SUMMITANALYTICAL	**CONFIDENTIAL**

Ocuphire Pharma, Inc.

STATISTICAL ANALYSIS PLAN

Protocol Title: Randomized, Parallel Arm, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to Reverse Pharmacologically-Induced Mydriasis in Healthy Subjects

Study Number: OPI-NYXRM-301 (MIRA-2)

Phase: Phase 3

Sponsor: Ocuphire Pharma, Inc.
37000 Grand River Avenue, Suite 120
Farmington Hills, MI 48335 USA

Author: Summit Analytical, LLC
8354 Northfield Blvd.
Bldg. G Suite 3700
Denver, CO 80238

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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
CCLRU	Cornea and Contact Lens Research Unit
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CRF	Case Report Form
CSR	Clinical Study Report
DD	Drug Dictionary
DCNVA	distance-corrected near visual acuity
EDC	electronic data capture
HR	heart rate
IOP	Intraocular pressure
ITT	Intention-To-Treat
LOCF	last observation carried forward
logMAR	logarithm of the minimum angle of resolution
LSM	least squares mean
MAR	missing at random
max	maximum
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-To-Treat
Nyxol	Phentolamine Mesylate Ophthalmic Solution 1% (Nyxol®)
OD	right eye
OR	odds ratio
OS	left eye

Abbreviation/Term	Definition
PD	pupil diameter
PP	Per Protocol
PT	preferred term
SAE	serious averse event
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model
SE	standard error
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event
TFL	tables, figures, and listings
VA	visual acuity
WHO	World Health Organization

3. INTRODUCTION

3.1. Preface

This document presents a statistical analysis plan (SAP) for Ocuphire Pharma, Inc. Protocol OPI-NYXRM-301 (MIRA-2) (*Randomized, parallel arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to reverse pharmacologically-induced mydriasis in healthy subjects*).

Reference materials for this statistical plan include the protocol OPI-NYXRM-301(20AUG2020) and as amended version 1 05FEB2021 and Case Report Forms (CRFs; Version 07NOV2020).

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 MIRA-2 study is a randomized, parallel arm, double-masked, placebo-controlled study of the safety and efficacy of Nyxol (0.75% Phentolamine Ophthalmic Solution) to reverse pharmacologically-induced mydriasis in healthy subjects.

The Sponsor intends to use this first Phase 3 registration study to evaluate Nyxol for the indication “the treatment of pharmacologically-induced mydriasis produced by adrenergic (phenylephrine) or parasympatholytic (tropicamide) agents, or a combination thereof.”

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 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:

- To evaluate the efficacy of Nyxol to expedite the reversal of pharmacologically-induced mydriasis across multiple mydriatic agents with an emphasis on phenylephrine
- To evaluate the efficacy of Nyxol to return subjects to baseline accommodation after worsening (with cycloplegic agents tropicamide and Paremyd)
- To evaluate the safety of Nyxol
- To evaluate any additional benefits of the reversal of pharmacologically-induced mydriasis

4.2. Study Endpoints

4.2.1. Primary Endpoints

The primary efficacy endpoint is the percentage of subjects' study eyes returning to ≤ 0.2 mm from baseline pupil diameter at 90 minutes.

The primary safety measures are:

- Conjunctival hyperemia
- Subjective ocular tolerability
- Adverse events (AEs)

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 and non-study eye unless otherwise indicated, and will include:

- Percentage of subjects returning to ≤ 0.2 mm from baseline **pupil diameter** at each remaining timepoint (30 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours)

- Change (in mm) from max **pupil diameter** (0 minutes) at each timepoint (30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours)
- Percentage of subjects with unchanged **accommodation** from baseline (-1 hour) at the following timepoints (0 min, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours)
- Change (in diopters) from baseline accommodation (-1 hour) at each time point (0 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours)
- Time (hours) to return to ≤ 0.2 mm from baseline pupil diameter (**time savings analysis**)

Safety and Tolerability:

