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Ocuphire Pharma, Inc.

STATISTICAL ANALYSIS PLAN

Protocol Title: Randomized, Double-Masked, Placebo-Controlled, Multicenter, Phase 3 Study of the Safety and Efficacy of Nyxol (Phentolamine Ophthalmic Solution 0.75%) as a Single Agent and With Adjunctive Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4% in Subjects With Presbyopia

Study Number: OPI-NYXP-301 (VEGA-2)

Phase: Phase 3

Sponsor: Ocuphire Pharma, Inc.
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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
CDISC	Clinical Data Interchange Standards Consortium
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
LDP	Low-Dose (0.4%) Pilocarpine Ophthalmic Solution
LSM	least-squares mean
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)

Abbreviation/Term	Definition
OR	odds ratio
OU	oculus uterque (both eyes)
PD	pupil diameter
POS	Phentolamine Ophthalmic Solution
PP	Per Protocol
PPS1	Per Protocol Stage 1
PPS2	Per Protocol Stage 2
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-301 (VEGA-2) (*Randomized, Double-Masked, Placebo-Controlled, Multicenter, Phase 3 Study of the Safety and Efficacy of Nyxol (Phentolamine Ophthalmic Solution 0.75%) as a Single Agent and With Adjunctive Low-Dose Pilocarpine Hydrochloride Ophthalmic Solution 0.4% in Subjects With Presbyopia*).

Reference materials for this statistical plan include Amendment 3 of the protocol OPI-NYXP-301 (29SEP2023) and Case Report Forms (CRFs; Version 09DEC2022).

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-2 study is a randomized, placebo-controlled, double-masked study of the safety and efficacy of Nyxol (POS 0.75%) in combination with low-dose Pilocarpine (LDP) 0.4% in subjects with presbyopia. The Sponsor intends [redacted]."

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 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 LDP as adjunctive therapy to Nyxol (Nyxol + LDP) to improve DCNVA without loss of BCDVA compared to placebo + LDP vehicle in subjects with presbyopia

Additional objectives

[redacted]

4.2. Study Endpoints

4.2.1. Primary Endpoints

The primary efficacy endpoint is the percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA from Baseline at 30 min post-LDP/vehicle comparing subjects treated with Nyxol + LDP to subjects treated with placebo + LDP vehicle at Visit 5 (Stage 2 Day 8).

This comparison will be analyzed first using a hierarchical testing method.

4.2.2. Secondary Endpoints

Efficacy:

[redacted]

[redacted]

Measurements:

- [redacted] Best-corrected distance VA measured [redacted]
- Distance-corrected intermediate VA [redacted]
- [redacted] Distance-corrected near VA [redacted]
- Pupil diameter [redacted]

All of the efficacy endpoints will be analyzed overall and by light/dark irides at all timepoints. Subjects will also be analyzed by binocular, study eye, and fellow eye, where applicable.

Safety:

Safety measures are AEs, ocular tolerability, intraocular pressure (IOP), BCDVA, biomicroscopy, ophthalmoscopy, heart rate (HR) and blood pressure (BP), and subject questionnaire. Urine pregnancy tests for females of childbearing potential will also be conducted.

Measurements:

- Biomicroscopy, ophthalmoscopy, and HR and BP measured in accordance with the site's standard practice
- Ocular tolerability [redacted]

- Intraocular pressure measured with a Tono-Pen or Goldmann Applanation tonometry
- Subject questionnaire completed by the subject

5. STUDY METHODS

5.1. General Study Design and Plan

This is a randomized, double-masked, placebo-controlled, multicenter, Phase 3 study in approximately 320 subjects with presbyopia. The study is performed in 2 stages, as described below.

Subjects are randomized 1:1:1:1 to treatments for both stages at Visit 1 (Screening/Baseline). Note that Visit 1 (Screening/Baseline) 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 assessments on the second day.

Stage 1 consists of 2 treatment groups (Nyxol or placebo [ie, Nyxol vehicle]), with approximately 160 subjects in each group. Stage 2 consists of 4 treatment groups (Nyxol + LDP, Nyxol + LDP vehicle, placebo + LDP, and placebo + LDP vehicle), with approximately 80 subjects per treatment group. Treatment assignments for Stage 1 and Stage 2 are assigned at randomization prior to Stage 1.

Randomization [redacted]. The subjects are recruited from approximately 30 investigational sites in the US.

Stage 1: Dosing of Treatment 1

Individuals who are potential subjects are identified by the study center to schedule the Screening Visit. If a subject successfully completes their Screening Assessments, then Visit 1 becomes the Baseline Visit.

