SUMMITANALYTICAL	**CONFIDENTIAL**

Ocuphire Pharma, Inc.

STATISTICAL ANALYSIS PLAN

Protocol Title: Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Eye Drops in Subjects with Presbyopia

Study Number: OPI-NYXP-201 (VEGA-1)

Phase: Phase 2

Sponsor: [redacted]

Author: [redacted]

SAP Date: 2021-06-10

Status: FINAL V1.0

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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
BCDVA	best-corrected distance visual acuity
BP	blood pressure
°C	degrees Celsius
CCLRU	Cornea and Contact Lens Research Unit
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CI	confidence interval
CSR	clinical study report
DCIVA	distance-corrected intermediate visual acuity
DCNVA	distance-corrected near visual acuity
DLD	dim light vision disturbances (also referred to as night vision disturbances or NVD)
eCRF	electronic Case Report Form
EDC	electronic data capture
ETDRS	Early Treatment Diabetic Retinopathy Study
°F	degrees Fahrenheit
FDA	Food and Drug Administration
HR	heart rate
IOP	intraocular pressure
IUD	intrauterine device
LCVA	low-contrast visual acuity
LDP	Low-Dose (0.4%) Pilocarpine Ophthalmic Solution
LDPE	low-density polyethylene
LSM	least-squares mean
MCMC	Markov Chain Monte Carlo

Abbreviation/Term	Definition
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-to-Treat
Nyxol	0.75% Phentolamine Ophthalmic Solution or 1% Phentolamine Mesylate Ophthalmic Solution
Nyxol + LDP	Nyxol dosed in the evening with LDP dosed during the day
OD	oculus dexter (right eye)
OR	odds ratio
OU	oculus uterque (both eyes)
PD	pupil diameter
POS	Phentolamine Ophthalmic Solution
PP	Per Protocol
PT	preferred term
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-DD	World Health Organization Drug Dictionary

3. INTRODUCTION

3.1. Preface

This document presents a statistical analysis plan (SAP) for Ocuphire Pharma, Inc. Protocol OPI-NYXP-201 (VEGA-1) (*Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Eye Drops in Subjects with Presbyopia*).

Reference materials for this statistical plan include the protocol OPI-NYXP-201 (30NOV2020) and Case Report Forms (CRFs; Version 01FEB2021).

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 VEGA-1 study is a randomized, parallel arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) with Low-Dose (0.4%) Pilocarpine Ophthalmic Solution (LDP) in subjects with presbyopia.

The Sponsor intends to use this Phase 2 study to evaluate Nyxol + LDP for the chronic indication of "temporary treatment of presbyopia."

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 wording of the first secondary objective was changed from "< 1 line of loss" to ">1 line of loss". An additional secondary endpoint for the percentage of subjects with distance-corrected near visual acuity (DCNVA) ≤ 20/15, ≤ 20/20, ≤ 20/25, ≤ 20/32, ≤ 20/40, ≤ 20/50, ≤ 20/63, ≤ 20/80, ≤ 20/100, ≤ 20/125, and ≤ 20/160 was added to Section 4.2.2. The secondary endpoints originally specified as the percentage of subjects with improvement in best-corrected distance visual acuity (BCDVA) was changed to improvement or loss in BCDVA. The Snellen ranges for secondary endpoints with discrete ranges were changed to 25/50 to 20/63, 20/80 to 20/100, and 20/125 to 20/160. Otherwise, the analyses described in this analysis plan are consistent with the analyses described in the study 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 objectives of this study are as follows:

Primary objective

- To evaluate the efficacy of Nyxol + LDP to improve distance-corrected near visual acuity (DCNVA) compared to Placebo alone in subjects with presbyopia

Secondary objectives

- To evaluate the efficacy of Nyxol + LDP to prevent >1 line of loss of best-corrected distance visual acuity (BCDVA)
- To evaluate the efficacy of Nyxol + LDP to improve DCNVA compared to Nyxol alone or LDP alone
- To evaluate the ability of Nyxol + LDP to produce the "pinhole" pupil size (approximately 1.6-2 mm)
- To evaluate the efficacy of Nyxol + LDP to improve distance-corrected intermediate visual acuity (DCIVA) and BCDVA
- To evaluate the effect of iris color on the efficacy of Nyxol + LDP
- To evaluate the ocular and systemic safety of Nyxol + LDP and each component individually

4.2. Study Endpoints

4.2.1. Primary Endpoints

Endpoints will be evaluated following administration of masked Nyxol or Placebo (Treatment 1) at or near bedtime for 3 to 4 days, with administration of LDP or No Treatment (Treatment 2) at the next visit (Visit 2) in the morning. Analysis will be completed for 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP (LDP alone)
- Placebo + No Treatment 2 (Placebo alone)

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. The improvement in binocular DCNVA for each subject is relative to the subject's own Baseline value (Visit 1). The analysis of the primary endpoint will be completed for the per-protocol (PP) population, as defined in Section 7.1.2 below.