- Best-corrected distance visual acuity (BCDVA)
- Distance corrected near visual acuity (DCNVA)
- Vital signs (heart rate [HR] and blood pressure [BP])
- Intraocular pressure (IOP)
- Subject questionnaire of symptoms
- Urine pregnancy tests for females of childbearing potential

5. STUDY METHODS

5.1. General Study Design and Plan

A randomized, parallel arm, double-masked, placebo-controlled Phase 3 study in at least 168 randomized subjects (160 evaluable for efficacy, where evaluable is defined as receiving one or two drops of study medication and have a pupil diameter measurement at the 90 minute time point at Visit 1), evaluating the safety and efficacy of Nyxol in subjects with pharmacologically-induced mydriasis.

Following the successful completion of screening, each subject will be stratified by eye color and simultaneously be randomized to mydriatic agent (unmasked) and treatment (masked).

At Visit 1 subjects will be screened for study eligibility. After screening, eligible subjects will be randomized 1:1 to one of the two treatment arms (Nyxol or Placebo).

At the same visit, subjects who have been randomized and stratified by irides type (1:1 [light/dark]) will receive one of three approved mydriatic agents approximately 1 hour prior to receiving study treatment. Randomization will be stratified 3:1:1 by mydriatic agent (2.5% phenylephrine, 1% tropicamide, and Paremyd), with randomization into each mydriatic agent capped at approximately 96:32:32 evaluable subjects.

Each subject will receive one drop of mydriatic agent in each eye. If the drop is missed, the Investigator should give the drop again.

At Visit 1, measurements will be completed before (-1 hour /baseline) and 60 minutes after (maximum/0 minutes) the mydriatic agent instillation in each eye (i.e., right before the study treatment is administered), and at 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, and 6 hours after treatment dosing.

At the Follow-Up Visit, which is one day after Visit 1, measurements will be measured 24 hours after treatment dosing.

The study eye is defined as the right eye (OD). The non-study eye is defined as the left eye (OS). The study and non-study eye will both be evaluated at all assessments. Adult subjects (≥ 18 years old) will have two drops (dosed 5 minutes apart) of treatment administered in the study eye (OD) and one drop of treatment administered in the non-study eye (OS) one hour after mydriatic drug instillation. Pediatric subjects (<18 years old) will have one drop of treatment administered in each eye one hour after mydriatic drug instillation.

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

Table 1 Screening and Mydriatic/Treatment Schedule

	[redacted]
Day (D)	
Visit	
Hour	
Informed Consent/ Assent	
Screening # assigned	
Med/Ophth History	
Demographics	
Prior/Concomitant Medications*	
Urine Pregnancy Test**	
HR/BP	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
[redacted]	
Adverse Events	
Ocular Tolerability^	
Mydriatic Agent	
IOP^	
Biomicroscopy	
Ophthalmoscopy^	
Questionnaire^	
Randomization # Assigned	
Treatment: Nyxol/ Placebo	

Screening Visit if subject qualifies becomes the first Treatment Visit. The mydriatic drug(s) will be given at -1 hour.
*Investigators to note changes to concomitant medications at any time throughout the visit.
**UPT is for females of childbearing potential.
^Measurements will be performed as follows:

[redacted]

IOP – Tono-Pen
Ophthalmoscopy – Direct or indirect ophthalmoscopy without dilation. Use of 90D lens (indirect) is allowed
Questionnaire – Subject Questionnaire will be a brief symptom survey.

5.2. Inclusion – Exclusion Criteria and General Study Population

The study population will be approximately 168 normal healthy subjects at least 12 years of age, with approximately 160 evaluable subjects. Written informed consent will be obtained from each adult subject. A signed assent form will be obtained for all minors ages 12-17, as well as a separate parental/Legal Guardian consent.

