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Opus Genetics, Inc.

STATISTICAL ANALYSIS PLAN

Protocol Title:	Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of POS (0.75% Phentolamine Ophthalmic Solution) in Subjects Who Have Previously Had Keratorefractive Surgery and Have Decreased Visual Acuity Under Mesopic Conditions
Study Number:	OPI-NYXDLD-302 (LYNX-2)
Phase:	Phase 3
Sponsor:	Opus Genetics, Inc. [redacted]
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2. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Only abbreviations and terms relevant to the SAP are repeated herein. The reader is referred to the protocol for the complete and comprehensive list of abbreviations and definitions of terms for the study.

Abbreviation/Term	Definition
ADaM	Analysis Data Model
AE	adverse event
ANCOVA	analysis of covariance
ARP	All Randomized Population
ATC	Anatomical Therapeutic Chemical
BAT	Brightness Acuity Tester
BCDVA	best-corrected distance visual acuity
BP	blood pressure
CCLRU	Cornea and Contact Lens Research Unit
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CRF	Case Report Form
CSR	Clinical Study Report
DD	drug dictionary
DLD	dim light vision disturbances
ECC	endothelial cell count
EDC	electronic data capture
ETDRS	Early Treatment Diabetic Retinopathy Study
HR	heart rate
IOP	Intraocular pressure
ITT	Intention-To-Treat
logMAR	logarithm of the minimum angle of resolution
LSM	least squares mean
MAR	missing at random
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
mHCVA	mesopic high-contrast visual acuity
mITT	Modified Intention-To-Treat
mLCVA	mesopic low-contrast visual acuity
OD	right eye

Abbreviation/Term	Definition
OS	left eye
OU	both eyes
PD	pupil diameter
PMM	pattern mixture model
POS	0.75% Phentolamine Ophthalmic Solution
PP	Per Protocol
PT	preferred term
QD	once daily
SAE	serious adverse event
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model
SE	standard error
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event
TFL	tables, figures, and listings
VA	visual acuity
WHO	World Health Organization

3. INTRODUCTION

3.1. Preface

This document presents a statistical analysis plan (SAP) for Opus Genetics, Inc. Protocol OPI-NYXDLD-302 (LYNX-2) (*Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of POS (0.75% Phentolamine Ophthalmic Solution) in Subjects Who Have Previously Had Keratorefractive Surgery and Have Decreased Visual Acuity Under Mesopic Conditions*).

Reference materials for this statistical plan include the protocol OPI-NYXDLD-302 (01FEB2024) and case report forms (CRFs; 15MAY2024).

The SAP described hereafter is an *a priori* plan. The SAP will be finalized and approved prior to unmasking of any study data.

For the reasons stated here, the conduct of the study in the field is considered to be independent of any study outcome that might materialize upon enactment of the currently proposed statistical plan.

3.2. Purpose of Analyses

The LYNX-2 study is a randomized, placebo-controlled, double-masked, multiple-dose study of the safety and efficacy of POS (0.75% Phentolamine Ophthalmic Solution). The Sponsor intends to use this Phase 3 registration study to evaluate POS for the indication of "treatment of decreased visual acuity under dim light conditions in post-refractive surgery patients."

Post-hoc exploratory analyses not identified in this SAP may be performed to further examine the study data. These analyses will be clearly identified, where appropriate, in the final clinical study report (CSR). Additional analyses not prospectively identified in this SAP may also be completed for publications, or regulatory or funding inquiries. These analyses, if performed, may not be reported in the CSR but will be fully detailed in the document containing the additional analyses.

3.3. Summary of Statistical Analysis Changes to the Protocol

The analyses described in this analysis plan are consistent with the analyses described in the protocol.

4. STUDY OBJECTIVES AND ENDPOINTS

Study objectives and endpoints defined in the protocol include safety and efficacy endpoints. Objectives and pre-specified endpoints are as follows:

4.1. Study Objectives

The primary objectives of this study are:

- To evaluate the efficacy of POS to improve mesopic low-contrast visual acuity (mLCVA) in subjects with post-refractive surgery visual disturbances
- To evaluate the efficacy of POS to improve visual acuity (VA) under various lighting conditions
- To evaluate the safety of POS in subjects with post-refractive surgery visual disturbances
- To evaluate tachyphylaxis of the therapeutic response to POS in subjects with post-refractive surgery visual disturbances

4.2. Study Endpoints

4.2.1. Primary Endpoints

The primary efficacy endpoint is the percent of subjects with ≥ 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (≥ 3 lines) of improvement in the study eye in mLCVA compared to Baseline at Day 15.

4.2.2. Secondary Endpoints

Secondary endpoints for efficacy and safety assessments include the following:

Efficacy:

Secondary efficacy endpoints for the study eye include:

- Percent of subjects with ≥ 10 and ≥ 15 ETDRS letters improvement in mLCVA compared to baseline (Day 1 pre-dose) at Days 3, 8, and 15 (≥ 10 ETDRS letters only) and Week 6
- Mean and change from baseline (Day 1 pre-dose) mLCVA at 0.5, 1, and 3 hours post-dose on Day 1
- Percent of subjects with ≥ 10, and ≥ 15 ETDRS letters improvement in mesopic high-contrast visual acuity (mHCVA) compared to baseline (Day 1 pre-dose) at Days 3, 8, 15, and Week 6
- Mean and change from baseline (Day 1 pre-dose) in mLCVA and mHCVA at Days 3, 8, 15, and Week 6

- Mean and change from baseline (Day 1 pre-dose) in pupil diameter (PD) at Days 3, 8, 15, and Week 6
- Change in subject questionnaire responses compared to baseline (Day 1 pre-dose) at Days 3, 8, 15, and Week 6

Efficacy Measurements:

- [redacted]
- [redacted]
- [redacted]
- [redacted]

Secondary Safety and Tolerability endpoints:

- Adverse events (AEs)
- Intraocular pressure (IOP)
- Percentage of subjects with ≥ 5, ≥ 10, and ≥ 15 letters of loss in BCDVA from Baseline (Day 1 pre-dose) at 12 hours post-dose
- Change from Baseline (Day 1 pre-dose) in BCDVA at 12 hours post-dose
- Biomicroscopy
- Corneal specular microscopy, if available
- Ophthalmoscopy
- Vital signs (heart rate [HR] and blood pressure [BP])

Safety Measurements:

- [redacted]
- [redacted]

4.2.3. Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include all of the secondary VA and PD efficacy endpoints with the analysis of VA data performed on the fellow eye and binocular test results, and the analysis of PD data performed on the fellow eye.