4.2.2. Secondary Endpoints

Secondary endpoints for efficacy and safety assessments include the following:

Efficacy:

Secondary efficacy endpoints will be analyzed by study eye, fellow eye, and binocular unless otherwise indicated, and will include:

- Percentage of subjects with improvement of ≥ 15 letters in **DCNVA** (photopic) at 1 hour and with < 5 letters of loss in photopic binocular **BCDVA** from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement of ≥ 5, ≥ 10, and ≥ 15 letters in **DCNVA** (photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Change in **DCNVA** (photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement in **DCNVA** (mesopic) from Baseline (Visit 1) to Visit 2 at 2 hours, 4 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Change in **DCNVA** (mesopic) from Baseline (Visit 1) to Visit 2 at 2 hours, 4 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement in **DCIVA** (photopic) from Baseline (Visit 1) to Visit 2 at 1 hour, 3 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Change in **DCIVA** (photopic) from Baseline (Visit 1) to Visit 2 at 1 hour, 3 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement or loss in **BCDVA** (photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone

- Change in **BCDVA** (photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement or loss in **BCDVA** (mesopic) from Baseline (Visit 1) to Visit 2 at 1 hour, 3 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Change in **BCDVA** (mesopic) from Baseline (Visit 1) to Visit 2 at 1 hour, 3 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Change and percent change in **Pupil Diameter (PD)** (mesopic and photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with a **PD** (mesopic and photopic) of < 2.4, < 2.2, < 2.0, < 1.8, < 1.6, and < 1.4 mm measured on Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with a **PD** (mesopic and photopic) of 1.6 to < 2 mm, 2 to < 2.4 mm, and 1.2 to < 1.6 mm measured at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with a ≥ 3-line improvement in photopic binocular **DCNVA** with a **PD** (photopic and mesopic) of 1.6 to < 2 mm at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with Baseline **DCNVA** (photopic and mesopic) ≤ 20/63, ≤ 20/80, ≤ 20/100, ≤ 20/125, and ≤ 20/160 with a ≥ 15-letter improvement in **DCNVA** on Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with Baseline **DCNVA** (photopic and mesopic) from 25/50 to 20/63, 20/80 to 20/100, and 20/125 to 20/160 with a ≥ 15-letter improvement in **DCNVA** on Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo alone
- Percentage of subjects with improvement of ≥ 5, ≥ 10, and ≥ 15 letters in **DCNVA** (photopic) from Baseline (Visit 1) to Visit 2 at 0 minutes, comparing Nyxol to Placebo treated subjects
- Change in **DCNVA** (photopic) from Baseline (Visit 1) to Visit 2 at 0 minutes, comparing Nyxol to Placebo treated subjects
- Percentage of subjects with DCNVA (photopic and mesopic) ≤ 20/15, ≤ 20/20, ≤ 20/25, ≤ 20/32, ≤ 20/40, ≤ 20/50, ≤ 20/63, ≤ 20/80, ≤ 20/100, ≤ 20/125, and ≤ 20/160

Safety and Tolerability:

- Conjunctival hyperemia
- Subjective ocular tolerability (Visit 2)
- Adverse events (AEs)
- Vital signs (heart rate [HR] and blood pressure [BP])
- Intraocular pressure (IOP)
- Biomicroscopy
- Ophthalmoscopy
- Urine pregnancy tests for females of childbearing potential

5. STUDY METHODS

5.1. General Study Design and Plan

This is a placebo-controlled, double-masked, randomized, 4-arm Phase 2 study in males and females between 40 to 64 years of age with presbyopia. A sample size of approximately 152 subjects will be randomized into 1 of 4 treatment arms with the expectation that approximately 140 subjects will be evaluable for efficacy, where evaluable is defined as having received at least one drop of Treatment 1, administered Treatment 2 (LDP or No Treatment), and completed at least one photopic binocular DCNVA measurement after Treatment 2.

Subjects are evaluated for safety and efficacy following administration of masked Nyxol or Placebo (Treatment 1) at or near bedtime for 3 to 4 days, with administration of LDP or No Treatment (Treatment 2) at the next visit (Visit 2) in the morning. Measurements are made at multiple visits and timepoints, and analysis compares 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP
- Placebo + No Treatment 2 (Placebo alone)

Subjects are randomized 4:3:3:4 into the above groups.

After randomization, masked Treatment 1 study medications (Nyxol or Placebo) is dispensed. Treatment 1 study medication is taken by the subject at or near bedtime (approximately 8PM-10PM) starting the night of Visit 1 (Screening/Baseline) or at the appropriate evening in order for Treatment 1 to be taken daily for 3 to 4 consecutive days immediately prior to Visit 2.

Treatment 2 (LDP or No Treatment) is unmasked and is administered at Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