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

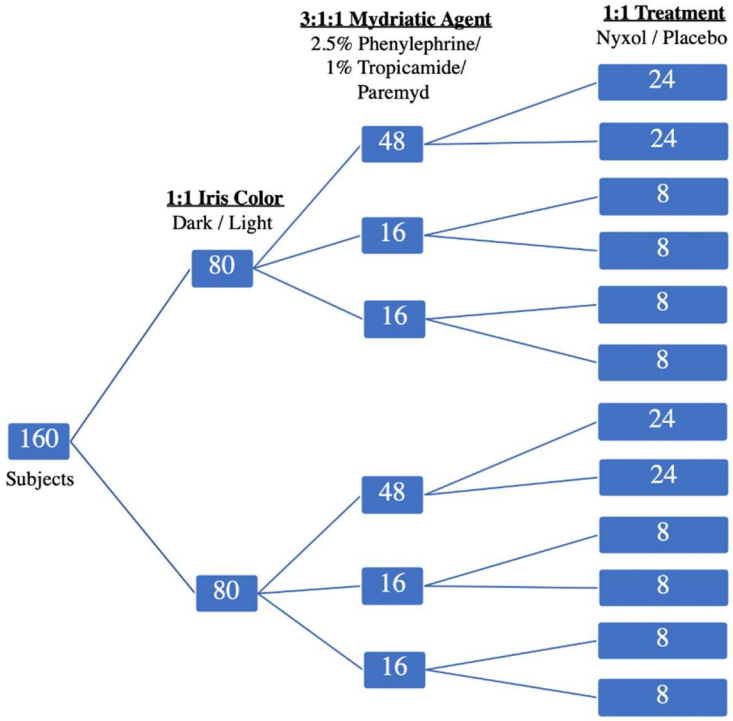
5.3. Randomization and Blinding

A randomization code for allocating subjects to treatment will be prepared by a masked biostatistician not connected with the study. At the initiation of study related procedures, every potential subject is assigned a Screening number in numerical order per strata. Once a subject is qualified for the study, the subject is assigned a randomization number in the order provided by the biostatistician.

[redacted]. The mydriatic agent randomization will be approximately 3:1:1 (2.5% phenylephrine, 1% tropicamide, and Paremyd). That is, approximately 60% of the randomized subjects will receive one drop of 2.5% phenylephrine 1 hour before treatment (96 evaluable subjects), approximately 20% will receive one drop of 1% tropicamide 1 hour before treatment (32 evaluable subjects), and approximately 20% will receive Paremyd 1 hour before treatment (32 evaluable subjects).

A randomization schema for evaluable subjects by investigational treatment, mydriatic agent, and irides type is are described in Figure 1.

Figure 1 **Randomization schema for evaluable subjects by investigational treatment, mydriatic agent, and irides type**



Treatment	Mydriatic Agent	Iris Color
Nyxol	2.5% Phenylephrine	Dark
Placebo	2.5% Phenylephrine	Dark
Nyxol	1% Tropicamide	Dark
Placebo	1% Tropicamide	Dark
Nyxol	Paremyd®	Dark
Placebo	Paremyd®	Dark
Nyxol	2.5% Phenylephrine	Light
Placebo	2.5% Phenylephrine	Light
Nyxol	1% Tropicamide	Light
Placebo	1% Tropicamide	Light
Nyxol	Paremyd®	Light
Placebo	Paremyd®	Light

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

5.4. Analysis Variables

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

Efficacy variables include:

- [redacted]
- [redacted]

Safety variables include:

- Conjunctival hyperemia (eye redness) measured with a Cornea and Contact Lens Research Unit (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
- DCNVA (i.e., Near VA)
- BCDVA (i.e., Distance VA)
- AEs
- Vital signs (HR and BP)
- IOP
- Subject questionnaire
- Urine pregnancy tests for females of childbearing potential

6. SAMPLE SIZE

A sample size of 160 evaluable subjects (80 per treatment group) is needed for the study.

