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-302 (MIRA-3)
Phase:	Phase 3
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SAP Date:	2022-03-14
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SUMMITANALYTICAL	**AD ST-07** **Statistical Analysis Plan Approval Form**

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SAP Version: Final V1.0
SAP Date: 14MAR2022

The statistical analysis plan has been reviewed and approved.

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
BAT	brightness acuity tester
BCDVA	best-corrected distance visual acuity
BP	blood pressure
CCLRU	Cornea and Contact Lens Research Unit
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CRF	case report form
CSR	clinical study report
DD	Drug Dictionary
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

Abbreviation/Term	Definition
OS	left eye
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-302 (MIRA-3) (*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-302 (03SEP2021) and Case Report Forms (CRFs; Version 27OCT2021).

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-3 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 Phase 3 registration study to evaluate Nyxol for the [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 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

A hierarchical analysis was added to formally test a family of endpoints beyond the primary efficacy endpoint. Pharmacokinetic parameter estimation methods were also added. 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:

- 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 systemic exposure of Nyxol based on pharmacokinetic (PK) sampling
- 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 (-1 hour) photopic pupil diameter at 90 minutes.

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 fellow eye unless otherwise indicated, and will include:

- [redacted]
- [redacted]

- [redacted]

Safety and Tolerability:

- Conjunctival hyperemia
- Impairment in visual acuity (best-corrected distance visual acuity [BCDVA] and distance-corrected near visual acuity [DCNVA])
- Subjective ocular tolerability
- Adverse events (AEs)
- Vital signs (heart rate [HR] and blood pressure [BP])
- Intraocular pressure (IOP)
- Subject questionnaire of symptoms

5. STUDY METHODS

5.1. General Study Design and Plan

This is a randomized, parallel arm, double-masked, placebo-controlled Phase 3 study in approximately 330 randomized subjects evaluating the safety and efficacy of Nyxol in subjects with pharmacologically induced mydriasis. Following the successful completion of screening, each subject will 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 2:1 to one of the two treatment arms (Nyxol or Placebo). 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).

Subjects will have 2 drops of study treatment (Nyxol or placebo) administered in the study eye (right eye [OD]). Each drop will be instilled 5 minutes apart. Subjects will have only 1 drop of study treatment administered in the fellow eye (left eye [OS]).

The study eye and fellow eye will both be evaluated at all assessments unless otherwise specified.

Blood sampling for Nyxol PK measurements will be conducted in a subset of approximately 30 adult subjects at approximately 2 select study sites.

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

At Visit 2 (Follow-Up Visit), which is 1 day after Visit 1, measurements will be made 24 hours after study treatment dosing.

The study eye is defined as the right eye (OD). The fellow eye is defined as the left eye (OS). The study and fellow eye will both be evaluated at all assessments. All subjects will have two drops (dosed 5 minutes apart) of treatment administered in the study eye (OD) and one drop of treatment administered in the fellow eye (OS) 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]	[redacted]
Day		
Visit		
Start time [b]		
Informed consent/assent		
Subject identification number assigned		
Medical/Ophthalmic history		
Demographics		
Prior/Concomitant medications [c]		
Urine pregnancy test [d]		
[redacted]		
AEs [c]		

AE, adverse event; BAT, Brightness Acuity Tester; BCDVA, best-corrected distance visual acuity; BP, blood pressure; DCNVA, distance-corrected near visual acuity; HR, heart rate; IOP, intraocular pressure; PK, pharmacokinetic.

5.2. Inclusion – Exclusion Criteria and General Study Population

The study population will be approximately 330 normal healthy subjects at least 12 years of age. 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.

Treatment randomization will be 2:1, Nyxol or Placebo (vehicle). Stratification by irides type will be 1:1, light or dark. 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 (198 subjects), approximately 20% will receive one drop of 1% tropicamide 1 hour before treatment (66 subjects), and approximately 20% will receive Paremyd 1 hour before treatment (66 subjects).

The study treatment (Nyxol or Placebo) 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:

- Pupil diameter
- Accommodation
- Pupillary light reflex

- BCDVA under glare conditions (BAT)
- Glare discomfort measured on a 4-point scale:
    - 0 – No discomfort
    - 1 – Mild discomfort
    - 2 – Moderate discomfort
    - 3 – Severe discomfort

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

PK variables include:

- Nyxol plasma concentration

6. SAMPLE SIZE

A sample size of approximately 330 subjects (approximately 220 treated with Nyxol) in this study will result in a total of > 300 subjects treated with Nyxol in the reversal of mydriasis program (including prior studies); this number of subjects is needed to meet the minimum number of subjects exposed to Nyxol to assess safety in this population. [redacted]

All subjects will be randomized into the study in a 2:1 ratio to 1 of the 2 treatment arms (Nyxol or placebo, respectively), with a 1:1 stratification by light/dark irides (equal number of light and dark irides stratified across treatment groups). Furthermore, subjects will be randomized into the study at a ratio of 3:1:1 to mydriatic agent (2.5% phenylephrine, 1% tropicamide, or Paremyd, respectively). Therefore, if 198 subjects are randomized to 2.5% phenylephrine, then 66 subjects will be assigned to 1% tropicamide and 66 subjects to Paremyd, resulting in 330 total subjects.