Once a subject arrives at the study center, a member of the Site Staff interviews the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form (ICF) is signed, and a subject number is assigned.

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

At Visit 1 (Stage 1 Day 1), eligible subjects were randomized, and the first dose of Treatment 1 was administered at the study site at Time 0 min (note: if Visit 1 is split into 2 separate days, then dosing occurs on the second day). Efficacy and safety measurements were made before dosing with Treatment 1 (Time 0 min) and at multiple post-dose timepoints from 30 min to 8 hours.

In Stage 1, the safety and efficacy of Nyxol as a single agent was evaluated after 1 day and 7 days of daily dosing.

Following Visit 2 (Stage 1 Day 8), subjects discontinued dosing Treatment 1 for 7 to 14 days (Washout Period). During the Washout Period, subjects returned for Visit 3 (Stage 1 Day 10 ± 1 day) to assess the resolution of drug treatment effects.

During Stage 1, Treatment 1 is dosed daily in the evenings near bedtime starting with the day after completion of Visit 1, except for Visit 1 [Stage 1 Day 1], when the subject is dosed during the day at the study site, and the evening before Visit 2, when Treatment 1 is dosed as close to 12 hours prior to Time 0 min at Visit 2.

Analysis will consist of comparison across the 2 arms:

- Nyxol
- Placebo

Stage 2: Dosing of Treatments 1 and 2

Visit 4 (Stage 2 Day 1) is the start of Stage 2. In Stage 2, the efficacy and safety of Nyxol + LDP was evaluated following 1 day and 7 days of dosing, in which Treatment 1 (Nyxol or placebo) is dosed daily in the evenings near bedtime, except the evening before Visits 4 and 5, when Treatment 1 is dosed as close to 12 hours prior to the scheduled time for those visits. Treatment 2 (LDP or LDP vehicle) is dosed daily in the mornings. Treatment 2 will be dosed at the study site at the beginning of Visits 4 and 5 (Stage 2 Days 1 and 8, respectively).

Analysis consists of comparison across the 4 arms:

- Nyxol + LDP
- Nyxol + LDP vehicle
- Placebo + LDP
- Placebo + LDP vehicle

Other design considerations:

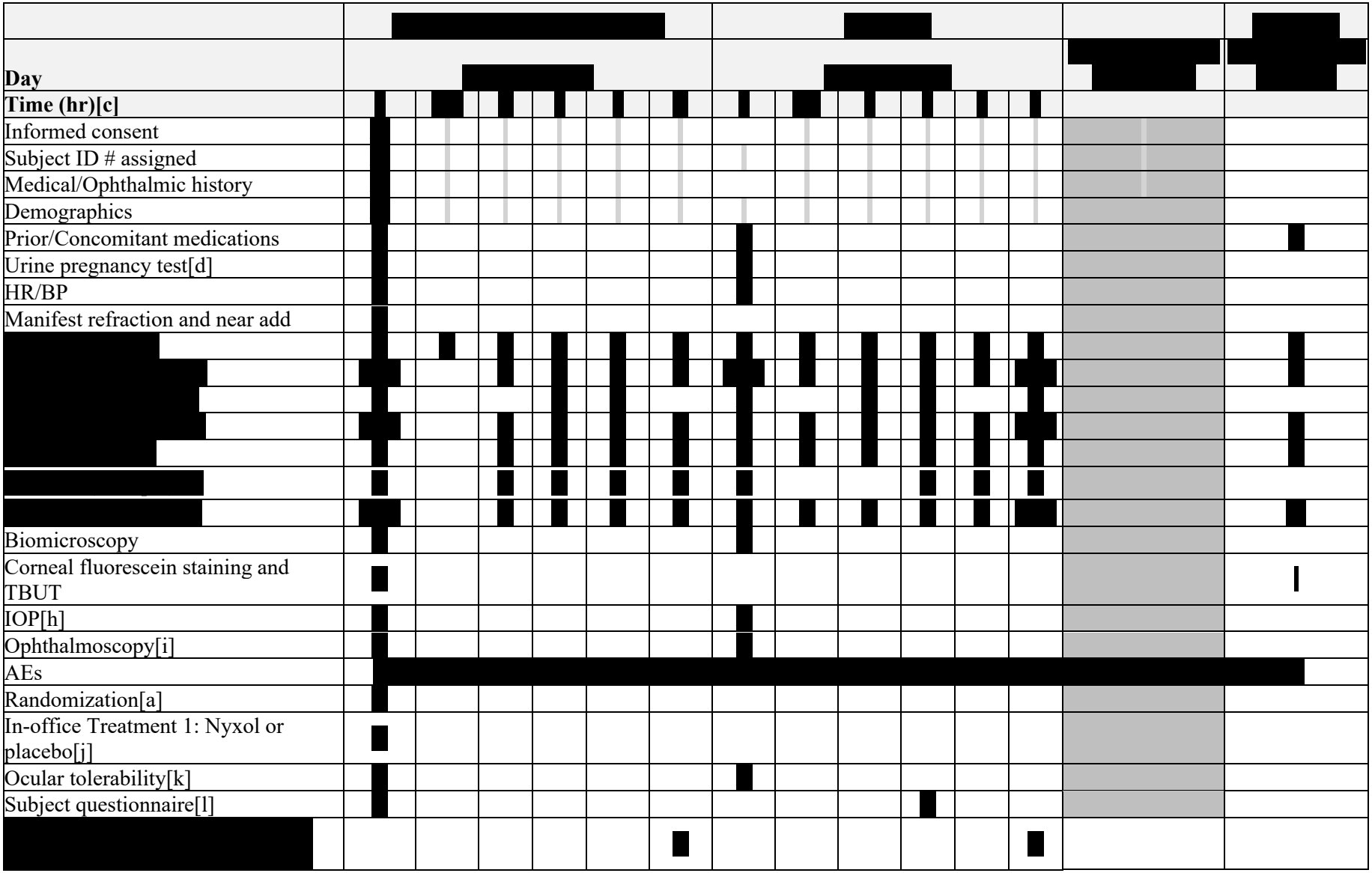
Study medications (Treatments 1 and 2) were each dosed as a single drop in each eye, with Treatment 1 dosed in the evenings near bedtime and Treatment 2 dosed in the mornings. Baseline for the study is defined as pre-treatment (prior to Time 0 min) at Visit 1 (Screening/Baseline).