The primary efficacy endpoint will be met if subjects show a positive effect for Nyxol (α = 0.05 significance, two-tailed). [redacted] The assumptions for this power calculation are estimated from the MIRA-1 (OPI-NYXRM-201) Phase 2 results. Data from this study on the effects of treatment to reverse pharmacologically induced mydriasis were adjusted to take into account the increased dose of Nyxol used in this MIRA-2 Phase 3 study (2 drops vs 1 drop) and adjusted for the 3:1:1 mydriatic agent groups, among other factors.

[redacted] Furthermore, subjects will be randomized into the study at a ratio of approximately 3:1:1 to one of the three mydriatic agents, 2.5% phenylephrine, 1% tropicamide, and Paremyd. Therefore, if 96 evaluable subjects are randomized to 2.5% phenylephrine, then 32 subjects will be assigned to 1% tropicamide and 32 subjects to Paremyd, resulting in 160 evaluable subjects.

It is assumed that there will be a 5% drop-out before the 90-minute efficacy assessment; therefore 168 subjects will be randomized to obtain 160 evaluable subjects for the primary analysis.

7. GENERAL CONSIDERATIONS

7.1. Analysis Populations

The following analysis populations will be defined for this study.

7.1.1. Modified Intention-to-Treat (mITT)

The mITT will include all randomized subjects who received one or two drops of study treatment and then had at least one scheduled pupil diameter measurement during Visit 1. The mITT will be used for the primary endpoint analysis and to analyze selected secondary 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 will include all subjects in the mITT who have two drops of study treatment in their Study Eye, have all scheduled pupil diameter measurements during Visit 1, had an increase of >0.2 mm in pupil diameter in the study eye at time 0 minutes compared to baseline (-1 hour), and have no major protocol deviations. The PP population will be used to analyze selected secondary 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. 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), and by mydriatic agent (phenylephrine, tropicamide, Paremyd) will be completed for efficacy endpoints. An additional mydriatic

agent subgroup, combining 1% tropicamide and the Paremyd subjects into a "tropicamide" group, will be used for efficacy endpoints.

Other possible subgroups include age, sex, and race. If there is sufficient sample, analysis of safety and efficacy endpoints will be completed for the subgroup of pediatric subjects.

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' study eyes returning to ≤ 0.2 mm baseline pupil diameter at 90 minutes in the study eye. 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. Confirmatory analyses will 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. If 5% or fewer data are missing at the 90-minute time point in all treatment groups, an analysis with last observation carried forward (LOCF) for missing data will be applied within a treatment group. Neither the baseline (-1 hour) nor max pupil diameter (0 minutes) time points will be used for imputation. If more than 5% of data are missing at the 90-minute time point in any treatment group, 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 pupil diameter 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: pupil diameter at Day 1 (30 minutes, 60

minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 24 hours). No imputation will be applied to the max pupil diameter (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 mixed models. See Section 9.1 for details on these models. The estimates and standard errors (SEs) of the differences in LSM (least squares means) based on the 500 imputed datasets are then combined by applying Rubin's rules for multiple imputed datasets. T-tests are also provided. SAS Proc MI, Proc MIXED, and Proc MIANALYZE will be utilized for these analyses. The averaged difference in LSM with the corresponding 95% CI will also be presented.

Example SAS code is provided below:

```
    select ParameterEstimates;
run;
```

7.3.3. Handling of Early Termination Visit Information

In the event that a subject is terminated early from this study on Day 1, the early termination data for safety variables will be assigned to the closest scheduled time point on Day 1. 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 22.0) system for reporting (preferred term and body system).

Prior and concomitant medications will be coded using WHO-DD (World Health Organization Drug Dictionary) (Version March 2019, 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 14.5.5.

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.

Figure 2 SDTM, ADaM, and TFL Development and Validation

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 or Placebo).

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. 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 prior to any drug administration on Day 1 (study drug or mydriatic agent), usually the -1 hour time point. If the Day 1 value is missing or the assessment is not completed at the -1 hour time point, the value at Screening 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.

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.