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 2 drops of study treatment in the study eye and had at least 1 scheduled post-treatment PD measurement during Visit 1. The mITT Population 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 includes all subjects in the mITT Population who had 2 drops of study treatment in the study eye, had all scheduled PD measurements during Visit 1, had an increase of > 0.2 mm in PD in the study eye at 0 minutes compared to baseline (-1 hour), and had no major protocol deviations considered to have significant impact on treatment outcome. 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 Intent-to-Treat (ITT) Population. The ARP may be used in confirmatory efficacy analyses, with subjects included in their randomized treatment regardless of the treatment they actually received.

7.1.4. Safety Population (SP)

The SP will include all randomized subjects who received at least 1 drop of study treatment. The SP will be used to summarize safety variables, using the treatment they actually received.

7.1.5. PK Population

The PK Population will include all Nyxol subjects who had at least one PK sample taken at any post-treatment timepoint. The PK population will be used to summarize PK variables, using the treatment they actually 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 [redacted]

Other possible subgroups include age, sex, and race. If there is sufficient sample, analysis of safety and efficacy endpoints may 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

Partial or Missing Dates

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

A. Start Dates
   1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.
   2) If the month is unknown, then:
      i) If the year matches the first dose date year, then impute the month and day of the first dose date.
      ii) Otherwise, assign 'January.'
   3) If the day is unknown, then:
      i) If the month and year match the first dose date month and year, then impute the day of the first dose date.
      ii) Otherwise, assign the first day of the month.

B. Stop Dates
   1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.
   2) If the month is unknown, then assign 'December.'
   3) If the day is unknown, then assign the last day of the month.

7.3.2.2. Missing Baseline Data

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

7.3.2.3. Imputation Methods

[redacted]

[redacted]

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 [redacted]. The observed outcome [redacted] for each imputed dataset will be [redacted]. See Section 9.1 for details on these models. The estimates and standard errors (SEs) of the odds ratios based on the [redacted]

Example SAS code is provided below:

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 24.1) system for reporting (preferred term and body system).

Prior and concomitant medications will be coded using WHO-DD (World Health Organization Drug Dictionary) (Global Version 2020-09-01).

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. Analysis of plasma samples for Nyxol concentration determinations will be performed by a central PK laboratory (MicroConstants now BioAgilytix).

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

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 at Screening or the -1 hour time point.

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, subjects in the PP Population, and subjects in the PK 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, PK population, 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 (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 fellow eye, using descriptive statistics:

- Pupil diameter (-1 Hour)
- Max pupil diameter (0 Minutes)
- Accommodation (-1 Hour)
- Accommodation (0 Minutes)
- BCDVA (-1 Hour)
- BCDVA (0 Minutes)
- DCNVA (-1 Hour)
- DCNVA (0 Minutes)
- IOP (Screening)

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 24.1. 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

[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. 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.

To formally test the significance of endpoints of interest beyond primary efficacy, endpoints will be tested in a predefined sequence using observed data only, each at the significance level 0.05, until the first nonsignificant test. The primary efficacy endpoint will be first in this sequence. The endpoints in the sequence are for various analysis populations, eye types (study eye, fellow eye, or binocular), and time points. Some endpoints in the sequence are restricted to subgroups of mydriatic agent or irides type. The sequence is specified in Section 13.3.

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:

[redacted]

[redacted]

By including [redacted] primary efficacy analysis model, the ability to detect a 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 logistic regression model with only treatment as a [redacted]

In addition, the primary efficacy endpoint [redacted] using the same model indicated above [redacted]. 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 fellow 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 fellow 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:

```
proc glimmix data=efficacydata;
        class subjid eyetype magent irides;
        model outcome = eyetype magent irides baseavg/dist=bin;
        lsmeans eyetype /adjust=tukey;
        random subjid / solution;
run;
```

Secondary Efficacy Endpoints:

Secondary efficacy endpoints are indicated in Section 4.2.2. Secondary efficacy endpoints will be analyzed by study eye and fellow 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, [redacted], 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 minutes).

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 minutes) 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 treatment, [redacted], and the respective baseline as a covariate. 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 for the PP Population using a Cox proportional hazards regression model with treatment, mydriatic agent, light/dark irides, and the baseline pupil diameter as a covariate. 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:

```
proc phreg data=efficacydata plots(overlay)=survival;
        class trtname agent irides;
        model timetoreturn*cnsr(1) = trtname agent irides base;
        hazardratio trtname;
        baseline covariates=efficacydata out=Pred1 survival=_all_/diradj group=trtname;
        ods output  hazardratios=hr;
run;
```

The output from the model will include the hazard ratio comparing treatment groups, its 95% CI and associated p-value. Survival plots, generated from the Cox model described above, 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] using the same model indicated above but without mydriatic agent or irides as a factor, 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. Accommodation will be presented using diopters, which will be converted from cm using diopters = 100/cm.