During Visit 2 (Stage 1 Day 8), Time 0 min is defined as the timing of the first efficacy assessments at the visit, approximately 12 hours after dosing with Nyxol the previous night (12 hour post-dose timepoint). During Visits 4 and 5 (Stage 2 Days 1 and 8, respectively), Time 0 min is defined as the time of Treatment 2 administration.

The study eye is defined as [redacted] 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 Schedule of Events for Stage 1



AE, adverse event; BCDVA, best-corrected distance visual acuity; BP, blood pressure; DCNVA, distance-corrected near visual acuity; HR, heart rate; ID, identification; IOP, intraocular pressure; OD, right eye; OS, left eye; PD, pupil diameter; VA, visual acuity.

Table 2 Schedule of Events for Stage 2

Time (hr)[b]														
Prior/Concomitant medications														
Urine pregnancy test[c]														
HR/BP														
Biomicroscopy														
IOP[g]														
Ophthalmoscopy[h]														
AEs														
In-office Treatment 2: LDP or LDP vehicle[i]														
Ocular tolerability[j]														
Subject questionnaire[k]														
Treatment 1: Nyxol or placebo and Subject diary dispensed / reviewed [l]														
Treatment 2: LDP or LDP vehicle and Subject diary dispensed / collected [i]														

5.2. Inclusion – Exclusion Criteria and General Study Population

5.3. Subject Inclusion Criteria

1. Males or females ≥ 40 and ≤ 64 years of age.
2. Able to comply with all protocol-mandated procedures independently and to attend all scheduled office visits.
3. Able and willing to give signed informed consent.
4. Able to self-administer study medication throughout the study period.

[redacted]

8. [redacted].

5.4. Subject Exclusion Criteria

Subjects excluded from the study will be individuals with the following characteristics:

Ophthalmic (in either eye):

1. Use of any topical prescription (including Vuity) or over-the-counter (OTC) ophthalmic medications of any kind within 7 days of Screening until study completion, with the exception of lid scrubs with OTC products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) and artificial tears as specified in Exclusion #2 below.
2. Use of any OTC artificial tears (preserved or unpreserved) during Visit days or 15 min before or after instillation of Treatment 1 or Treatment 2.
3. Current use of any dry eye product such as topical ophthalmic therapy for dry eye (eg, generic cyclosporine, Restasis, Xiidra, Cequa, and Eysuvis) or intranasal dry eye product (eg, Tyrvaya) or other devices.
4. Tear break-up time of < 5 seconds or corneal fluorescein staining Grade ≥ 2 in the inferior zone or Grade ≥ 1 in the central zone using the National Eye Institute scale.
5. Clinically significant ocular disease (eg, cataract, glaucoma, corneal edema, uveitis, retinal degeneration, loss of visual field, or any macular pathology) that might interfere with the study as deemed by the Investigator.