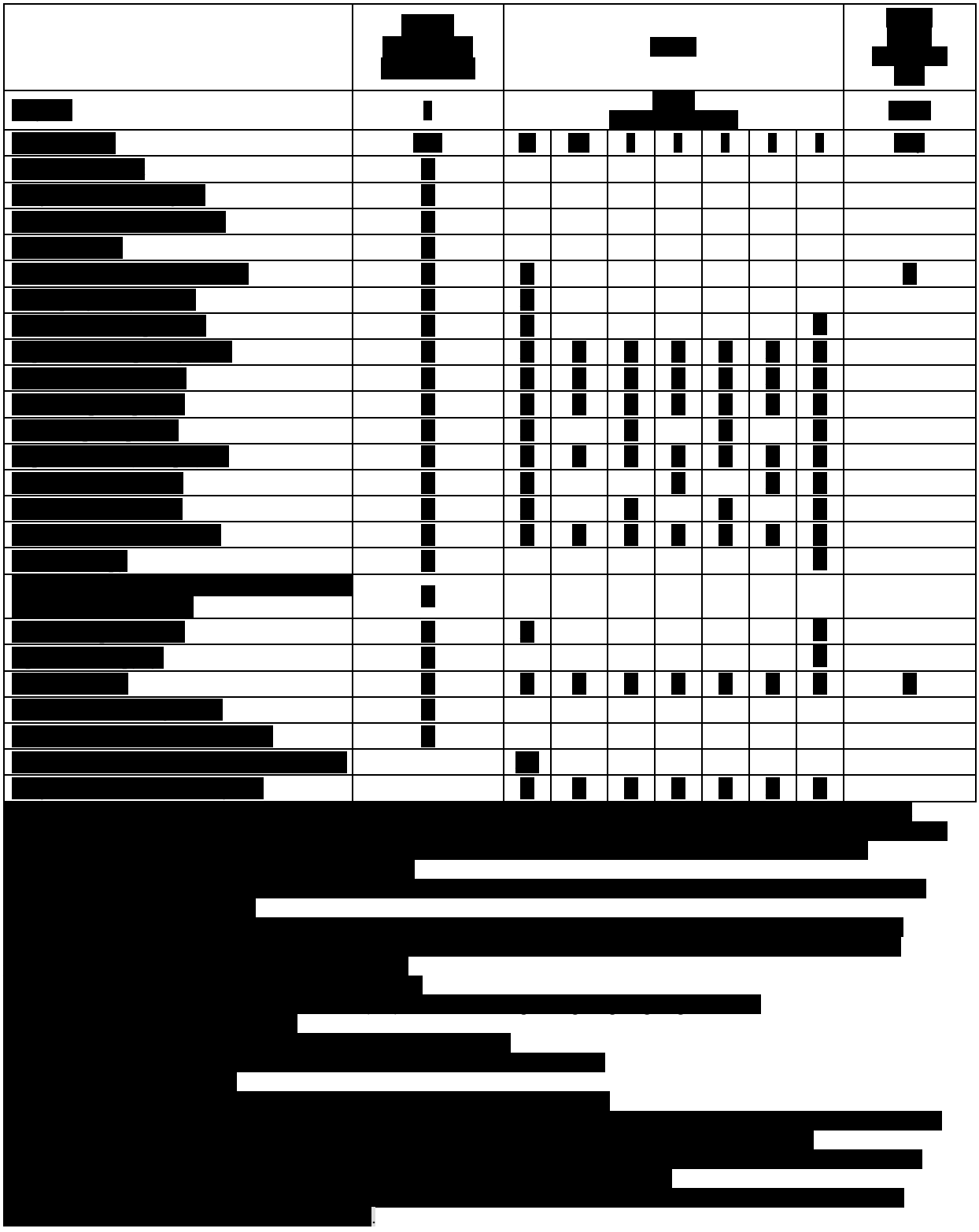
Treatment 1 (Nyxol or Placebo) is administered to both eyes (OU) by the subject.

Treatment 2 (LDP or No Treatment) is administered OU by the Site Staff.

The study eye is defined as the eye with worse Baseline photopic DCNVA. In the case where both eyes have the same Baseline photopic DCNVA, the study eye will be the right eye (OD). The non-study eye will be the fellow eye. The study eye and fellow eye will both be evaluated at all assessments.

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

Table 1 Visit Schedule



5.2. Inclusion – Exclusion Criteria and General Study Population

The study population will be approximately 152 subjects between 40 to 64 years of age with presbyopia, with approximately 140 evaluable subjects.

Individuals who are potential subjects are identified by the study center to schedule the Screening Visit. The Screening Visit shall occur the same day as the Baseline Visit.

Once a subject arrives at the study center, a member of the Site 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 number is assigned.

The start of Screening includes an explanation of the study, a medical and ophthalmic history, and demographics. Eligibility for Inclusion Criteria #1 to 4 as well as Exclusion Criteria #1 to 3, 5 to 13, and 15 to 19 shall be determined and only eligible subjects will continue. This shall be followed by a urine pregnancy test for females of childbearing potential (Exclusion Criterion #20), and HR/BP (Exclusion Criteria #21-22).

The subject will then undergo several VA measurements, including photopic BCDVA as well as mesopic and photopic DCNVA (Inclusion Criteria #5-7). In addition, subjects will undergo measurement of photopic DCIVA and mesopic BCDVA.

The screening assessment will also include an ophthalmic examination that includes assessment of PD, biomicroscopy, dry eye examination with tear break-up time testing and corneal fluorescein staining (Exclusion Criterion #4), intraocular pressure (IOP) measurement (using a Tono-Pen), and direct or indirect ophthalmoscopy without dilation.

In addition, conjunctival hyperemia will be assessed visually with a 4-point grading scale using images from the Cornea and Contact Lens Research Unit (CCLRU) (Exclusion Criterion #14).

If all eligibility criteria are met, the subject will be randomized into the study.

5.3. Randomization and Masking

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 number within numerical order within each site. Once a subject is qualified for the study, the subject is assigned a randomization number in the order provided by the biostatistician.