Exploratory analyses may be performed to compare efficacy endpoints between the study eye and fellow 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 fellow 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 24.1.

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. 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 of TEAEs by SOC and PT
- Summary 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 of TEAEs by SOC, PT, and maximum severity (mild, moderate, severe)
- Summary of serious TEAEs by SOC and PT
- Summary of TEAEs leading to withdrawal from the study by SOC and PT

- Summary 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 (-1 hour, 0 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 24 hours) for the observed value and change from baseline (-1 hour). Separate summaries will be created for the study eye and the fellow eye. The categorical summary will be repeated for pediatric subjects.

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 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 fellow eye.

10.5. Visual Acuity

Visual acuity assessments will be summarized at the timepoints -1 hour, 0 minutes, 60 minutes, 2 hours, 6 hours, and 24 hours for BCDVA and -1 hour, 0 minutes, 90 minutes, 3 hours, 6 hours, and 24 hours for DCNVA, using letters and logMAR units. Only letters will be recorded in the CRF and will be converted to logMAR programmatically as follows: logMAR = 0.02*(S - # Letters), where S = 55 for BCDVA and S = 70 for DCNVA. The values of S are the number of letters read equivalent to a Snellen Acuity of 20/20. For example, for BCDVA, if the # letters = 55, then LogMAR = 0.02*(55 - 55) = 0.00; if # letters > 55, then LogMAR is negative. As a reference 5 letters is equivalent to 1 line. Separate summaries will be created for the study eye, the fellow eye, and both eyes. Treatments will be compared for the Safety Population using the same ANCOVA model proposed for the continuous secondary efficacy endpoints for both change from baseline (-1 hour) and change from maximum (0 minutes).

In addition, DCNVA will be analyzed by mydriatic agent, and BCDVA and DCNVA will be analyzed by age group (< 18, 18–30, 30–44, 45–64, and ≥ 65), using the same models as for the efficacy variables. All DCNVA tables will be repeated for the PP Population.

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. The summary will be repeated for pediatric subjects.

10.7. IOP

Observed values and change from baseline in IOP at 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. The summary will be repeated for pediatric subjects.

10.8. Subject Questionnaire

Subject questionnaire values will be summarized for each timepoint (-1 hour, 0 minutes, 60 minutes, 2 hours, 4 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. PK ANALYSES

All PK analyses will be conducted using the PK population. All PK analyses will be completed using the actual treatment a subject received. Observed case data will be used; no imputation will be performed for missing PK data.

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

Blood sampling for Nyxol PK measurements will be conducted in a subset of approximately 30 adult subjects at approximately 2 select study sites. Analysis of plasma samples for Nyxol concentration determinations will be performed by a central PK laboratory. Nyxol plasma concentrations will be summarized by time point (15 minutes, 60 minutes, and 3 hours) using descriptive statistics. For concentrations that are below the lower limit of quantification (LLOQ), the summary will use one-half the LLOQ as the analysis value.

The following PK parameters will be calculated for phentolamine using a model-independent approach (i.e., noncompartmental analysis) by a validated pharmacokinetic software (Phoenix WinNonlin Professional v 8.3 or higher); T_{max}, C_{max}, $AUC_{(0\text{-}T)}$, and $AUC_{(0\text{-}3)}$. The apparent terminal elimination rate constant λ (the rate for the log linear portion of the plasma concentration profile terminal phase) will not be calculated since a minimum of three concentrations in the terminal phase are required for calculation of λ. Consequently, the $T_{1/2}$, $AUC_{(0\text{-inf})}$, CL/F, and Vd/F will not be calculated.

Any tables and listings of PK parameters will be completed by a PK vendor and will not be included in the SAP TLFs.

Definitions for Plasma PK Parameters:

$AUC_{(0\text{-}3)}$ is the area under the plasma concentration-time curve from time zero to 3 hours as calculated by the trapezoidal rule; linear-up log-down.

$AUC_{(0\text{-}T)}$ is the area under the plasma concentration-time curve from time zero to the last measurable plasma concentration (CT) as calculated by the trapezoidal rule; linear-up log-down.

$AUC_{(0\text{-inf})}$ is the area under the plasma concentration-time curve from time zero to infinity. It is calculated as the sum of the area from time zero to the time of the last quantifiable plasma concentration (C_T) and the area from T to infinity, calculated as the last quantifiable plasma concentration divided by λ, where λ is the terminal elimination rate constant as follows:

$$AUC_{(0-\text{inf})} = AUC_{(0-T)} + \frac{C_T}{\lambda}$$

CL/F is clearance divided by bioavailability. It is determined by dividing the total dose by $AUC_{(0\text{-inf})}$.