6. Recent or current evidence of ocular infection or inflammation in either eye (such as current evidence of clinically significant blepharitis, conjunctivitis, keratitis, etc.). Subjects must be symptom free for at least 7 days.
7. Any history of herpes simplex or herpes zoster keratitis.
8. Known allergy, hypersensitivity, or contraindication to any component of the phentolamine, pilocarpine, or vehicle formulations.
9. Prior participation in a study involving the use of Nyxol for the treatment of presbyopia.
10. History of cauterization of the punctum or punctal plug (silicone or collagen) insertion or removal.
11. Ocular trauma within 6 months prior to Screening.
12. Ocular surgery or any ocular laser treatment within 6 months prior to Screening.
13. Subjects with surgical monovision, multifocal or extended depth of focus IOLs are excluded (monofocal IOLs are accepted if in place > 6 months prior to Screening).
14. History of any traumatic (surgical or nonsurgical) or nontraumatic condition affecting the pupil or iris (eg, irregularly shaped pupil, neurogenic pupil disorder, iris atrophy, iridotomy, iridectomy, iritis, etc.).
15. Unwilling or unable to discontinue use of contact lenses at Screening until study completion.

Systemic:

16. Known hypersensitivity or contraindication to alpha- and/or beta-adrenoceptor antagonists (eg, chronic obstructive pulmonary disease or bronchial asthma; abnormally low BP or HR; second- or third-degree heart blockage or congestive heart failure).
17. Known hypersensitivity or contraindication to any systemic cholinergic parasympathomimetic agent.
18. Clinically significant systemic disease (eg, uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the judgment of the Investigator.
19. Initiation of treatment with, or any changes to, the current dosage, drug, or regimen of any systemic adrenergic or cholinergic drugs within 7 days prior to Screening or during the study; however, Flomax (tamsulosin) is specifically excluded.
20. Participation in any investigational study within 30 days prior to Screening.
21. Females of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. A female is considered to be of childbearing potential unless she is 1 year

postmenopausal or 3 months post-surgical sterilization. All females of childbearing potential, including those with post−tubal ligation, must have a negative urine pregnancy test result at Visit 1 (Screening/Baseline).

22. Resting HR outside the range of 50 to 110 beats per minute following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Heart rate may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position.
23. Hypertension with resting diastolic BP > 105 mmHg or systolic BP > 160 mmHg following at least a 5-min rest period in the sitting position at Visit 1 (Screening/Baseline). Blood pressure may be repeated **only once** if outside the specified range, following another 5-min rest period in the sitting position.

5.5. Randomization and Masking

A randomization code for allocating subjects to each treatment group will be prepared by an unmasked biostatistician not connected with the study. Subjects will be then randomized 1:1:1:1 into 1 of 4 treatment arms at Visit 1:

- Nyxol in Stage 1; Nyxol + LDP in Stage 2
- Nyxol in Stage 1; Nyxol + LDP vehicle in Stage 2
- Placebo in Stage 1; Placebo + LDP in Stage 2
- Placebo in Stage 1; Placebo + LDP vehicle in Stage 2

Randomization will [redacted]

The Treatment 1 (Nyxol/placebo) and Treatment 2 (LDP/LDP vehicle) study medications was 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.

5.6. 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:

- Photopic and mesopic DCNVA (i.e., Near VA)
- Photopic and mesopic BCDVA (i.e., Distance VA)
- Photopic DCIVA (i.e., Intermediate VA)

- Photopic and mesopic PD
- Subject questionnaire responses related to change in near vision and satisfaction with near vision

Safety variables include:

- AEs
- Subjective ocular tolerability [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- IOP
- BCDVA
- Biomicroscopy
- Ophthalmoscopy
- Subject questionnaire
- Vital signs (HR and BP)
- Urine pregnancy tests for females of childbearing potential

6. SAMPLE SIZE

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 1 drop of Treatment 1 (Nyxol or placebo). The mITT Population will be used to analyze the primary endpoint as well as other efficacy endpoints, with subjects included in their randomized treatment regardless of the treatment they actually received.

7.1.2. Per Protocol Stage 1 Population (PPS1)

The PP Stage 1 (PPS1) Population will include all subjects in the mITT Population who receive 1 drop of Treatment 1 the day prior to Visit 2, have binocular DCNVA and BCDVA in photopic conditions at Baseline (Visit 1) and at 12 hours post-dose at Visit 2, and have no major protocol deviations considered to have significant impact on treatment outcome in Stage 1. The PPS1 Population will be used for analysis of select efficacy endpoints.