The demographics consist of age (year), sex, race, ethnicity, and study eye (OD), 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), irides type (light or dark), eyeglasses-wearing status (yes or no) (distance vision or near vision), and mydriatic agent (phenylephrine, tropicamide, or Paremyd). 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, irides type, distance vision/near vision correction status, and mydriatic agent 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 and non-study eye, using descriptive statistics:

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

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 22.0. 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 the study drug and the mydriatic agent on Day 1 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. If the analysis using the mITT Population shows a positive effect for Nyxol at the 0.05 level of significance, the primary endpoint will be considered met.

Confirmatory analysis of the primary efficacy endpoint will be performed using the ARP, 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. If there is sufficient sample, analysis of efficacy endpoints will be completed for the subgroup of pediatric subjects.

9.1. Clinical Efficacy

For all efficacy endpoints, Baseline is defined as -1 hour prior to treatment on Day 1. This is the time when the mydriatic agent is administered, and the pupil diameter measurement is considered normal. Max timepoint is defined as time 0 minutes, during which maximum pupil diameter is expected; this is also the timepoint at which the treatment is administered (Nyxol or Placebo).

All efficacy data will be summarized by treatment group and timepoint (-1 hour [baseline], 0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours), as appropriate.

Primary Efficacy Endpoint:

The primary efficacy endpoint is the percentage of subjects returning to ≤ 0.2 mm baseline pupil diameter at 90 minutes post-treatment in the study eye. [redacted] The primary efficacy endpoint will be analyzed using a logistic regression model with [redacted]. 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:

[redacted]

By including the mydriatic agent and irides type as factors in the primary efficacy analysis model, the model efficiency as well as a change in the treatment effect will be increased. Including these factors 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]. Additionally, a sensitivity analysis will be applied to the primary efficacy endpoint for the mITT and PP populations, which uses a [redacted].

In addition, the primary efficacy endpoint will be analyzed by mydriatic agent and by light/dark irides using the same model indicated above but without mydriatic agent or irides as a factor, as appropriate. For these subgroup analyses, observed case data only will be used; that is, missing values will not be imputed. Each mydriatic agent will be analyzed individually, and an additional mydriatic agent subgroup, combining 1% tropicamide and the Paremyd subjects into a "tropicamide" group, will be analyzed.

In addition, the primary efficacy endpoint will be analyzed for the pediatric population, as well as by mydriatic agent.

A comparison of the study and non-study eye for each subject will be completed for the primary efficacy endpoint, as well as by mydriatic agent. This analysis will be by treatment, and will be analyzed using a logistic regression model with eye type (study eye or non-study eye), mydriatic agent, and light/dark irides as fixed effects, subject as a random effect, and the average baseline pupil diameter across eye type as a covariate. The percentage of eyes meeting the criteria, the OR with 95% CI, and p-value will be provided. Example SAS code is as follows:

Secondary Efficacy Endpoints:

Secondary efficacy endpoints are indicated in Section 4.2.2. Secondary efficacy endpoints will be analyzed by study eye and non-study eye, unless otherwise indicated. Binocular accommodation will be analyzed separately.

Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (ANCOVA), with change from baseline as the dependent variable, treatment, mydriatic agent, and light/dark irides as factors, and the respective baseline value included as the covariate. Note that most secondary efficacy endpoints are in relation to baseline (-1 hour), whereas some pupil diameter endpoints are in relation to max (0 minute).

Each ANCOVA will be performed using the mITT and PP populations. The output from each ANCOVA will include the LSM and SE for both treatment groups, along with the placebo-corrected LSM, its 95% CI and associated p-value. Line graphs displaying the mean and SE of the change from maximum (0 minute) pupil diameter value will be presented.