C_{max} is the maximum observed concentration.

$T_{1/2}$ is terminal half-life calculated by ln(2)/λ where λ is the rate constant for the log-linear portion of the terminal phase. A minimum of three values in the post-distribution phase of the plasma concentration-time curve are required for calculation of λ.

T_{max} is the time to reach C_{max}.

Vd/F is the volume of distribution based on the terminal phase divided by bioavailability. It is determined by dividing CL/F by λ.

λ is the apparent terminal elimination rate constant.

12. REFERENCES

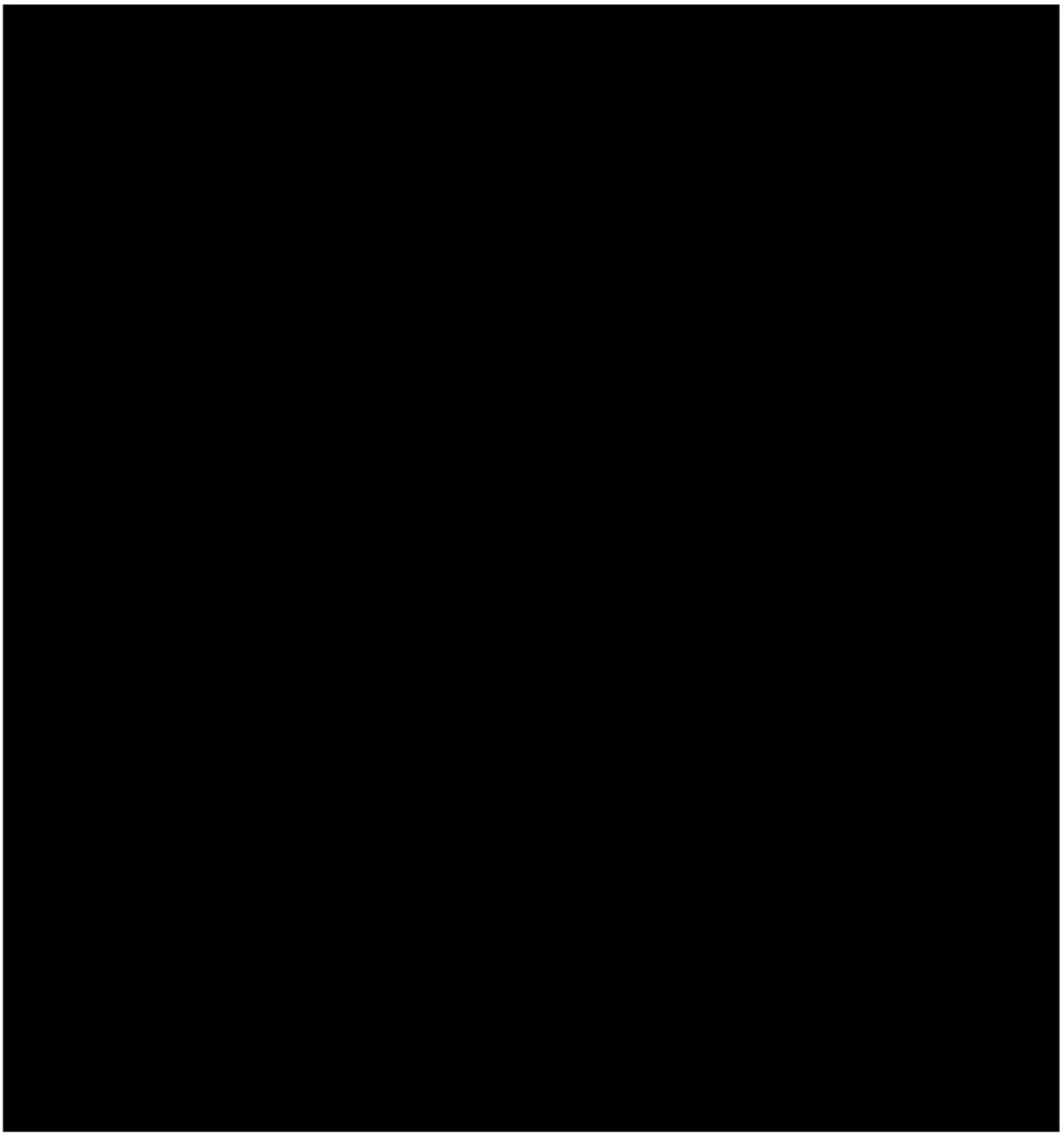
[1] ICH E9 Expert Working Group. Statistical Principles for Clinical Trials: ICH Harmonized Tripartite Guideline, September 1998