7.1.3. Per Protocol Stage 2 Population (PPS2)

The PP Stage 2 (PPS2) Population will include all subjects in the mITT Population who receive 1 drop of Treatment 1 the day prior to Visit 4 and Visit 5, receive 1 drop of Treatment 2 (LDP or LDP vehicle) at Visit 4 and Visit 5, have binocular DCNVA and BCDVA in photopic conditions at the 30 Minute timepoint at Visit 5 (after Treatment 2 dosing), and have no major protocol deviations considered to have significant impact on treatment outcome in Stage 2. The PPS2 Population will be used for analysis of select efficacy endpoints.

7.1.4. 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.

7.1.5. Safety Population (SP)

The SP will include all randomized subjects who have received at least 1 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 [redacted]

7.3. Management of Analysis Data

7.3.1. Data Handling

Data from unscheduled visits may be used for the primary efficacy analysis as described in Section 7.3.2.3 below. Otherwise, unscheduled visit data 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 percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA from Baseline comparing Nyxol-treated subjects to placebo-treated subjects at 12 hours post-dose at Visit 2 (Stage 1 Day 8). For the analysis of the primary efficacy and key secondary endpoints, 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 either PP population, 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 that is required for the primary efficacy analyses, 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

7.3.3. Handling of Early Termination Visit Information

In the event that a subject is terminated early from the study, the early termination data will be assigned to the closest scheduled visit and/or time point. If the closest visit or time point has valid data, the early termination data will be assigned to the next available visit or 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 25.1) system for reporting (preferred term and body system).

Prior and concomitant medications will be coded using WHO-DD (World Health Organization Drug Dictionary) (Version Global C3, September 1, 2022).

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

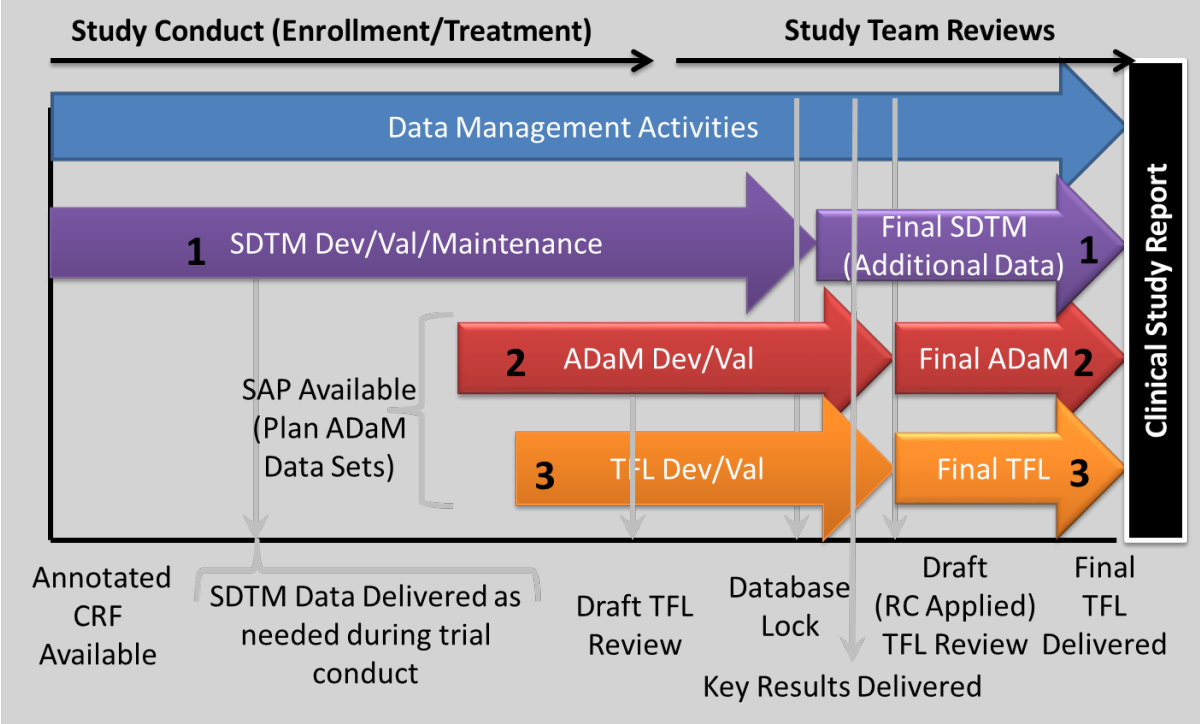
Study data identified in the schedules for time and events (

Table 1 and Table 2) are collected, and source verified, on the electronic data capture (EDC) Fountayn Version 1.0.0.