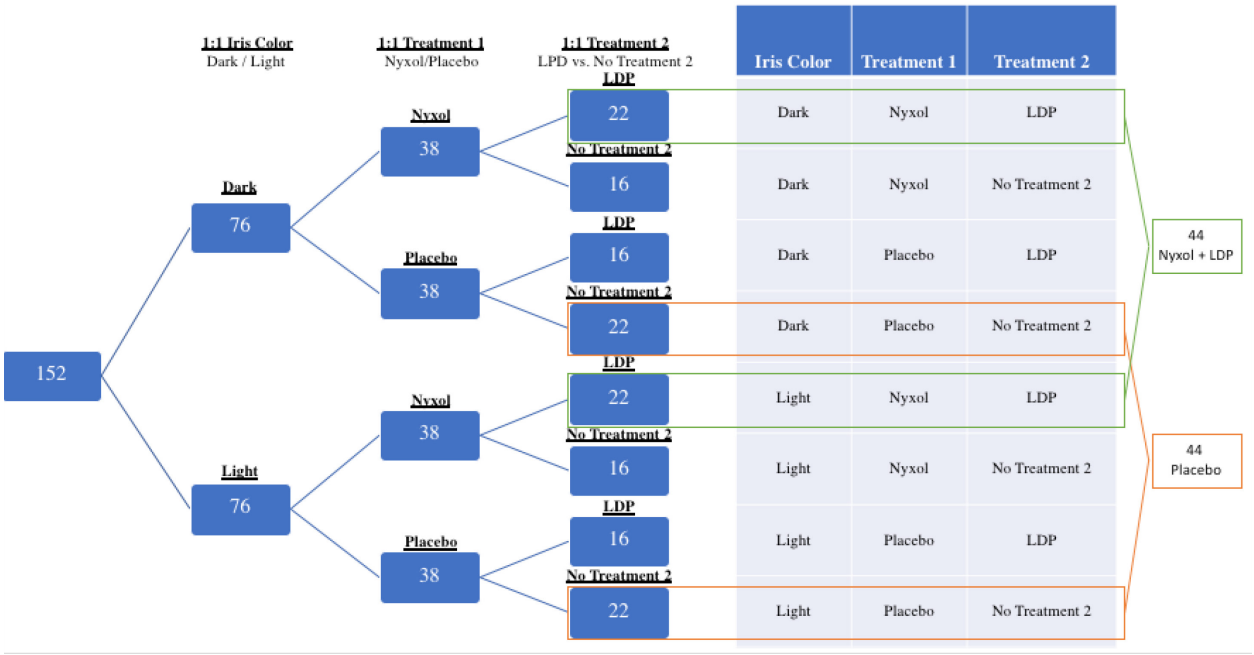
Subjects will be then randomized 4:3:3:4 into 1 of 4 treatment arms:

- Nyxol + LDP
- Nyxol + No Treatment 2 (Nyxol alone)
- Placebo + LDP (LDP alone)
- Placebo + No Treatment 2 (Placebo alone)

Randomization will be stratified 1:1 by iris color (light/dark irides).

A randomization schema for evaluable subjects by investigational treatment (Treatment 1 and Treatment 2) and irides type is described in Figure 1.

Figure 1 Randomization schema by investigational treatment and irides type



The Treatment 1 study medications will be masked to Investigators, Site Staff, study subjects, Ocuphire, and all other study personnel involved in the conduct of the study. Only in case of medical emergency or occurrence of SAEs will the randomization code be unmasked by the Medical Monitor and made available to the Investigator, Ocuphire, and/or other personnel involved in the monitoring or conduct of this study.

Treatment 2 (LDP or No Treatment) is unmasked and will be administered on Visit 2 by a designated, unmasked, Site Staff member, distinct from the Site Staff member recording measurements/assessments.

Randomization for both Treatments will occur at Visit 1.

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:

- DCNVA (i.e., Near VA)
- BCDVA (i.e., Distance VA)

- DCIVA (i.e., Intermediate VA)
- Pupil diameter (PD)

Safety variables include:

- Conjunctival hyperemia (eye redness) measured with a CCLRU card 4-point scale:
  - None (0) = Normal Appears white with a small number of conjunctival blood vessel easily observed.
  - Mild (+1) = Prominent, pinkish-red color of both the bulbar and palpebral conjunctiva.
  - Moderate (+2) = Bright, scarlet red color of the bulbar and palpebral conjunctiva.
  - Severe (+3) = Beefy red with petechiae, dark red bulbar and palpebral conjunctiva with evidence of subconjunctival hemorrhage.
- Subjective ocular tolerability measured on a 4-point scale:
  - 0 – No discomfort
  - 1 – Mild discomfort
  - 2 – Moderate discomfort
  - 3 – Severe discomfort
- AEs
- Vital signs (HR and BP)
- IOP
- Biomicroscopy
- Ophthalmoscopy
- Urine pregnancy tests for females of childbearing potential

6. SAMPLE SIZE

A sample size of approximately 140 subjects (40 for Nyxol + LDP and Placebo alone arms and 30 for each of the other 2 arms) who are evaluable for efficacy is needed for the study. The primary treatment comparison will be Nyxol + LDP compared to Placebo alone. [redacted]

Subjects will be randomized 4:3:3:4 to Nyxol + LDP, Nyxol + No Treatment 2 (Nyxol alone), Placebo + LDP, and Placebo + No Treatment 2 (Placebo alone), respectively.

It is assumed that there will be approximately 7% drop-out between Visit 1 and end of Visit 2. To account for this drop-out, a total of approximately 152 subjects will be randomized into the study.

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 population will include all randomized subjects who received at least one drop of Treatment 1 and were administered Treatment 2 (LDP or No Treatment). The mITT population will be used to analyze selected 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 included all subjects in the mITT population who received one drop of Treatment 1 the day prior to Visit 2 and were administered Treatment 2 at Visit 2, had binocular DCNVA and BCDVA in photopic conditions at Baseline (Visit 1) and at Visit 2 time 1 hour, and had no major protocol deviations. The PP population will be used for the primary endpoint analysis and to analyze all 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 will 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 drop of study treatment (Treatment 1 or Treatment 2). 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 baseline values for the given assessment.