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 [redacted] For each analysis, the percentage of subjects in each treatment group meeting the criteria, the OR with 95% CI and p-value will be provided. The analysis of the time (hours) to return to ≤ 0.2 mm from baseline pupil diameter (time savings analysis) endpoint will be performed using a Cox proportional hazards regression model with [redacted]. Subjects who do not return to ≤ 0.2 mm from baseline pupil diameter by the 6 hour time point will have their time to return censored at 8 hours. Example SAS code is as follows:

[redacted]

The output from the model will include the hazard ratio comparing treatment groups, its 95% CI and associated p-value. Survival plots will also be generated. Time to return to baseline (-1 hour) pupil diameter will be measured beginning at the Max (0 hour) time point.

In addition, each secondary efficacy endpoint will be analyzed by [redacted], as appropriate. Each mydriatic agent will be analyzed individually, and an additional mydriatic agent subgroup, combining 1% tropicamide and the Paremyd subjects into a "tropicamide" group, will be analyzed. Analyses of endpoints related to accommodation will only be completed by mydriatic agent. [redacted]

Exploratory analyses may be performed to compare efficacy endpoints between the study eye and non-study eye within the same subject. Categorical variables will be analyzed as described above for the primary efficacy endpoint. Continuous variables will be analyzed using a mixed model with eye type (study eye or non-study eye), mydriatic agent, and light/dark irides as fixed effects, subject as a random effect, and the average baseline pupil diameter across eye type as a covariate.

10. SAFETY ANALYSES

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

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

10.1. Adverse Events

AEs will be coded using MedDRA, Version 22.0.

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. A separate listing of AEs for pediatric subjects will be provided.

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. A separate summary by SOC, PT, and treatment group will be completed by mydriatic agent.

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

The following summary tables will be presented for TEAE data:

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

- Summary table of serious TEAEs by SOC and PT
- Summary table of TEAEs leading to withdrawal from the study by SOC and PT
- Summary table of TEAEs leading to study medication discontinuation by SOC and PT

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

10.2.1. Deaths

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

10.2.2. Serious Adverse Events

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

10.2.3. Adverse Events Leading to Withdrawal from the Study

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

10.2.4. Adverse Events Leading to Discontinuation of Study Medication

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

10.3. Conjunctival Hyperemia

Results from the conjunctival hyperemia assessment, measured with a CCLRU card 4-point scale, [redacted] e (-1 hour). Separate summaries will be created for the study eye and the non-study eye.

Additionally, [redacted]

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 the 0 minute time point. 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. Separate summaries will be created for the study eye and the non-study eye.

10.5. Visual Acuity

Visual acuity assessments (BCDVA and DCNVA) will be summarized at the following timepoints (0 minutes, 90 minutes, 6 hours, and 24 hours), using letters and logMAR units. Only letters will be recorded in the CRF and will be converted to logMAR programmatically as follows: [redacted] Separate summaries will be created for the study eye, the non-study eye, and both eyes. Treatments will be compared using the same ANCOVA model proposed for the continuous secondary efficacy endpoints. In addition, DCNVA will be analyzed by mydriatic agent using the same model as for the efficacy variables.

10.6. Vital Signs

Descriptive statistics of observed values will be presented for vital sign data at each time point (Screening, 6 hours, and 24 hours), including systolic BP (mmHg), diastolic BP (mmHg), and HR (bpm) by treatment group and overall. Changes from baseline to each scheduled post-baseline time point will be presented.

10.7. IOP

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

10.8. Subject Questionnaire

Subject questionnaire values will be summarized for each timepoint (-1 hour, 0 minutes, 60 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours) by treatment group. A separate summary will be created for each mydriatic agent.

10.9. Other Safety Measures

Urine pregnancy tests for females of childbearing potential will be presented in by-subject listings. Results from biomicroscopic and ophthalmoscopic examinations, which are completed only at Screening, will also be presented in by-subject listings.