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



[redacted]

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 and placebo for Stage 1, and Nyxol + LDP, Nyxol + LDP vehicle, Placebo + LDP, and Placebo + LDP vehicle for Stage 2.

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, unless the endpoint is used for the hierarchical testing method. 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

A hierarchical analysis will formally test a family of endpoints beyond the primary efficacy endpoint. The percent of subjects with ≥ 15 letters of improvement in photopic binocular DCNVA and with < 5 letters of loss in photopic binocular BCDVA will be analyzed at 30 min post-LDP/vehicle at Visit 5 (Stage 2 Day 8 [Day 22-29]), comparing Nyxol + LDP to placebo + LDP vehicle, Nyxol + LDP vehicle, and placebo + LDP. These comparisons will be analyzed second, third, and fourth in the testing hierarchy. The full hierarchy is in Section 12.3.

Otherwise, 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, 0 Hour (Time 0 min), prior to any treatment administration. If the Visit 1, 0 Hour assessments are 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 Stage 1 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. A similar summary will be presented by Stage 2 treatment group.

End of trial information will also be summarized in these tables, 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 Stage 1 treatment group, Stage 2 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 type (light, 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 iris 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 Stage 1 treatment group, Stage 2 treatment group, and overall. Non-ocular and ocular medical history will be summarized separately.

Medical history will be coded using the MedDRA Version 25.1 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 Concomitant Medications

The number and percentages of all concomitant medications will be summarized by Stage 1 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 Stage 1 treatment group. A similar summary will be presented by Stage 2 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 Stage 1 treatment group, Stage 2 treatment group, and overall.

8.6. Treatment Exposure and Compliance

Exposure to Treatment 1 for the evening of Stage 1 Day 2 through the evening prior to Visit 2 (Stage 1 Day 8) will be [redacted]

For Stage 2, exposure to Treatment 1 will be assessed [redacted]

[redacted]

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.

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 for the Baseline assessment; for Visit 1 (Stage 1 Day 1) post-treatment time points (0.5 hours, 1 hour, 3 hours, 5 hours, and 8 hours); Visit 2 (Stage 1 Day 8) time points (12 hour post-Treatment 1 dose, 12.5 hours, 13 hour, 15 hours, 17 hours, and 20 hours); Visit 3 (Stage 1 Day 10); Visit 4 (Stage 2 Day 1) time points (12 hour post-Treatment 1 dose, 0.5 hours, 1 hour, 3 hours, 5 hours, and 8 hours post-Treatment 2); and Visit 5 (Stage 2 Day 8) time points (12 hour post-Treatment 1 dose, 0.5 hours, 1 hour, 3 hours, 5 hours, and 8 hours post-Treatment 2).

9.1.1. Primary Efficacy Analysis and Key Secondary Analyses

[redacted]

[redacted] 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 [redacted]. 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 the PPS1 and PPS2 populations, 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 3 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, [redacted] 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 vs. Placebo for Stage 1 time points, and Nyxol + LDP vs. Nyxol + LDP vehicle, Placebo + LDP, and Placebo + LDP vehicle for Stage 2 time points. [redacted]

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 vs. Placebo for Stage 1 time points, and Nyxol + LDP vs. Nyxol + LDP vehicle, Placebo + LDP, and Placebo + LDP vehicle for Stage 2 time points. 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 [redacted].

Finally, select questions from the Subject Questionnaire will be analyzed. The results for Question 1 (change in near vision) and Question 5 (satisfaction with near vision) will be analyzed at Visit 2 (Stage 1 Day 8) and Visit 5 (Stage 2 Day 8) using an ANCOVA model with numeric observed response as the dependent variable ([redacted] and treatment as a factor. Only observed subject questionnaire data will be used; no imputation will be performed for missing data.

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 25.1.

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.

TEAEs that start during Stage 1 will only be counted in the Stage 1 tables using the Treatment 1 that was actually taken, regardless of the TEAE stop date. TEAEs that start during Stage 2 will be counted in both Stage 1 and Stage 2 tables.

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. 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 the 0 hour time point for each visit. 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 vs. Placebo for Stage 1 time points, and Nyxol + LDP vs. Nyxol + LDP vehicle, Placebo + LDP, and Placebo + LDP vehicle for Stage 2 time points. Separate summaries will be created for the study eye and the fellow eye.

10.4. Vital Signs

Descriptive statistics of observed values will be presented for vital sign data at baseline, Visit 2 (Stage 1 Day 8) at the 12 hour post-dose time point, and Visit 5 (Stage 2 Day 8) at the 8 hour time point by treatment group. Changes from baseline to each time point will be presented.