5. STUDY METHODS

5.1. General Study Design and Plan

The LYNX-2 study is a placebo-controlled, double-masked, 2-stage, multiple-dose, multicenter, Phase 3 study in approximately 200 randomized subjects with post-refractive surgery visual disturbances under mesopic lighting conditions. Safety and efficacy of POS will be evaluated following self-administration of POS once daily (QD) at or near bedtime in each eye for approximately 6 weeks during Stage 1. During optional Stage 2, the subject will continue to self-administer study medication in each eye QD in the evening near bedtime for an additional 42 weeks.

Following the successful completion of screening, each subject will be stratified by iris color (light/dark irides) and will then be randomized 1:1 to 1 of 2 treatment arms:

- Daily evening (PM) POS (n=100)
- Daily PM placebo (ie, POS vehicle; n=100)

The study eye for the primary endpoint will be the eye that meets Inclusion Criteria #6, #7, and #8 at Screening:

6. Pupil diameter ≥ 5 mm under mesopic conditions in at least 1 eye. This test may be repeated once, following an additional 5 minutes of dark adaptation to the mesopic light conditions if the initial results do not meet this criterion

7. mLCVA ≤ 30 ETDRS letters (20/63 Snellen or worse) in at least 1 eye using the Precision Vision Illuminator Cabinet with 5% translucent contrast chart and a mesopic filter at 4 m

[redacted]

In the case in which both eyes meet Inclusion Criteria #6, #7, and #8, the study eye will be the eye with the worse mLCVA. In the case where both eyes have the same Screening mLCVA score, the study eye shall be the right eye (OD). Visual acuity assessments will be made on the study eye, fellow eye, and binocularly.

Individuals who are potential subjects are identified by the study center to schedule the Screening/Baseline Visit (Visit 1). Note that Visit 1 assessments are recommended to be performed on the same day; however, in instances in which subjects are unable to complete the full day of assessments for Visit 1, subjects may be scheduled to return for the completion of Visit 1 within 1 week of Screening. If Visit 1 is split over 2 separate days, all visit assessments up to and including randomization must occur on the first day, and then the subject should return to complete their post-randomization (Baseline) assessments starting with treatment dosing on the second day of the visit.

Once a subject arrives at the study center, a member of the study center staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed, and a subject identification number is assigned.

For subjects passing all eligibility criteria, the Screening Visit becomes the Baseline Visit. The subject will then be randomized into the study. The first dose of study medication will be administered at the study site at the Baseline Visit (Day 1) and mLCVA assessments will be made at 0.5, 1, and 3 hours post-dose. The subject diary for Stage 1 will be dispensed.

The first dose of at-home study medication will be administered by subjects as 1 drop OU on Day 2 as close as possible to 12 hours prior to the scheduled time for Visit 2/Day 3.

There will be 5 office visits in Stage 1: Visit 1 (Screening/Baseline)/Day 1, Visit 2/Day 3, Visit 3/Day 8, Visit 4/Day 15, and Visit 5/Week 6. mLCVA evaluations shall be performed on each of these days.

The study is also designed to examine tachyphylaxis of the therapeutic response to POS for mLCVA. This will be achieved by comparing change from Baseline at Week 6 in the POS group to the best change from baseline achieved during the first month of treatment for mLCVA.

At Visit 5, subjects agreeing to continue to Stage 2 will be reconsented. The length of optional Stage 2 will continue for an additional approximately 42 weeks, with 7 office visits at 12, 18, 24, 30, 36, 42, and 48 weeks.

The schedule for assessments and timing of events in Stage 1 is presented in Table 1. The schedule of events in Stage 2 is presented in Table 2.

Table 1 Stage 1 Schedule of Visits and Procedures

Visit number	1 [a]				2	3	4	5
Visit timing (window)	Screening/Baseline Day 1				Day 3	Day 8 (± 1 day)	Day 15 (± 1 day)	End of Stage 1 Week 6 (± 3 days)
Hours (window)	0	0.5 (± 5 min)	1 (± 5 min)	3 (± 10 min)	NA	NA	NA	NA
Informed consent	X							X
Medical/Ophthalmic history	X							
Demographics	X							
Prior/Concomitant medications [b]	X				X	X	X	X
Urine pregnancy test [c]	X				X	X	X	X
HR/BP [d]	X				X	X	X	X
Manifest refraction and near add	X							
Subject questionnaire [e]	X				X	X	X	X
BCDVA [f,g]	X				X	X	X	X
PD [h]	X				X	X	X	X
mLCVA [g]	X	X	X	X	X	X	X	X
[redacted]	X							
mHCVA [g]	X				X	X	X	X
Slit lamp biomicroscopy	X				X	X	X	X
Automated corneal specular microscopy, if available	X				X	X	X	X
Dry eye test [i]	X							
IOP [j]	X				X	X	X	X
Ophthalmoscopy [k]	X				X	X	X	X
Adverse events	X				X	X	X	X
Treatment dosing [l]	X							
Treatment dispensed/collected	X [a]							X
Subject diary dispensed/reviewed [m]	X				X	X	X	X
Study medication reminder [n]	X				X	X	X	

[redacted] BCDVA, best-corrected distance visual acuity; BP, blood pressure; D, diopter; HR, heart rate; IOP, intraocular pressure; mHCVA, mesopic high-contrast visual acuity (BCDVA); mLCVA, mesopic low-contrast visual acuity (BCDVA); NA, not applicable; PD, pupil diameter; VA, visual acuity.