7.2.2. Planned Subgroups

Subgroup analyses by irides type (light, dark) will be completed for efficacy endpoints.

Other possible subgroups include age, sex, and race.

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.

7.3.2. Missing Data

The primary efficacy endpoint is the percentage of subjects with ≥ 15 letters of improvement from baseline in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone. 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, as well as the PP, if required. Confirmatory analyses may be performed using the ARP, also using imputation for missing data.

Otherwise, there will be no substitutions made to accommodate missing data points for efficacy data. All data recorded on the CRF 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

<u>Partial or Missing Dates</u>

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 using the mITT and ARP, as well as the PP, if required. If 5% or fewer data are missing at the Visit 2, 1 hour time point in the Nyxol + LDP and Placebo alone treatment groups, an analysis with last observation carried forward (LOCF) for missing data will be applied within a treatment group. Neither the baseline (Visit 1) nor the Visit 2, 0 minutes time points will be used for imputation. If more than 5% of data are missing at the Visit 2, 1 hour time point in the Nyxol + LDP and Placebo alone treatment groups, 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 (1987) 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.

Missing values for DCNVA 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: binocular photopic DCNVA at Visit 2 (30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours). No imputation will be applied to the Visit 2, 0 minutes time point.

The number of imputations will be set to 500. The outcomes of interest (change from baseline) will be calculated from these imputed datasets. The treatment difference for each

imputed dataset will be evaluated using logistic regression. See Section 9.1 for details on these models. The estimates and standard errors (SEs) of the odds ratios based on the 500 imputed datasets are then combined by [redacted] The odds ratios with the corresponding 95% CI will also be presented.

Example [redacted]

[redacted]

7.3.3. Handling of Early Termination Visit Information

In the event that a subject is terminated early from this study on Visit 2, the early termination data for safety variables will be assigned to the closest scheduled time point on Visit 2. If the closest time point has valid data, the early termination data will be assigned to the next available time point.

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 (MedDRA Version 23.1) system for reporting (preferred term and body system).

Prior and concomitant medications will be coded using WHO-DD (World Health Organization Drug Dictionary) (Version September 2020, C Format).

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) Datatrak One version 14.6.2.

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 2.

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: Nyxol + LDP, Nyxol + No Treatment 2 (Nyxol alone), Placebo + LDP, Placebo + No Treatment 2 (Placebo alone).

All statistical tests will be two-sided and a difference resulting in a p-value of less than or equal to 0.05 will be considered statistically significant. The p-values for the analysis of secondary efficacy endpoints and safety endpoints will be considered descriptive. 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

No formal interim analysis or safety monitoring committee is planned for this study.

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

There will be no adjustments for multiplicity and no formal multiple testing procedures are to be implemented with this analysis plan.

7.6. Baseline Values

Baseline values are the values obtained at Visit 1, prior to any treatment administration. If the Visit 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 the study, the number of subjects who prematurely discontinued the study with reasons for withdrawal, the number of subjects completing the study medication dosing, 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, 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 and/or exclusionary.

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, and study eye, iris color (light blue, dark blue, blue with peripupillary brown, uniform green, green with brown iris ring, central brown and peripheral green, brown with some peripheral green, or brown), 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, iris color, and irides type will also 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, fellow eye, and binocular (if applicable), using descriptive statistics:

- BCDVA (photopic and mesopic)
- DCNVA (photopic and mesopic)
- DCIVA (photopic)
- 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 the MedDRA Version 23.1 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, collected from up to 30 days prior to Screening. 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 Administration

Treatment administration data for both Treatment 1 and Treatment 2 will be presented in by-subject listings.

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. Confirmatory analysis of the primary efficacy endpoint may be performed using the ARP, if different from the mITT, also using imputation for missing data. For the analysis of the secondary efficacy endpoints, only observed case data will be used.

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

9.1. Clinical Efficacy

For all efficacy endpoints, Baseline is defined as Visit 1.

All efficacy data will be summarized by treatment group for the Baseline assessment and for Visit 2 timepoints (0 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours), as appropriate.

9.1.1. Primary Efficacy Analysis

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA on Visit 2 at 1 hour with Nyxol + LDP compared to Placebo alone in the study eye. If the analysis using the PP Population shows a positive effect for Nyxol at the 0.05 level of significance, the primary endpoint will be considered met. The primary efficacy endpoint will be analyzed using a logistic regression model with treatment and light/dark irides as factors and the baseline DCNVA as a covariate. The percentage of subjects in each treatment group meeting the criteria, the odds ratio (OR) with 95% CI, and p-value will be provided. Example SAS code is as follows:

By including irides type as a factor in the primary efficacy analysis model, the model efficiency as well as a change in the treatment effect will be increased. Including this factor in the model will also make the results more generalizable to other studies in which the sample characteristics may differ from the current study [2].

In addition, the primary efficacy endpoint will be analyzed by light/dark irides using the same model indicated above but without irides as a factor. For this subgroup analyses, observed case data only will be used; that is, missing values will not be imputed.

9.1.2. Secondary Efficacy Analyses

Secondary efficacy endpoints are indicated in Section 4.2.2. Secondary efficacy endpoints will be analyzed by study eye, fellow eye, and binocular (if available) using the PP population, and the mITT population for selected endpoints (see the list of planned tables, Section 12.1, for details).