11. REFERENCES

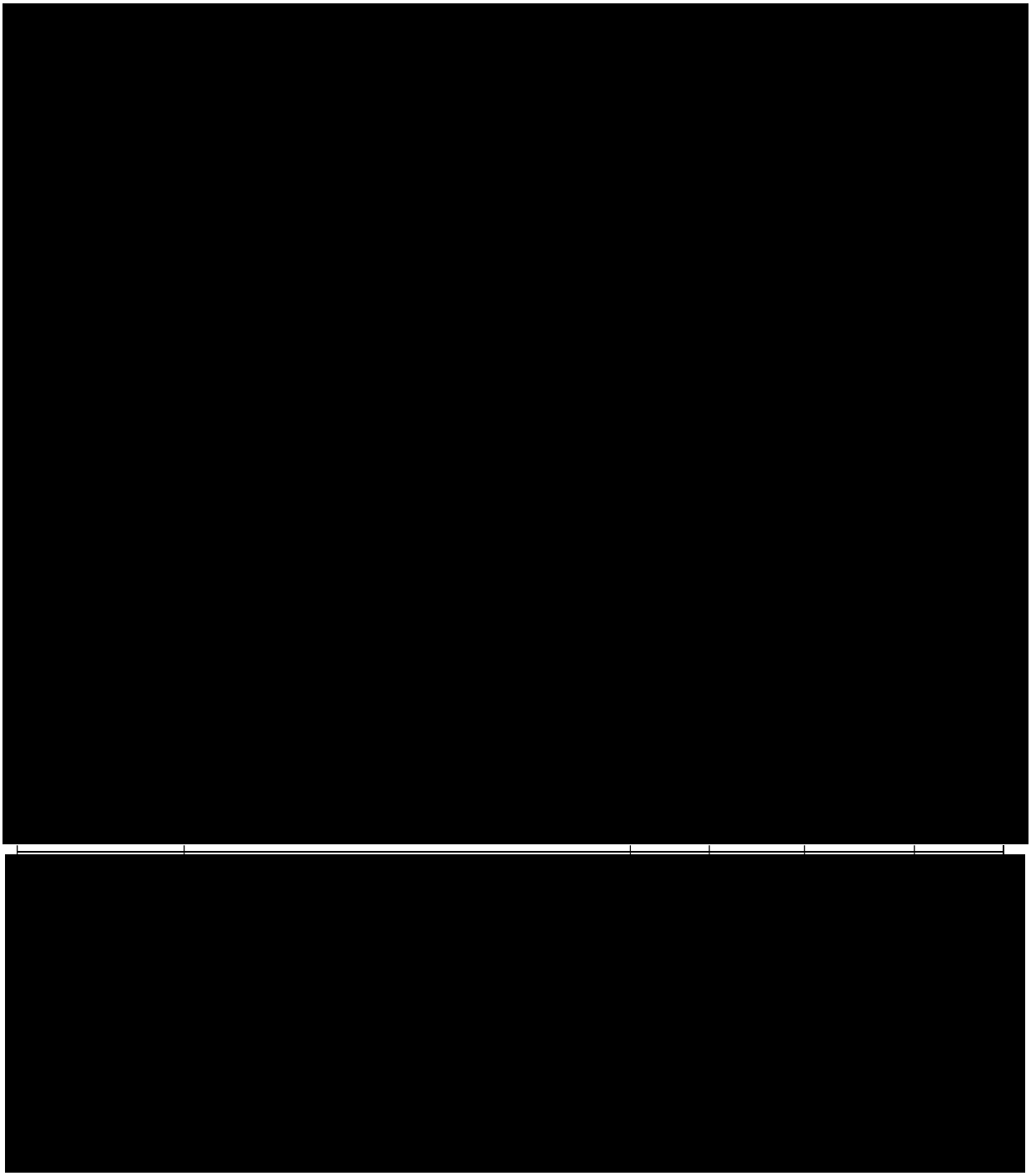
[1] ICH E9 Expert Working Group. Statistical Principles for Clinical Trials: ICH Harmonized Tripartite Guideline, September 1998