[a] If the subject meets all the inclusion criteria and none of the exclusion criteria, this Screening Visit becomes the Baseline Visit. A randomization number is assigned after the subject is qualified.

[b] This includes asking if subjects have been diagnosed with dry eye, take prescription drops for dry eye, or take artificial tear drops routinely for dry eye. Investigators will note changes to concomitant medications at any time throughout the study.

[c] Urine pregnancy test is for females of childbearing potential only.
[d] HR and BP can be measured manually or with available digital devices.
[e] Subject questionnaire will be a brief symptom survey.
[f] Photopic assessments will be conducted before mesopic assessments.
[g] Binocular VA assessments will be conducted first, followed by assessment of each eye individually at Screening; thereafter, study eye will be assessed first, followed by fellow eye and then binocular.
[redacted]
[i] Dry eye test will include both tear break-up test (performed first) and corneal fluorescein staining test.
[redacted]
[k] Direct or indirect ophthalmoscopy will be performed without dilation. Use of 90 D lens (indirect) is allowed.
[l] Treatment dosing will occur in the office at Visit 1.
[m] Subject diary will be provided to subjects at Visit 1, and a new subject diary will be provided to subjects consenting to participate in optional Stage 2 at Visit 5.
[n] All subjects will be reminded to instill treatment approximately 12 hours prior to the scheduled time of their next office visit.

Table 2 Optional Stage 2 Schedule of Visits and Procedures

Visit number	6	7	8	9	10	11	12
Visit timing (window)	Week 12 (± 3 days)	Week 18 (± 3 days)	Week 24 (± 3 days)	Week 30 (± 3 days)	Week 36 (± 3 days)	Week 42 (± 3 days)	End of Study Week 48 (± 3 days)
Prior/Concomitant medications [a]	X	X	X	X	X	X	X
Urine pregnancy test [b]	X	X	X	X	X	X	X
HR/BP [c]	X	X	X	X	X	X	X
Subject questionnaire [d]	X	X	X	X	X	X	X
BCDVA [e]	X	X	X	X	X	X	X
Slit lamp biomicroscopy	X	X	X	X	X	X	X
Automated corneal specular microscopy, if available	X	X	X	X	X	X	X
IOP [f]	X	X	X	X	X	X	X
Ophthalmoscopy [g]	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X
Treatment dispensed/collected	X	X	X	X	X	X	X
Subject diary reviewed	X	X	X	X	X	X	X

BCDVA, best-corrected distance visual acuity; BP, blood pressure; D, diopter; HR, heart rate; IOP, intraocular pressure.
[a] This includes asking if subjects have been diagnosed with dry eye, take prescription drops for dry eye, or take artificial tear drops routinely for dry eye. Investigators will note changes to concomitant medications at any time throughout the study.
[b] Urine pregnancy test is for females of childbearing potential only.
[c] HR and BP can be measured manually or with available digital devices.
[d] Subject questionnaire will be a brief symptom survey.
[e] Study eye BCDVA will be assessed first, followed by fellow eye and then binocular.
[redacted]
[g] Direct or indirect ophthalmoscopy will be performed without dilation. Use of 90 D lens (indirect) is allowed.

5.2. Inclusion – Exclusion Criteria and General Study Population

The study population will be approximately 200 subjects with post-refractive surgery visual disturbances under mesopic lighting conditions. Written informed consent will be obtained from each subject.

The inclusion and exclusion criteria defined in the protocol apply to all subjects and are not repeated herein the SAP. Reference is made to the final protocol for the specific inclusion and exclusion criteria for study subjects.

5.3. Randomization and Blinding

A randomization code for allocating subjects to treatment will be prepared by a masked biostatistician not connected with the study. At the initiation of study-related procedures, every subject who is screened is assigned a subject identification number in numerical order within site. Once a subject is qualified for the study, the subject is assigned a randomization number in the order provided by the biostatistician, and randomized into the study.

Treatment randomization will be 1:1, POS or Placebo (vehicle), with a stratification by light/dark irides. It is assumed that there will be less than 5% drop-out between Day 1 and Day 15.

The study medication will be masked to investigators, study staff, study subjects, the clinical research organization, and Opus Genetics. Only in case of medical emergency or occurrence of serious adverse events (SAEs) will the randomization code be unmasked by the study pharmacist and made available to the Investigator, Opus Genetics, and/or other personnel involved in the monitoring or conduct of this study. Rules for unmasking a subject for safety reasons are fully described in the protocol and not repeated herein this SAP.

5.4. Analysis Variables

Variables to be summarized include demographics and baseline characteristics, medical (non-ocular) and ocular history, concomitant medications, and study drug accountability.

Efficacy variables include:

- Mesopic low-contrast BCDVA (mLCVA)
- Mesopic high-contrast BCDVA (mHCVA)
- Photopic pupil diameter
- Mesopic pupil diameter
- Subject questionnaire

Safety variables include:

- AEs
- IOP
- Biomicroscopy
- Corneal specular microscopy
- Ophthalmoscopy
- Vital signs (HR and BP)
- Urine pregnancy tests for females of childbearing potential

6. SAMPLE SIZE

A total of approximately 200 subjects will be enrolled and randomized into the study in a 1:1 ratio to 1 of the 2 treatment arms (POS or placebo), with a stratification by light/dark irides.

The primary efficacy endpoint will be met if subjects show a positive effect for POS (α = 0.05 significance, 2-sided) at Day 15 in the study eye. A total of 200 subjects will provide 89% power to detect a difference of 16% between the POS and placebo groups in percent of subjects with ≥ 15 ETDRS letters improvement in monocular mLCVA at Day 15 compared to Baseline. [redacted]

To test for tachyphylaxis, a sample size of 100 subjects receiving POS provides greater than 90% power (α = 0.05 significance) to detect a 5-letter or more non-inferiority margin based on the change in monocular mLCVA after 6 weeks of daily dosing compared to the best change in mLCVA during the first month of the study, assuming a standard deviation of 9 letters.