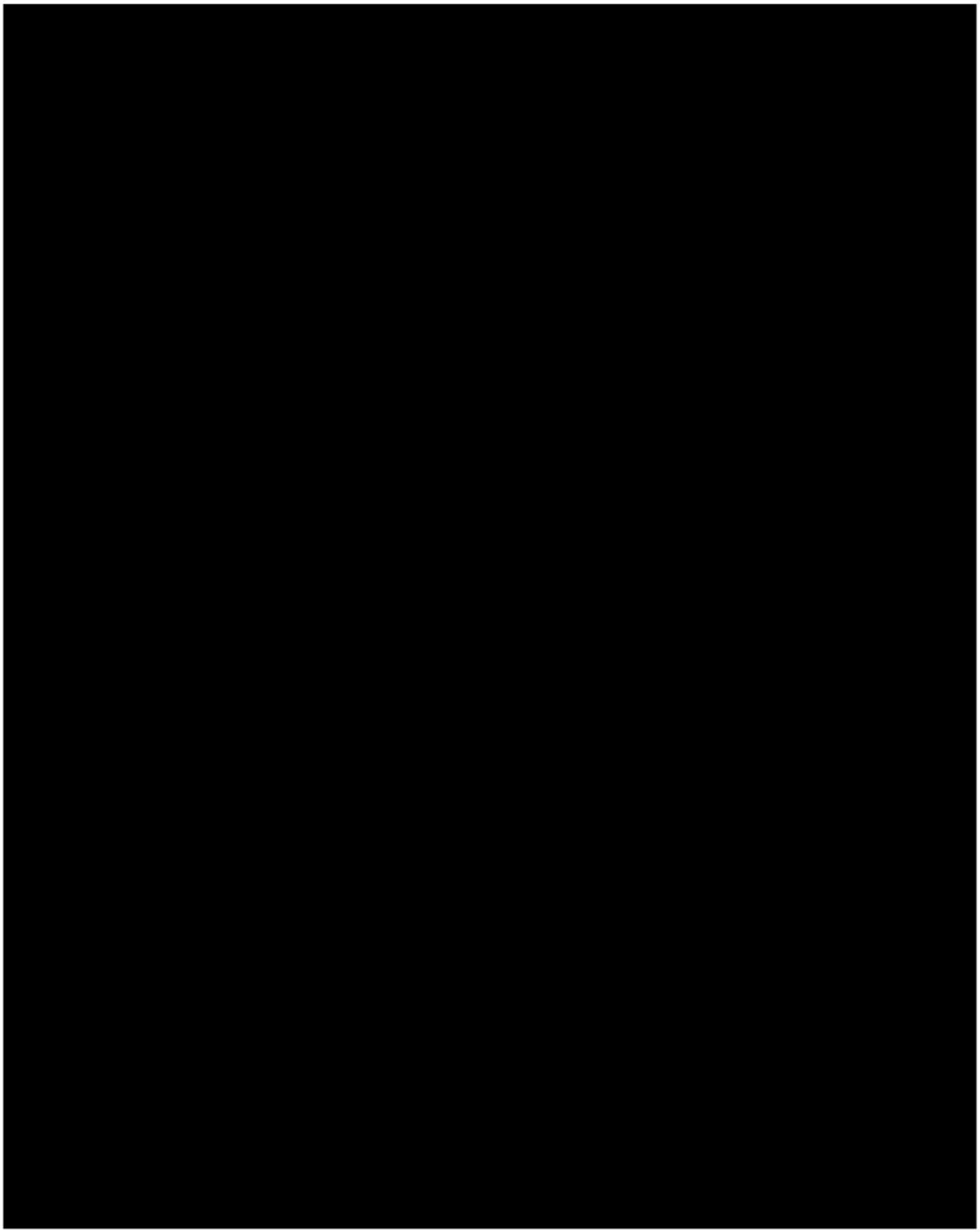
[2] Hauck WM, Anderson S, and Marcus SM, Should We Adjust for Covariates in Nonlinear Regression Analyses of Randomized Trials? *Controlled Clin Trials* 1998;19:249–256

