monocular and binocular BCDVA at 40 cm will be assessed at 0.5h after Refresh® Plus Eye Drops instillation. At Visit 3, post-treatment monocular and binocular BCDVA at 40 cm will be assessed at 0.5, 1 and 3 hours post treatment.

[b]At Visit 2 and Visit 4, conjunctival redness will be assessed at 3 and 10 hours post-treatment. At Visit 3, conjunctival redness will be assessed at 3 hours post-treatment only.

[c]At Visit 5, no treatment will be administered. These assessments will be performed once.

[d]AEs will be asked at the beginning of Visits 2, 3, 4, and 5 and at 3 hrs post treatment at visits 2 and 4, and at the end of Visits 1, 2, 3, 4, and 5

[e]All randomized subjects will be provided instructions on how to instill two (2) drops bilaterally, one drop in each eye followed by 2 minutes later, another drop in each eye

Note: Pre-treatment indicates that an assessment will be performed prior to any treatment during that study visit.

14.2 Appendix 2: Examination Procedures, Tests, Equipment, and Techniques

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14.4 Appendix 4: Investigator's Signature

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

Protocol Number: 22-150-0015

[redacted]

Signed:_____ Date:_____

[Name]

Principal Investigator

[Affiliation]