All subjects will be randomized into the study in a 1:1 ratio to 1 of the 2 treatment arms (POS or placebo), with a stratification by light/dark irides.

7. GENERAL CONSIDERATIONS

7.1. Analysis Populations

The following analysis populations will be defined for this study.

7.1.1. Modified Intention-to-Treat (mITT)

The mITT will include all randomized subjects who received at least one dose of study medication. The mITT will be used for the primary endpoint analysis and to analyze efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

7.1.2. Per Protocol Population (PP)

The PP population includes all subjects in the mITT who received at least 80% of expected doses of study medication through Day 15, have a Baseline and a Day 15 mLCVA measurement, and have no major protocol deviations considered to have significant impact on treatment outcome. The PP population will be used to analyze efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

7.1.3. All Randomized Population (ARP)

The ARP will include all randomized subjects. This population is also known as the Intention-To-Treat (ITT) population. The ARP may be used in confirmatory efficacy analyses, with subjects included in their randomized treatment regardless of the treatment they actually received.

7.1.4. Safety Population (SP)

The SP will include all randomized subjects who have received at least one dose of study medication. The SP will be used to summarize safety variables, using the actual treatment a subject received.

7.2. Covariates and Subgroups

7.2.1. Planned Covariates

Planned covariates include irides type (light, dark) and baseline values for the given assessment.

7.2.2. Planned Subgroups

Subgroup analyses by irides type (light and dark) will be completed for efficacy endpoints. In addition, analyses by age group (<18, 18-29, 30-44, 45-64, and >=65), birth sex, race, and ethnicity will be completed for the primary efficacy endpoint.

7.3. Management of Analysis Data

7.3.1. Data Handling

Data from unscheduled visits will not be included in the analysis of efficacy or safety but will be listed. Nominal visits will be used, with no additional analysis visit windowing except for early termination data (Section 7.3.3).

7.3.2. Missing Data

The primary efficacy endpoint is the percent of subjects with ≥ 15 ETDRS letters (≥ 3 lines) of improvement in the study eye compared to baseline in monocular mLCVA at Day 15. For the analysis of the primary efficacy endpoint, imputation will be performed for missing efficacy data as specified in Section 7.3.2.3 for the analysis using the mITT. Missing data for select secondary efficacy endpoints may also be imputed.

Otherwise, there will be no substitutions made to accommodate missing data points for efficacy data. All data recorded on the CRFs will be included in data listings that will accompany the CSR.

Safety data will be imputed in limited situations. If the severity of an AE is missing, then the severity will remain missing. If relationship of the AE to study drug is missing, the relationship will remain missing. Missing or partial dates for AEs or concomitant medications will be imputed as described in Section 7.3.2.1. Otherwise, all summaries of safety endpoints will be completed using observed cases in the SP; no imputation will be completed.

7.3.2.1. Handling of Missing Date Values

Partial or Missing Dates

The following conventions will be used to impute missing portions of dates for AEs and concomitant medications, if warranted. Note that the imputed values outlined here may not always provide the most conservative date. In those circumstances, the imputed value may be replaced by a date that will lead to a more conservative analysis.

A. Start Dates
   1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.
   2) If the month is unknown, then:

i) If the year matches the first dose date year, then impute the month and day of the first dose date.

ii) Otherwise, assign 'January.'

3) If the day is unknown, then:

i) If the month and year match the first dose date month and year, then impute the day of the first dose date.

ii) Otherwise, assign the first day of the month.

B. Stop Dates

1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.

2) If the month is unknown, then assign 'December.'

3) If the day is unknown, then assign the last day of the month.

7.3.2.2. Missing Baseline Data

Every effort will be made to ensure that accurate baseline information on the subjects is collected. In the event that a subject is missing baseline information, the subject will be included in the SP for assessment of safety and excluded from the primary analyses. Each case of missing baseline data will be evaluated for potential inclusion in the exploratory endpoints. All baseline data will be observed cases, without imputation.

7.3.2.3. Imputation Methods

Imputation for efficacy data will only be performed for the primary efficacy endpoint and select secondary efficacy endpoints using the mITT. Multiple imputation will be employed to analyze incomplete data sets under the assumption that the mechanism responsible for the missing data is at worst characterized as missing at random (MAR).

Multiple imputation is a simulation-based approach where missing values are replaced by multiple Bayesian draws from the conditional distribution of missing data given the observed data and covariates, creating multiple completed data sets. These completed datasets can then be analyzed using standard analysis methods. Rubin [2] presented rules for how to combine the multiple sets of estimates to produce overall estimates, confidence intervals (CIs), and tests that adequately incorporate missing data uncertainty.

To implement this approach for the primary endpoint, missing values for mLCVA will be imputed simultaneously based on an underlying joint normal distribution using a Markov Chain Monte Carlo (MCMC) method.

The imputations will be done separately for each treatment group and will include the following variables in the imputation model: mLCVA at baseline, Day 3, Day 8, Day 15, and Week 6.

The number of imputations will be set to 20. The change from baseline will be calculated from these imputed datasets. The observed outcome (yes/no) for each imputed dataset will be

evaluated using logistic regression. See Section 9.1 for details on these models. The estimates and 95% CIs of the difference of proportions based on the 20 imputed datasets are then combined by applying Rubin's rules for multiple imputed datasets. T-tests are also provided. SAS Proc MI, Proc NLMIXED, and Proc MIANALYZE will be utilized for these analyses. The difference of proportions with the corresponding 95% CI will also be presented.

This approach will be implemented for secondary endpoints in a similar fashion, with the variable and visits changed to fit the endpoint definition. Continuous secondary endpoints will be analyzed using analysis of covariance (ANCOVA) instead of logistic regression; see Section 9.1 for details on these models.

A sensitivity analysis will be employed on the primary efficacy endpoint using a control-based Pattern-Mixture Model (PMM). In this analysis, intermittent missing mLCVA data (Day 3, Day 8, and Day 15) will be imputed using MCMC methods, assuming MAR, within each treatment group. SAS PROC MI will be utilized for this step using the MCMC impute=monotone option. In accordance with the PMM method, all data from the placebo group and only data from subjects in the POS group who have missing data will be included in the multiple imputation used to fill in the intermittent missing data. Treatment will not be included as a factor in the multiple imputation.