10.5. IOP

Observed values and change from baseline in IOP at baseline, Visit 2 (Stage 1 Day 8) at the 12 hour post-dose time point, and Visit 5 (Stage 2 Day 8) at the 8 hour time point will be summarized for the study eye and the fellow eye by treatment group. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints.

10.6. Subject Questionnaire

Results from the subject questionnaire at baseline, and the 3 hour time point for Visit 2 (Stage 1 Day 8), Visit 4 (Stage 2 Day 1) and Visit 5 (Stage 2 Day 8) will be summarized by treatment group.

10.7. Biomicroscopy and Ophthalmoscopy

Results from biomicroscopic and ophthalmoscopic examinations at baseline, Visit 2 (Stage 1 Day 8) at the 12 hour post-dose time point, and Visit 5 (Stage 2 Day 8) at the 8 hour time point will be summarized by treatment group for the study eye and the fellow eye by treatment group.

10.8. Other Safety Measures

The percent of subjects who lose ≥ 5 letters from Baseline in photopic BCDVA will be summarized within the summary of secondary efficacy endpoint Percentage of subjects with loss or improvement in photopic and mesopic BCDVA from baseline. Treatments will be compared using the same logistic regression model proposed for the secondary efficacy endpoints.

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

The list of planned tables includes all of the *main* tables to be presented for the study. Cells that are highlighted in yellow are what we are considering for the top-line report.

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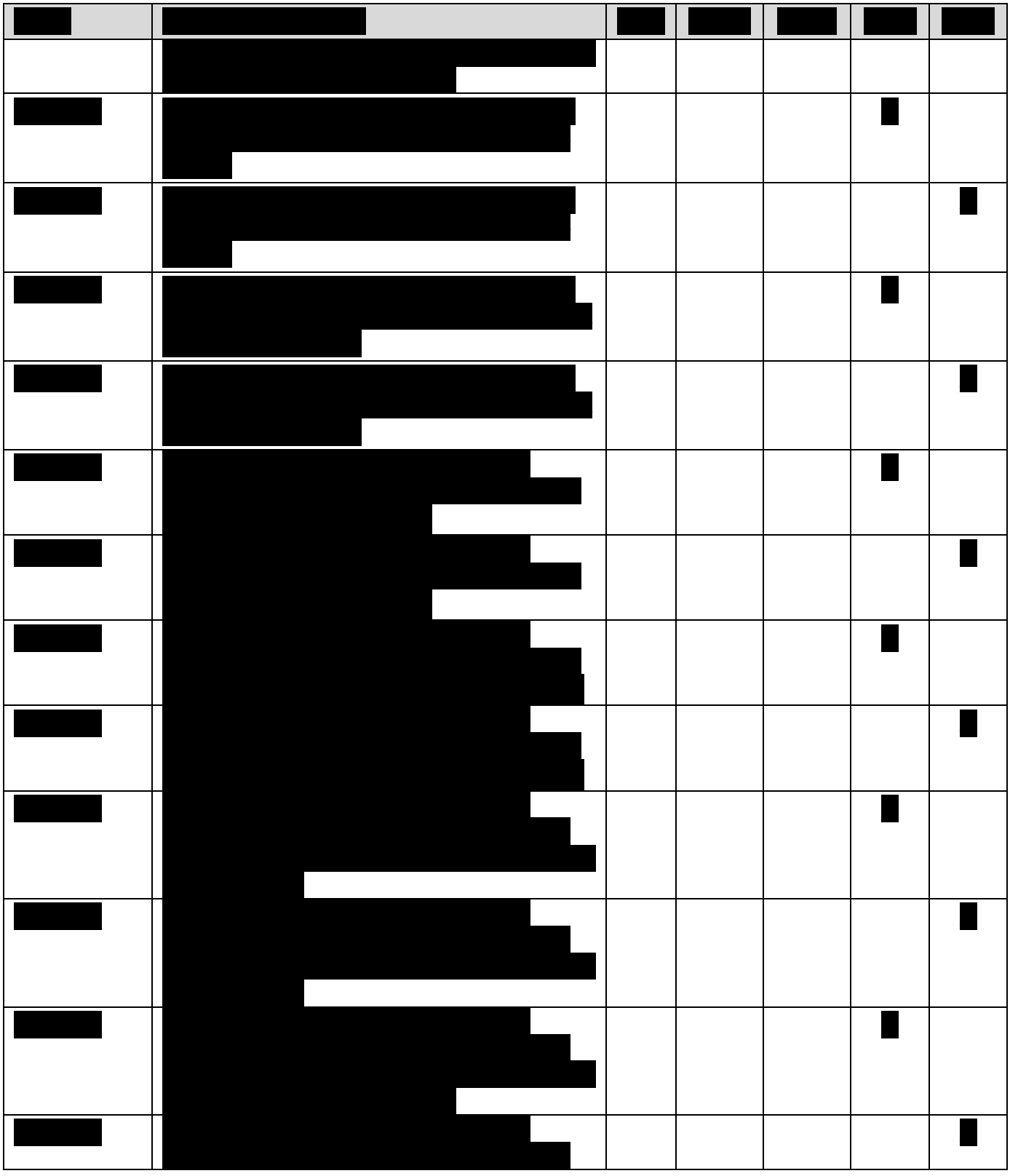