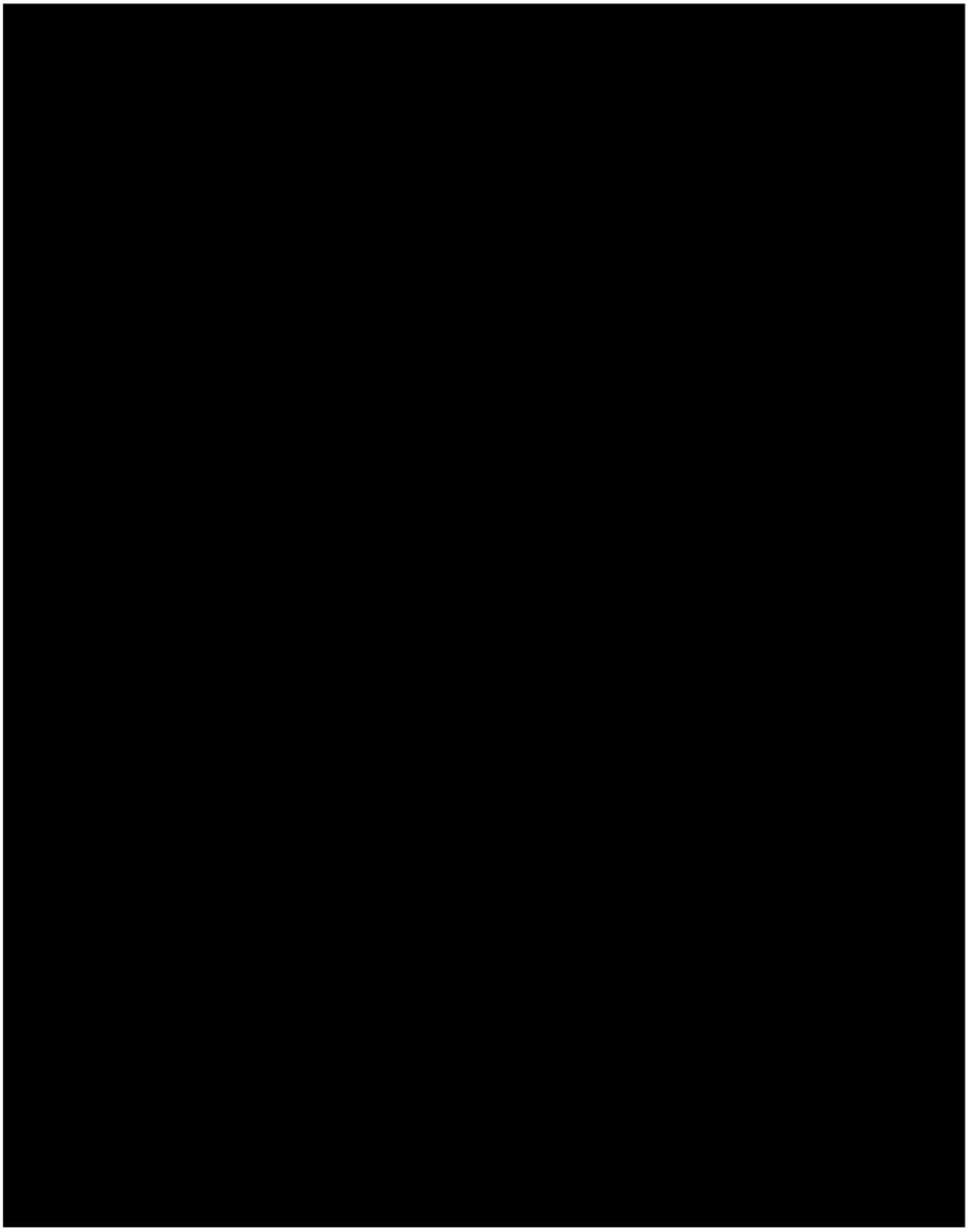
13. APPENDICES

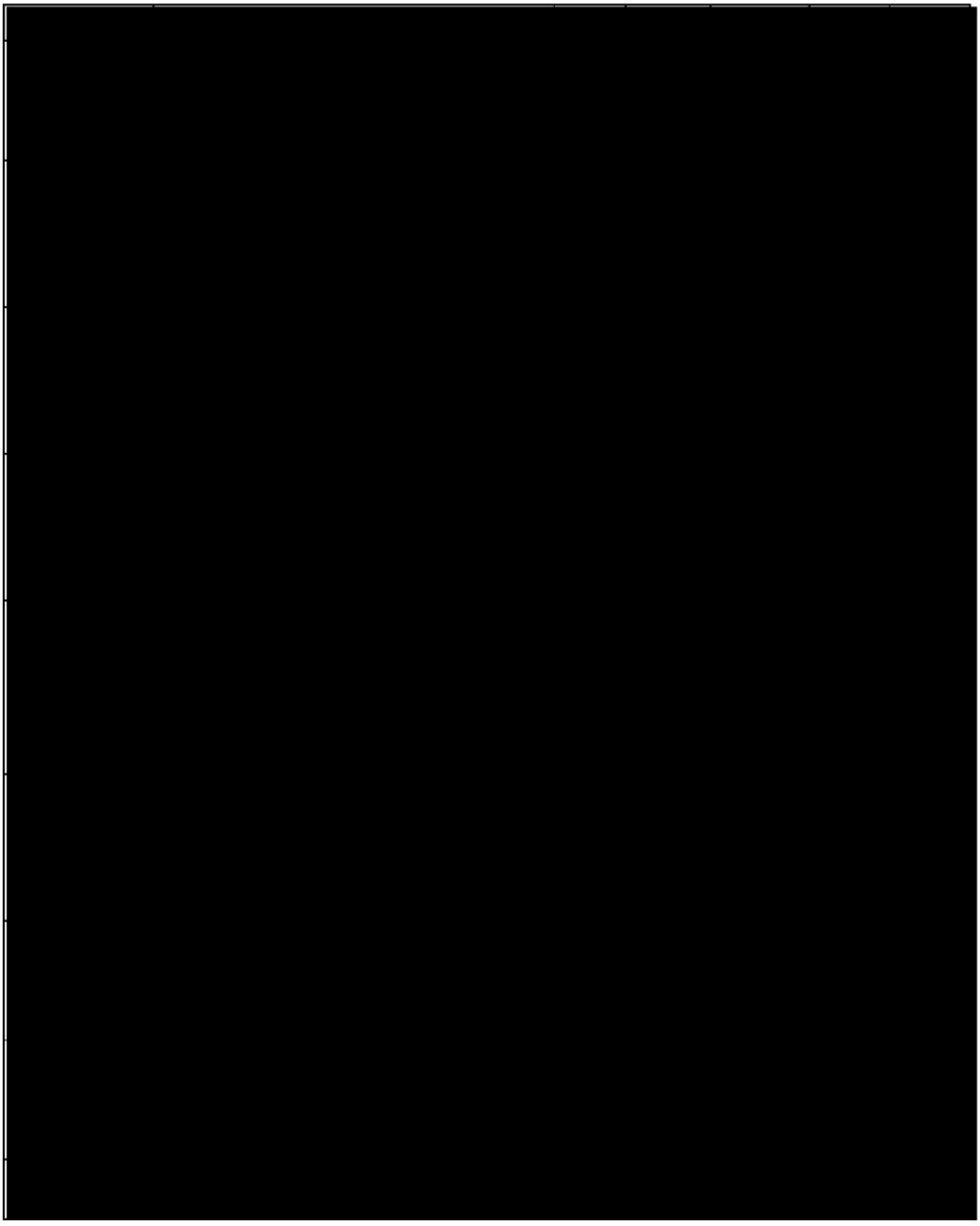
13.1. List of Planned Tables