The remaining missing mLCVA values with a monotone missing data pattern will then be imputed. The missing values in the POS group will not be constructed from the observed data in the POS group, but rather from the observed data in the placebo group. This model will also be used to impute missing efficacy measurements in the placebo group. The MNAR statement in SAS PROC MI will be used to impute all missing values. The imputed values will be converted into responder (yes/no) variables according to the definition of the primary efficacy endpoint. This will be performed for 20 datasets.

The observed outcome (yes/no) at Day 15 for each imputed dataset will be evaluated using logistic regression. The estimates and 95% CIs of the difference of proportions based on the 20 imputed datasets are then combined by applying Rubin's rules for multiple imputed datasets (as described above), and the difference of proportions with the corresponding 95% CI will be presented.

Assuming the primary efficacy analysis shows a significant difference between treatment groups at Day 15, a tipping point analysis will also be used as a sensitivity analysis. This analysis will apply multiple imputation assuming MAR, but will also employ increasingly negative shifts to be added to imputed mLCVA data from the POS group, crossed with increasingly positive shifts to be added to the imputed mLCVA data from the placebo group. The imputed values will be converted into responder (yes/no) variables according to the definition of the primary efficacy endpoint, and analyzed as described above. The analysis with no shifts will correspond to the original multiple imputation strategy assuming MAR described above. The most extreme shift will ensure that subjects with missing mLCVA data in the POS arm are considered as nonresponders while similar subjects in the placebo arm are considered as responders.

7.3.3. Handling of Early Termination Visit Information

In the event that a subject is terminated early from this study, the early termination data for safety and efficacy variables will be assigned to the closest visit. If the closest visit has valid data, the early termination data will be assigned to the next available visit.

7.3.4. Pooling of Investigational Sites

The data from all study centers will be pooled together for all planned analyses.

7.3.5. Coding Conventions for Events and Medications

All AEs and medical history will be mapped to the Medical Dictionary for Regulatory Activities using Medical Dictionary for Regulatory Activities (MedDRA) Version 26.1 for reporting (preferred term and body system).

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD) Version 2023Sep.

7.3.6. Analysis Software

Data manipulation, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations will be performed primarily using SAS (release 9.4 or higher) for Windows. If the use of other software is warranted, the final CSR will detail what software was used and for what purposes.

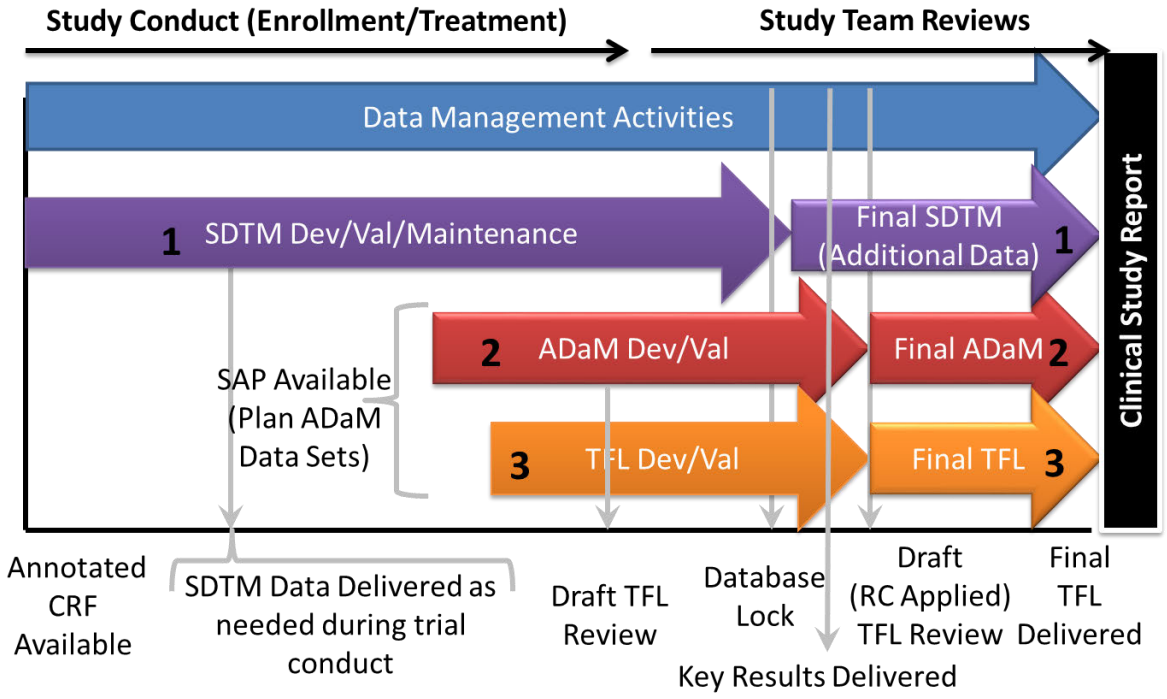
7.3.7. Study Data

Study data identified in the schedule for time and events (Table 1) are collected, and source verified, on the electronic data capture (EDC) system.

All study data will be formulated into regulatory-compliant data sets to provide transparency, traceability, and integrity of trial analysis results from the collection source. Observed study data will be mapped to the Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) and serve as the source data from the trial. All study analyses will be completed using analysis data sets that are derived from the SDTM and follow the CDISC Analysis Data Model (ADaM) architecture.

The methods for programming the CDISC SDTM and ADaM data sets are described in Figure 1.

Figure 1 SDTM, ADaM, and TFL Development and Validation



Where:

1. Development, validation, and maintenance of SDTM domains
2. Development and validation of ADaM data sets, with input source the appropriate SDTM domains.
3. Development and validation of tables, figures, and listings (TFL), with input data source the SDTM domains and analysis specific ADaM data sets.