All continuous secondary endpoints derived from VA assessments, such as change in DCNVA, DCIVA, and BCDVA, will be analyzed using Early Treatment Diabetic Retinopathy Study (ETDRS) letters correctly read. Any secondary endpoints using Snellen measurements will be converted from letters read as follows:

Table 2 Snellen Equivalents to VA Assessment Letters Read

DCNVA/DCIVA letters	Snellen	BCDVA letters
≥75	20/15	≥60
70 - 74	20/20	55 - 59
65 - 69	20/25	50 - 54
60 - 64	20/32	45 - 49
55 - 59	20/40	40 - 44
50 - 54	20/50	35 - 39
45 - 49	20/63	30 - 34
40 - 44	20/80	25 - 29
35 - 39	20/100	20 - 24
30 – 34	20/125	15 - 19
25 - 29	20/160	10 - 14
<25	20/200	<10

Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (ANCOVA), with observed value as the dependent variable, treatment, and light/dark irides as factors, and the respective baseline value included as the covariate. Another ANCOVA will be applied with change from baseline as the dependent variable using the same factors and covariates.

The output from each ANCOVA will include the LSM and SE for all treatment groups, along with each LSM, the 95% CI and associated p-value for Nyxol + LDP vs. Nyxol alone, Placebo + LDP, and Placebo alone. Continuous pupil diameter endpoints will be assessed using the study eye, fellow eye, and best eye, which is defined as the eye with the greatest reduction in diameter.

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 will be presented, and the OR with 95% CI and p-value will be provided for Nyxol + LDP vs. Nyxol alone, Placebo + LDP, and Placebo alone. Categorical pupil diameter endpoints will be assessed using the study eye, fellow eye, and either eye, which is defined as achieving a given criterion in either eye.

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.

10. SAFETY ANALYSES

All safety analyses will be conducted using the SP unless specified otherwise below. 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 MedDRA, Version 23.1.

Treatment-emergent adverse events (TEAEs) are defined as any AE that begins or worsens after initiation of the investigational product and through the subject's last study visit (study completion or early termination).

If the onset of an AE is on or after the date of first dose of study medication or is increasing in severity after first dose of study medication, then the AE will be considered treatment emergent.

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 (completed for both the SP and mITT population)
- 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. Conjunctival Hyperemia

Results from the conjunctival hyperemia assessment, measured with a CCLRU card 4-point scale, will be summarized descriptively using counts and percentages for each treatment group at each time point (baseline, 0 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours) for the observed value and change from baseline. Separate summaries will be created for the study eye and the fellow eye.

Additionally, conjunctival hyperemia will be summarized as a continuous variable. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints.

10.4. Subjective Ocular Tolerability

Results from the subjective ocular tolerability assessment, measured on a 4-point scale, will be summarized descriptively using counts and percentages for each treatment group at each time point (0 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours). Additionally, the categories “No Discomfort” and “Mild Discomfort” will be pooled into a single category and summarized descriptively, as will the categories “Moderate Discomfort” and “Severe Discomfort”.

Treatments will be compared for the two pooled categories using a Fisher's exact test for Nyxol + LDP vs. Nyxol alone, Placebo + LDP, and Placebo alone. Separate summaries will be created for the study eye and the fellow eye.

10.5. Vital Signs

Descriptive statistics of observed values will be presented for vital sign data at each time point (baseline, 0 minutes, and 6 hours), including systolic BP (mmHg), diastolic BP (mmHg), and HR (bpm) by treatment group. Changes from baseline to each scheduled post-baseline time point will be presented.

10.6. IOP

Observed values and change from baseline in IOP at 0 minutes and 6 hours will be summarized for the study eye and the fellow eye. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints.

10.7. Biomicroscopy and Ophthalmoscopy

Results from biomicroscopic and ophthalmoscopic examinations at baseline and 6 hours will be summarized by treatment group for the study eye and the fellow eye.

10.8. Other Safety Measures

The efficacy endpoint "Percentage of subjects with improvement or loss in **BCDVA** (photopic) from Baseline (Visit 1) to Visit 2 at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours of ≥ 5, ≥ 10, and ≥ 15 letters comparing Nyxol + LDP to Nyxol alone, Placebo + LDP, and Placebo" will be analyzed using the Safety Population for photopic BCDVA.

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

11. REFERENCES

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

[2] Hauck WM, Anderson S, and Marcus SM, Should We Adjust for Covariates in Nonlinear Regression Analyses of Randomized Trials? *Controlled Clin Trials* 1998;19:249–256