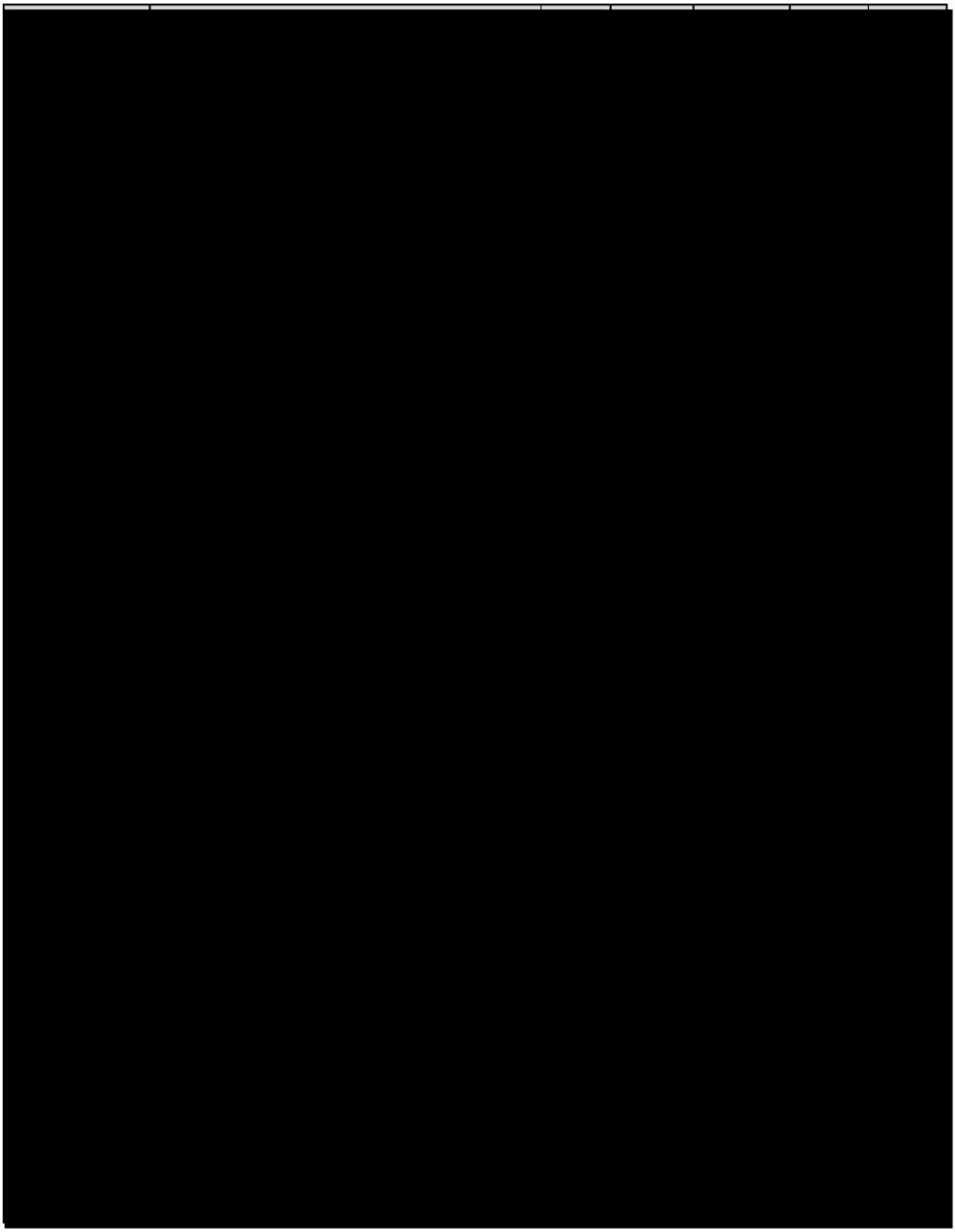
The list of planned tables includes all of the *main* tables to be presented for the study.