7.4. Planned Study Analyses

7.4.1. Statistical Summaries: Descriptive and Inferential

Categories for data presentation and analysis will consist of each treatment group (POS or Placebo).

All p-values will be rounded to and displayed in four decimals. If a p-value less than 0.0001 occurs, it will be shown in tables as <0.0001.

Descriptive summaries of variables will be provided where appropriate. For continuous variables, the number of non-missing values (n), mean, standard deviation, median, minimum, and maximum will be tabulated by treatment group. For categorical variables, the counts and proportions of each value will be tabulated by treatment group. Expansion of descriptive table categories within each treatment may occur if such elaborations are thought to be useful.

All study-related data collected will be presented in listings. Study-related data not subject to analysis according to this plan will not appear in any tables or graphs but will be included in the data listings.

7.4.2. Interim Analyses and Data Monitoring

Primary analysis of efficacy data will be conducted when all clinical data through the Week 6 visit are available and monitored for all subjects.

Most study staff, including the study biostatisticians and statistical programmers, will be unmasked when all clinical data through the Week 6 visit are collected and cleaned, and will perform the primary analysis. Other study personnel, including investigational site staff, the study monitor, and medical monitor will remain masked to individual treatment assignments until database lock for final analysis.

7.4.3. Final Analysis and Publication of Study Results

The final analysis will be completed after all subjects have completed the study.

7.5. Multiple Testing Procedures

Testing for the primary efficacy endpoint and select secondary endpoints will be completed using a hierarchical method to ensure that the family of tests in the hierarchy will simultaneously be tested at the 0.05 level of significance. See Section 9 for more information.

7.6. Baseline Values

Baseline values are the values obtained prior to any drug administration on the evening of the Baseline Visit (Day 1). For subjects who fail the eligibility criteria for pupil diameter in the first Day 1 measurement, and pass eligibility criteria on the repeat measurement, the repeat measurement will be used for the baseline value. If the Day 1 value is missing, any value collected prior to treatment administration will be treated as the baseline.

8. SUMMARY OF STUDY DATA

8.1. Subject Disposition

A summary of the analysis sets includes the number and percentage of subjects by treatment group and overall for the following categories: subjects in the ARP, subjects in the SP, subjects in the mITT Population, and subjects in the PP Population. All percentages will be based on the number of subjects in the ARP.

End of trial information will also be summarized in this table, including the number of subjects completing Day 15, the number of subjects completing the study, the number of subjects who prematurely discontinued the study with reasons for withdrawal, and the number of subjects who prematurely discontinued the study medication with reasons for study medication discontinuation.

A by-subject data listing of study completion information including the reason for premature study withdrawal, if applicable, will be presented.

8.2. Protocol Deviations

Major protocol deviations considered to have significant impact on treatment outcome, as determined by a Sponsor blinded review of the data prior to database lock and unblinding of the study, may result in the removal of a subject's data from the PP Population. The Sponsor or designee will be responsible for producing the final deviation file; this file will include a description of the protocol deviation and clearly identify whether this violation warrants exclusion from the PP Population. This file will be finalized prior to database lock.

All protocol deviations will be presented in a by-subject data listing, with a flag to indicate if a deviation was considered major.

8.3. Demographics and Baseline Characteristics

Subject demographic data and baseline characteristics will be tabulated and summarized descriptively by treatment group and overall. The demographic data and baseline characteristics will be summarized for the mITT Population, PP Population, SP, and ARP. If the mITT population is equivalent to any of the other populations, then only the mITT version will be generated rather than repeating equivalent summaries.

The demographics consist of age (year), sex, race, ethnicity, study eye (right [OD] or left [OS]), and irides type (light or dark). A subject's age in years is calculated using the date of the informed consent and date of birth. Age will be summarized using descriptive statistics. The number and percentage of subjects by sex, race, ethnicity, study eye, and irides type, will be reported. Percentages will be based on the total number of subjects in the study population presentation.

The following baseline characteristics will be summarized for study eye and fellow eye, and binocularly for visual acuity assessments, using descriptive statistics:

- mLCVA
- mHCVA
- Pupil diameter (photopic and mesopic)
- IOP

All demographic and baseline information will be presented in by-subject listings.

8.4. Medical History

The number and percent of subjects with individual medical histories will be summarized for all subjects by treatment group and overall. Non-ocular and ocular medical history will be summarized separately.

Medical history will be coded using MedDRA Version 26.1. The number and percentage of subjects with any medical history will be summarized overall and for each system organ class (SOC) and preferred term (PT). Percentages will be calculated based on number of subjects in the SP.

Subject medical history data including specific details will be presented in by-subject listings.

8.5. Prior and Concurrent Medications

The number and percentages of all concomitant medications will be summarized by treatment group, Anatomical Therapeutic Chemical (ATC) level 4, and PT. The total number of concomitant medications and the number and percentages of subjects with at least 1 concomitant medication will be summarized by treatment group. All summaries will be performed using the SP.

A concomitant medication is defined as any medication taken on or after the day of first exposure to study drug.

Prior medications are defined as any medication that has a start and stop date prior to the day of first exposure to any study drug. The total number of prior medications and the number and percentages of subjects with at least 1 prior medication will be summarized by treatment group.

8.6. Treatment Exposure and Compliance

Continuous descriptive summaries for total exposure to study medication (in days) and percentage compliance through Week 6, and for the entire study will be presented for the SP by treatment group. Categorical summaries of exposure will also be presented by treatment

group. Additionally, categorical summaries of compliance (subjects with compliance rates of <80% and ≥80%) will be presented by treatment group. Exposure to study drug will be determined by the visit date at the end of the treatment period – date of first study medication administration + 1. For subjects who discontinued study medication during the treatment period, the visit date at the end of the period will be replaced by the medication discontinuation date. Compliance will be determined by dividing the number of used vials returned by the exposure to study medication during the treatment period.