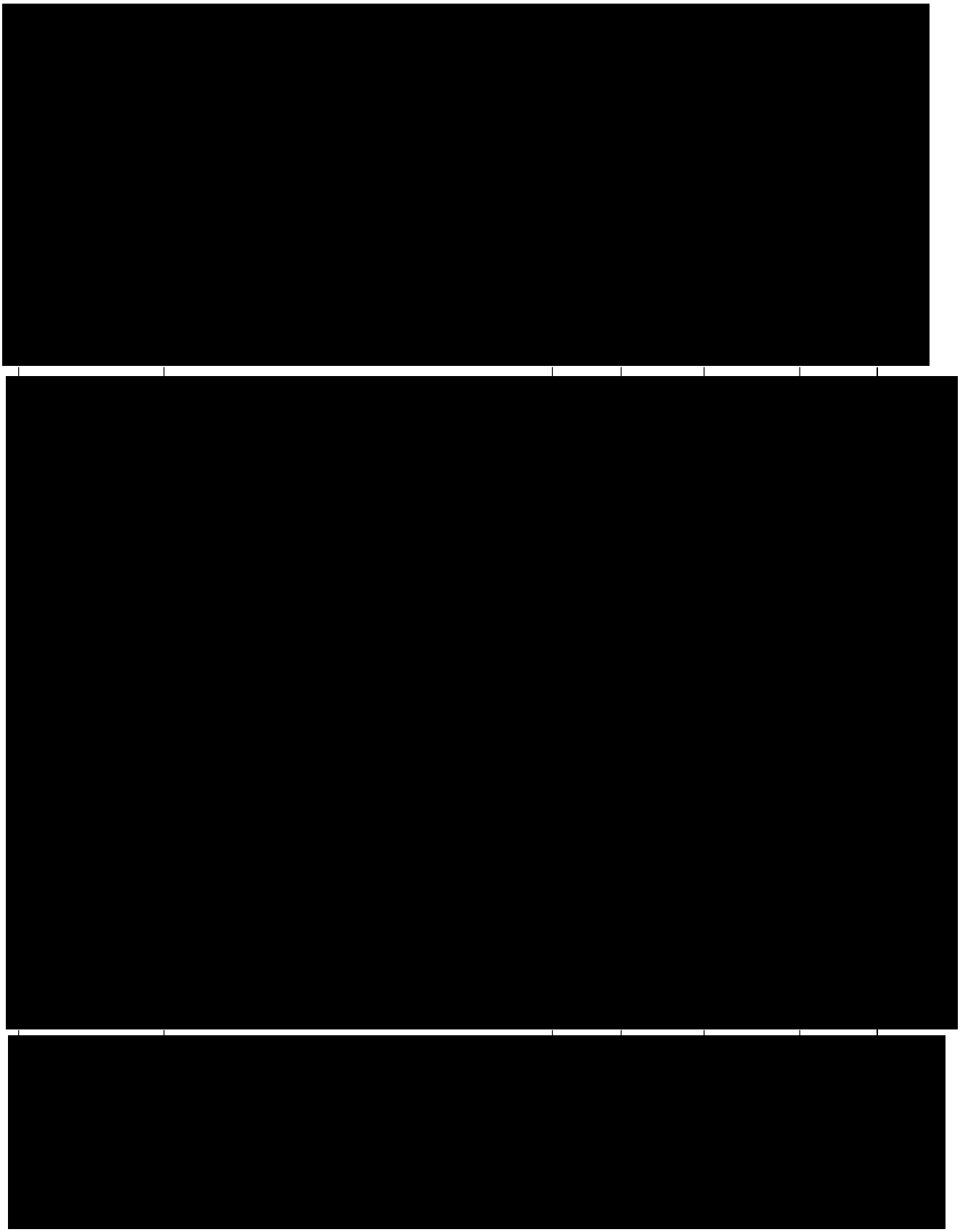
[2] Hauck WM, Anderson S, and Marcus SM, Should We Adjust for Covariates in Nonlinear Regression Analyses of Randomized Trials? *Controlled Clin Trials* 1998;19:249–256

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.





12.2. List of Planned Listings

Listing	Description of Listing
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

12.3. List of Planned Figures

[redacted]