12. APPENDICES

12.1. List of Planned Tables

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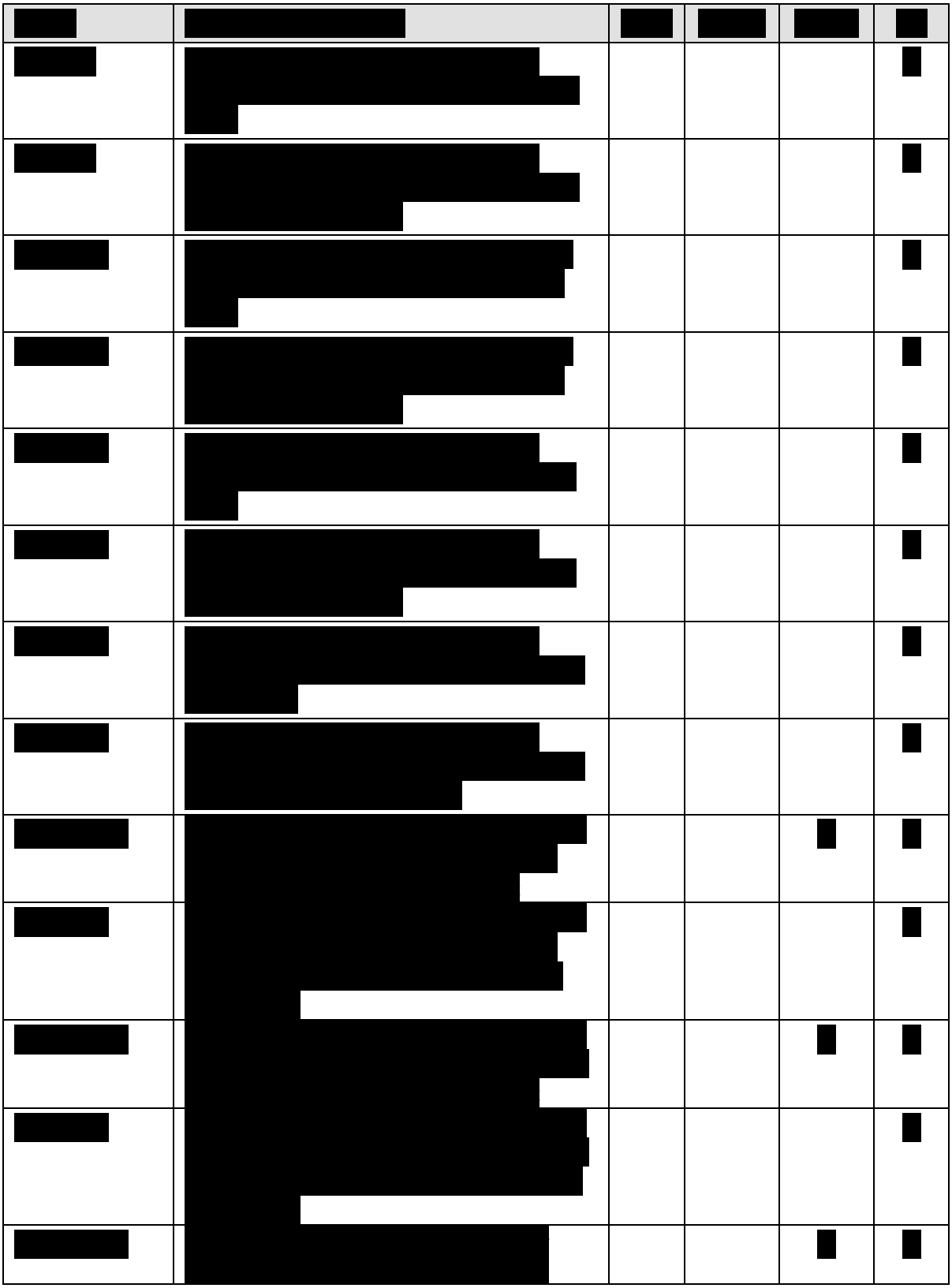