9. EFFICACY ANALYSES

Unless otherwise noted, efficacy will be assessed using the mITT and PP populations, with subjects included in their randomized treatment regardless of the treatment they actually received. For the analysis of the primary efficacy endpoint, imputation will be performed for missing data as described in Section 7.3.2.3. If the analysis using the mITT Population shows a positive effect for POS at the 0.05 level of significance, the primary endpoint will be considered met.

For the analysis of the other efficacy endpoints except the primary efficacy endpoint, only observed case data will be used.

A hierarchical approach will be applied to formally test the significance of endpoints of interest beyond primary efficacy. Select efficacy endpoints will be tested in a predefined sequence until the first nonsignificant test. The primary efficacy endpoint will be first in the sequence. See Section 12.2 for the full sequence of endpoints.

All efficacy assessment data, regardless of whether they are included in the analysis, will be presented in by-subject listings.

9.1. Clinical Efficacy

All efficacy data will be summarized by treatment group (POS or placebo) and visit.

9.1.1. Primary Efficacy Estimand

The primary efficacy estimand, corresponding to the primary efficacy endpoint, is defined by the following components:

1. The *population* is restricted to the mITT Population
2. The *variable* for the primary endpoint is percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15
3. The *intercurrent events* of treatment discontinuation or use of prohibited concomitant medications will be handled according to the treatment policy strategy, irrespective of premature discontinuation of randomized treatment
4. The *population-level summary* is the difference in percent of subjects with ≥ 15 ETDRS letters improvement in the study eye in mLCVA compared to Baseline (Day 1 pre-dose) at Day 15, analyzed using a logistic regression model with treatment and light/dark irides as factors and the baseline mLCVA as a covariate
5. *Treatment:* There will be 1 active treatment arm (POS) and 1 control arm (placebo)

9.1.2. Primary Efficacy Analysis

The primary efficacy endpoint will be analyzed using a logistic regression model with treatment and light/dark irides as factors and the standardized baseline mLCVA as a covariate, which is derived from the baseline mLCVA by subtracting the mean of all baseline mLCVA values and dividing by the standard deviation. The percentage of subjects in each treatment group meeting the criteria, the difference of proportions with 95% CI, and p-value will be provided. Example SAS code is as follows:

```
PROC NLMIXED DATA=MLCVA_RESP;
        BY _IMPUTATION_;
        PARMS B0=0 B2=1 B2=1 B3=1;
        XB = B0 + B1*TRTN + B2*IRIDES + B3*B_MLCVA;
        P = EXP(XB)/(1+EXP(XB));
        MODEL RESP ~ BINARY(P);
        ESTIMATE 'DIFF_PROP' LOGISTIC(B0+B1+0.5*B2+0*B3) – LOGISTIC(B0+0.5*B2+0*B3);
RUN;
```

In addition, the primary efficacy endpoint will be analyzed using the same model indicated above, but without irides as a factor. Analyses of subgroups, including by irides type, will be performed (see Section 7.2.2 for all subgroups).

9.1.3. Secondary Efficacy Estimands

Secondary efficacy estimands will have the same population, intercurrent events, and treatment as the primary estimand. For secondary categorical endpoints, the population-level summary will also match the primary estimand; only changes to the variable (eg, visit, type of assessment) will be required to match the particular secondary endpoint.

Secondary efficacy estimands for continuous endpoints will also vary depending on the assessment, visit, and time point. For change from baseline endpoints, the population-level summary is the change from Baseline in the assessment at the required visit, analyzed using ANCOVA with treatment and light/dark irides as factors and the baseline assessment as a covariate.

The following is an example of a secondary efficacy estimand with a change from baseline endpoint:

1. The *population* is restricted to the mITT Population
2. The *variable is* change from Baseline (Day 1 pre-dose) in mLCVA in the study eye at Day 15
3. The *intercurrent event* of treatment discontinuation or use of prohibited concomitant medications will be handled according to the treatment policy strategy, irrespective of premature discontinuation of randomized treatment
4. The *population-level summary* is the change from Baseline in mLCVA in the study eye at Day 15, analyzed using ANCOVA with treatment and light/dark irides as factors and the baseline mLCVA as a covariate

5. *Treatment:* There will be 1 active treatment arm (POS) and 1 control arm (placebo)

9.1.4. Secondary and Exploratory Efficacy Analysis

Secondary and exploratory efficacy endpoints are indicated in Section 4.2.2. Secondary and exploratory efficacy endpoints will be analyzed by study eye, fellow eye, and binocularly, unless otherwise indicated.

Each of the continuous secondary efficacy endpoints will be analyzed using the ANCOVA model specified in the secondary efficacy estimand, with change from baseline as the dependent variable, treatment, and light/dark irides as factors, and the respective baseline value included as the covariate.

Each ANCOVA will be performed using the mITT and PP populations. The output from each ANCOVA will include the LSM and SE for both treatment groups, along with the placebo-corrected LSM, its 95% CI and associated p-value.

For each of the secondary endpoints related to percent of subjects achieving certain criteria, the analysis will be performed using a logistic regression model with treatment and light/dark irides as factors, and the respective baseline as a covariate. For each analysis, the percentage of subjects in each treatment group meeting the criteria, the difference in percentage with 95% CI and p-value, will be provided.

In addition, each secondary efficacy endpoint will be analyzed by light/dark irides using the same model indicated above but without irides as a factor.

Select questions from the questionnaire, including starburst, glare, and halo severity, will be analyzed as continuous secondary variables. The Night Vision Questionnaire composite score, an average of the responses to the starburst, glare, and halo severity questions, will also be analyzed as a continuous secondary variable. Other questions from the subject questionnaire, including those questions for which it is not appropriate to be analyzed using the methods above, will be summarized categorically for each treatment group and visit, as well as by treatment group, visit, and irides type.