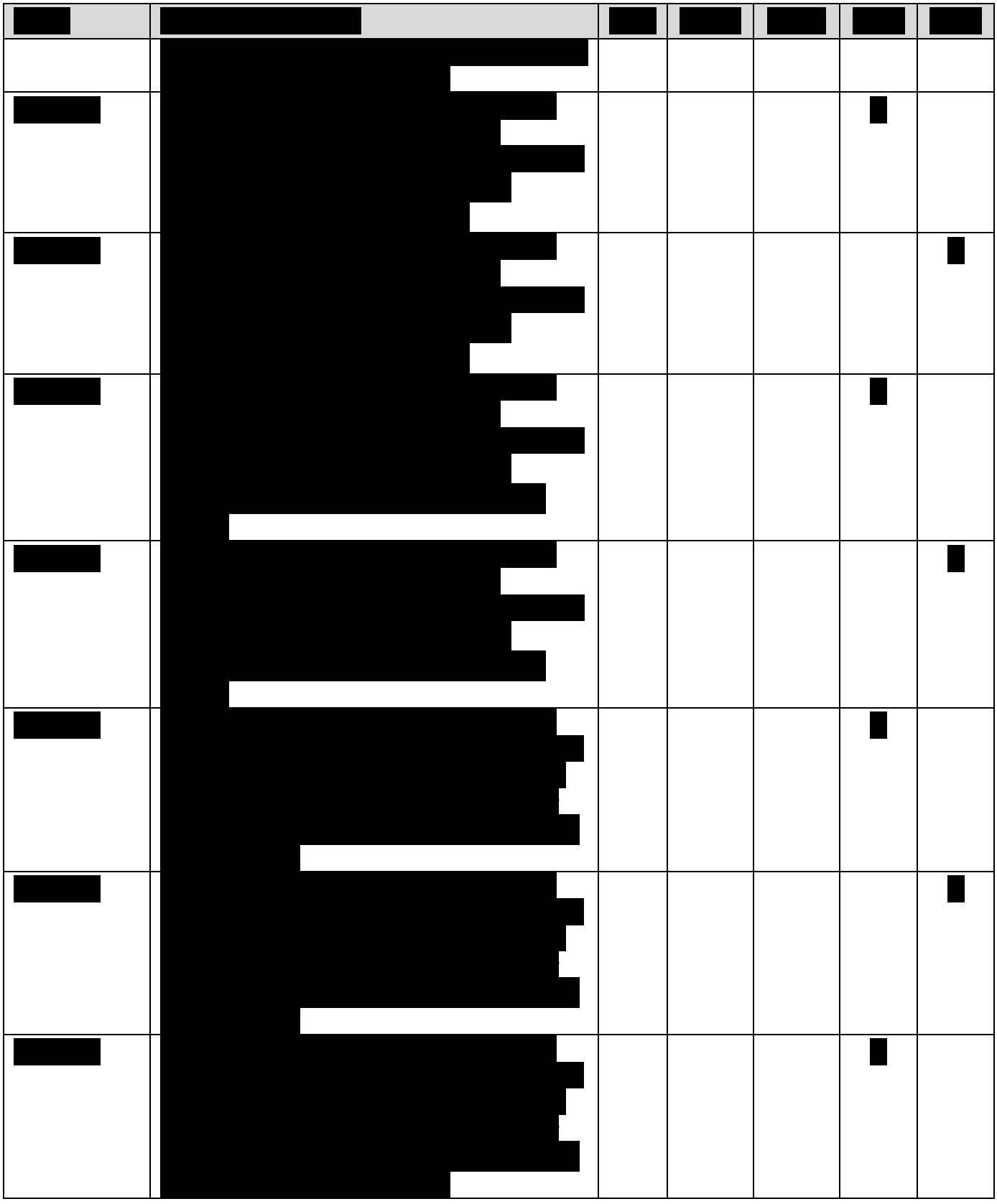

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