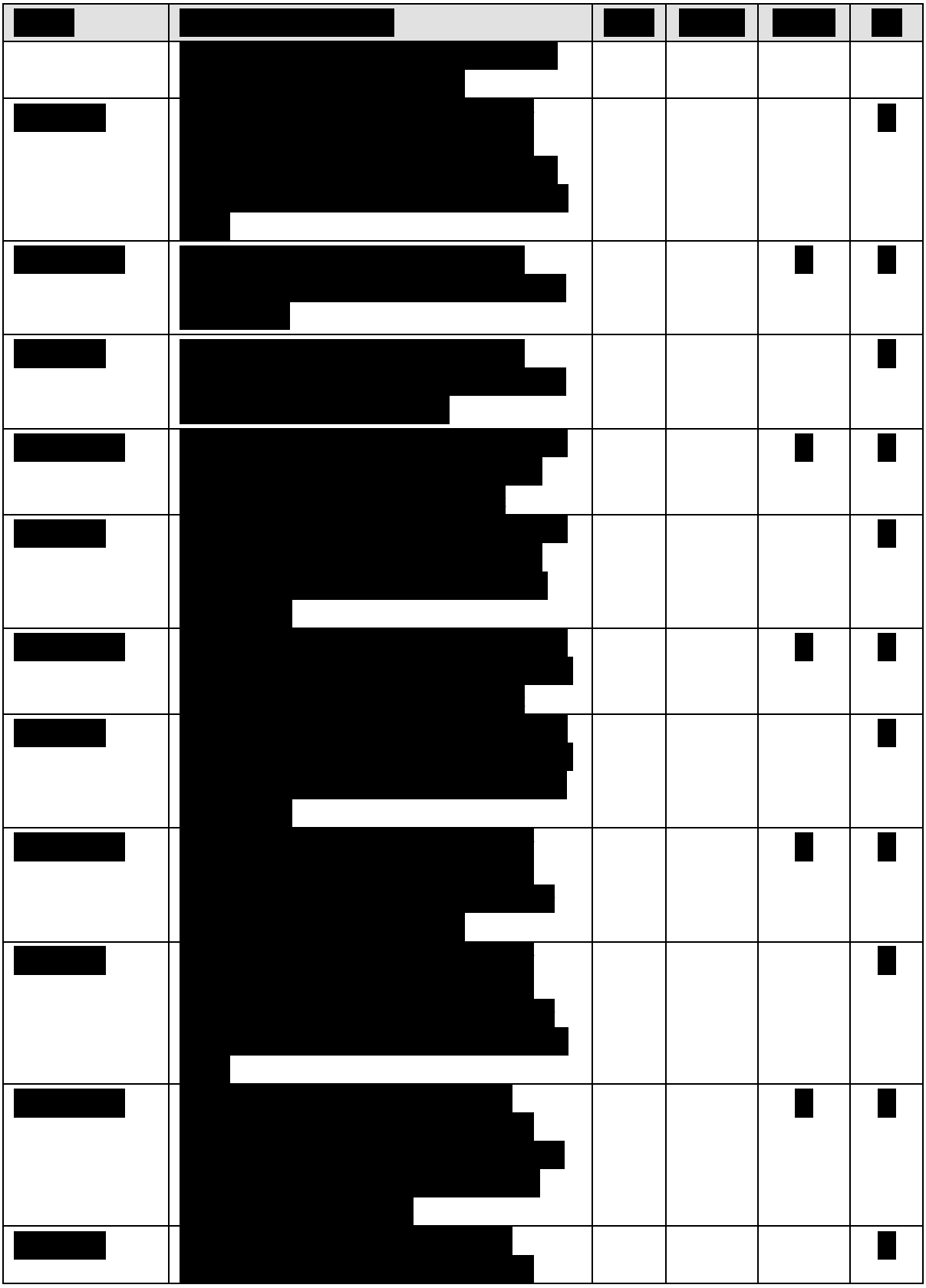

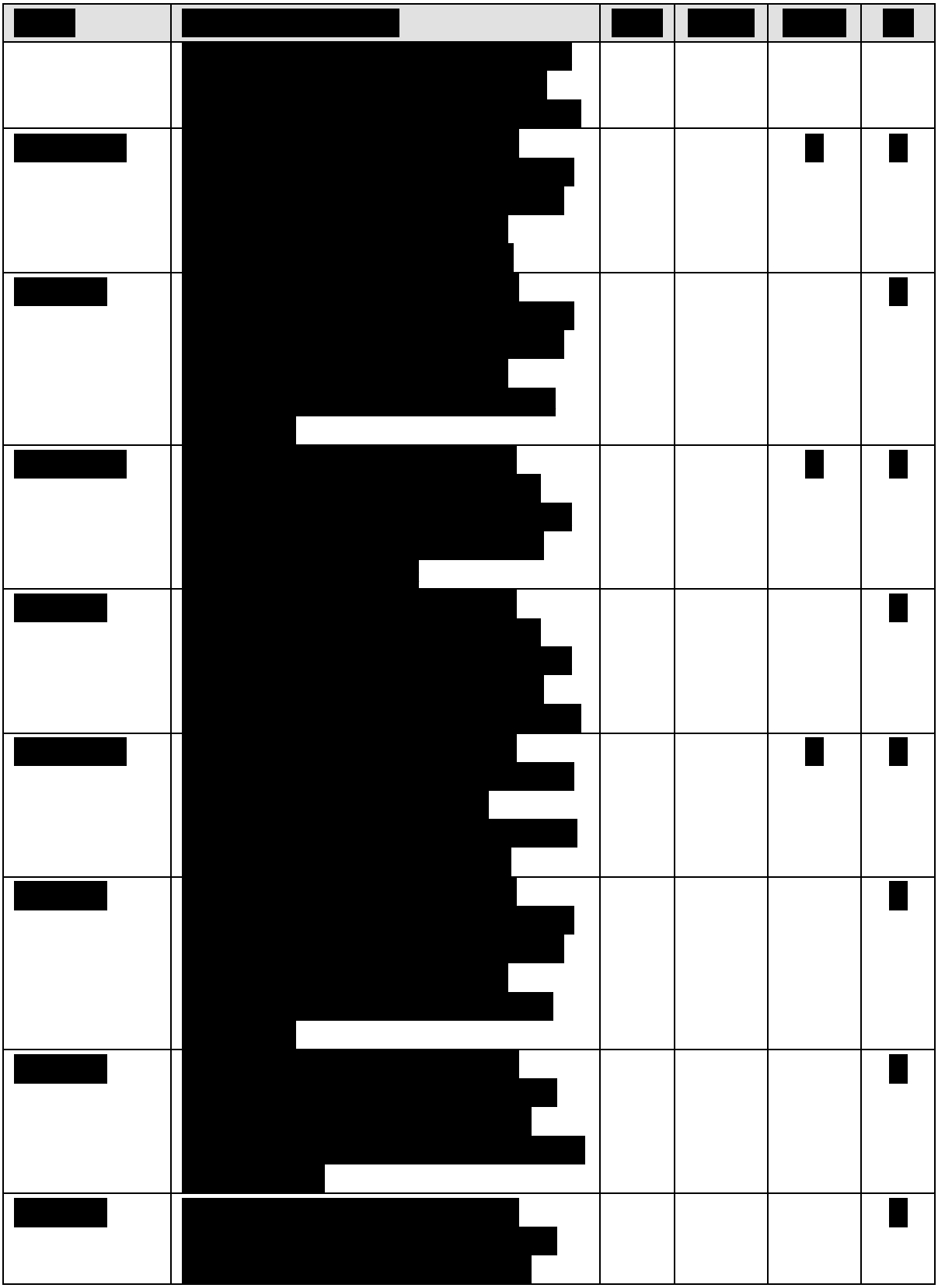

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12.2. List of Planned Listings

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