To test for tachyphylaxis, a mixed model for repeated measures will be applied with change from baseline in mLCVA as the dependent variable, treatment, visit, and light/dark irides as factors, and the respective baseline value included as the covariate. All visits through Week 6 where mLCVA is assessed will be included in the model. An unstructured covariance type will be used. Interactions between factors will be tested, and, if significant, interaction terms may be added to the model. The LSM of the difference between the change from baseline in mLCVA at Week 6 and the best change in mLCVA during the first month of the study, the 90% CI, and associated p-value from a test with null hypothesis of mLCVA at Week 6 – mLCVA at the visit/time point ≥ -5 and alpha = 0.05, will be presented.

10. SAFETY ANALYSES

All safety analyses will be conducted using the SP. All safety analyses will be completed using the actual treatment a subject received. Observed case data will be used; no imputation will be performed for missing safety data except for the limited situations described in Section 7.3.2.

All safety data will be presented in by-subject listings. Unscheduled assessments will not be summarized but will be included in the listings.

10.1. Adverse Events

AEs will be coded using the MedDRA Version 26.1.

Treatment-emergent adverse events (TEAEs) are defined as any AE that begins or worsens after initiation of the study drug and through the subject's last study visit (study completion/follow-up phone call, or early termination).

Only TEAEs will be summarized; all AEs (TEAE, non-TEAE) will be included in a by-subject listing.

The number and percent of subjects with any TEAEs will be summarized by SOC and PT by treatment group and overall. At each level of tabulation (e.g., at the PT level), subjects will be counted only once if they had more than one such event reported during the AE collection period.

Note that in MedDRA, ocular events are coded to the SOC of "Eye Disorders". Thus, using SOC in the summaries will provide a separation of ocular and non-ocular adverse events.

The following summary tables will be presented for TEAE data:

- Overall summary of TEAEs
- Summary table of TEAEs by SOC and PT
- Summary table of TEAEs by SOC, PT, and by greatest relationship level to study drug (not related, unlikely related, possibly related, probably related, definitely related, or unknown)
- Summary table of TEAEs by SOC, PT, and maximum severity (mild, moderate, severe)
- Summary table of serious TEAEs by SOC and PT
- Summary table of TEAEs leading to withdrawal from the study by SOC and PT
- Summary table of TEAEs leading to study medication discontinuation by SOC and PT

10.2. Deaths, Serious Adverse Events and Other Significant Adverse Events

10.2.1. Deaths

The AE listing will include all AEs, including deaths, regardless of causality; one of the columns in the listing will specify whether the AE was fatal.

10.2.2. Serious Adverse Events

The AE listing will include all AEs, including SAEs; one of the columns in the listing will specify whether the AE was an SAE.

10.2.3. Adverse Events Leading to Withdrawal from the Study

The AE listing will include all AEs, including AEs leading to withdrawal from the study; one of the columns in the listing will specify whether the AE led to withdrawal from the study.

10.2.4. Adverse Events Leading to Discontinuation of Study Medication

The AE listing will include all AEs, including AEs leading to discontinuation of study medication; one of the columns in the listing will specify whether the AE led to discontinuation of study medication.

10.3. IOP

Observed values and changes from baseline in IOP will be summarized using descriptive statistics at each visit for each treatment group and overall. Separate summaries will be created for the study eye and the fellow eye.

10.4. BCDVA

The percentage of subjects with ≥ 5, ≥ 10, and ≥ 15 letters of improvement or loss in BCDVA from baseline at each visit will be summarized by treatment group. In addition, descriptive statistics of observed values and changes from baseline will be presented for BCDVA data at each visit by treatment group and overall. Separate summaries will be created for the study eye, the fellow eye, and binocular.

10.5. Biomicroscopy and Ophthalmoscopy

Results from biomicroscopic and ophthalmoscopic examinations at each visit will be summarized by treatment group. Separate summaries will be created for the study eye and the fellow eye.

10.6. Corneal Specular Microscopy

Observed values and change from baseline in median endothelial cell count (ECC) from the automated corneal specular microscopy will be summarized by treatment group at each visit (if available). Separate summaries will be created for the study eye and the fellow eye.

10.7. Vital Signs

Descriptive statistics of observed values will be presented for vital sign data at each visit including systolic BP (mmHg), diastolic BP (mmHg), and HR (bpm) by treatment group and overall. Changes from baseline (Day 1) to each scheduled post-baseline time point will be presented.

10.8. Other Screening and Safety Measures

Urine pregnancy tests for females of childbearing potential will be presented in by-subject listings. Dry eye tests (tear break-up time and corneal fluorescein staining), performed only at Day 1, will also be listed.

11. REFERENCES

[1] ICH E9 Expert Working Group. Statistical Principles for Clinical Trials: ICH Harmonized Tripartite Guideline, September 1998

[2] Rubin, D.B. *Multiple Imputation for Nonresponse in Surveys*. John Wiley & Sons Inc., New York, 1987.

12. APPENDICES

12.1. List of Planned Tables, Figures, and Listings

The list of planned TFLs will be provided in a separate document, with shells for each planned output.

12.2. Hierarchy of Sequential Testing

1. Percentage of subjects gaining ≥15 letters mLCVA at Day 15
2. Mean change in mLCVA at Day 15
3. Mean change in mesopic PD at Day 15
4. Percentage of subjects gaining ≥10 letters mLCVA at Day 15
5. Mean change in subject questionnaire Item #4 (difficulty seeing the road because of oncoming headlights) at Day 15
6. Mean change in subject questionnaire Item #5 (difficulty seeing because of glare when driving at dawn or dusk) at Day 15
7. Mean change in mLCVA at Day 8
8. Mean change in mesopic PD at Day 8
9. Percentage of subjects gaining ≥15 letters mLCVA at Day 8
10. Percentage of subjects gaining ≥10 letters mLCVA at Day 8
11. Mean change in mLCVA at Week 6
12. Mean change in mesopic PD at Week 6
13. Percentage of subjects gaining ≥15 letters mLCVA at Week 6
14. Percentage of subjects gaining ≥10 letters mLCVA at Week 6
15. Percentage of subjects gaining ≥15 letters mHCVA at Day 15
16. Mean change in mHCVA at Day 15
17. Percentage of subjects gaining ≥15 letters mHCVA at Week 6