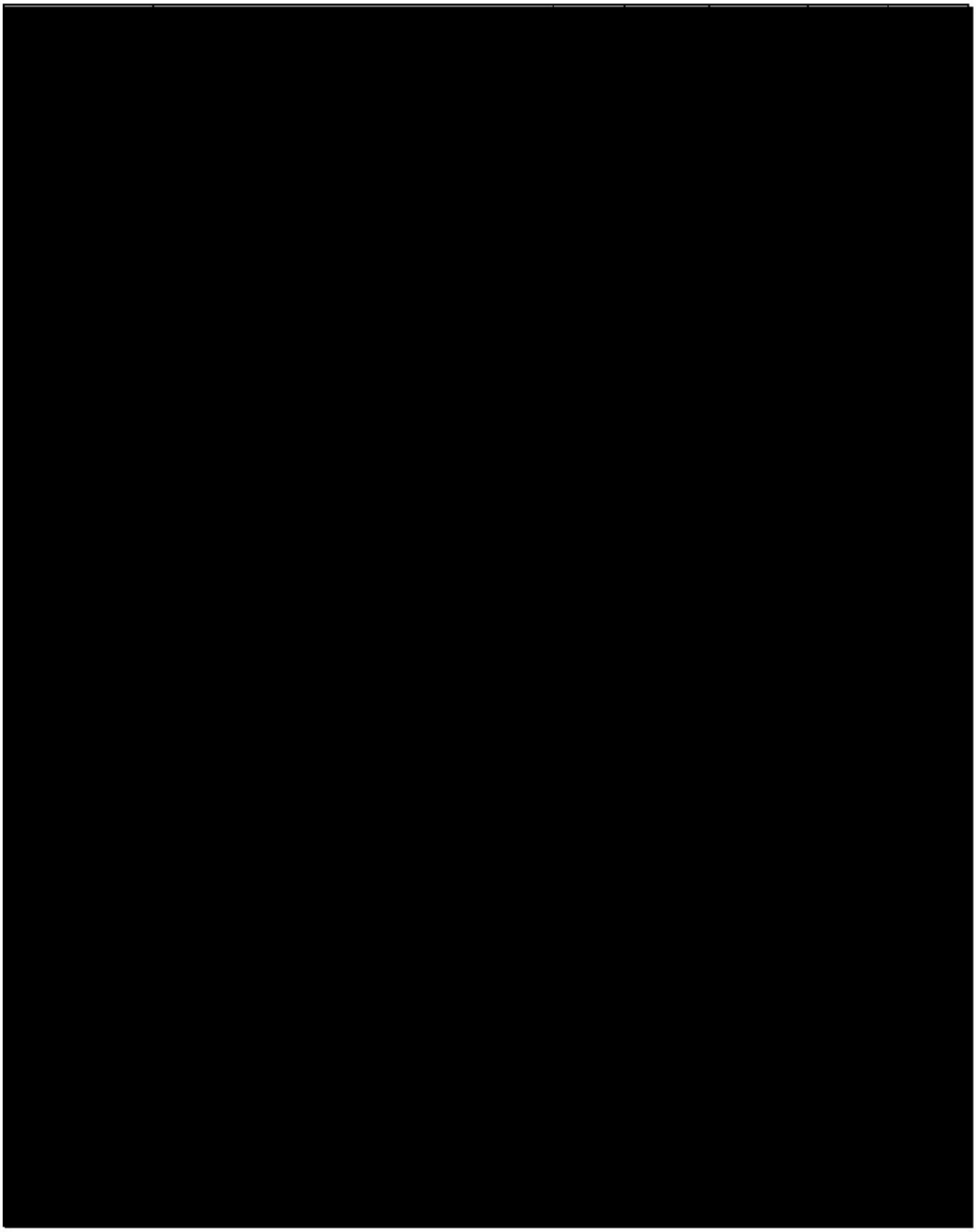


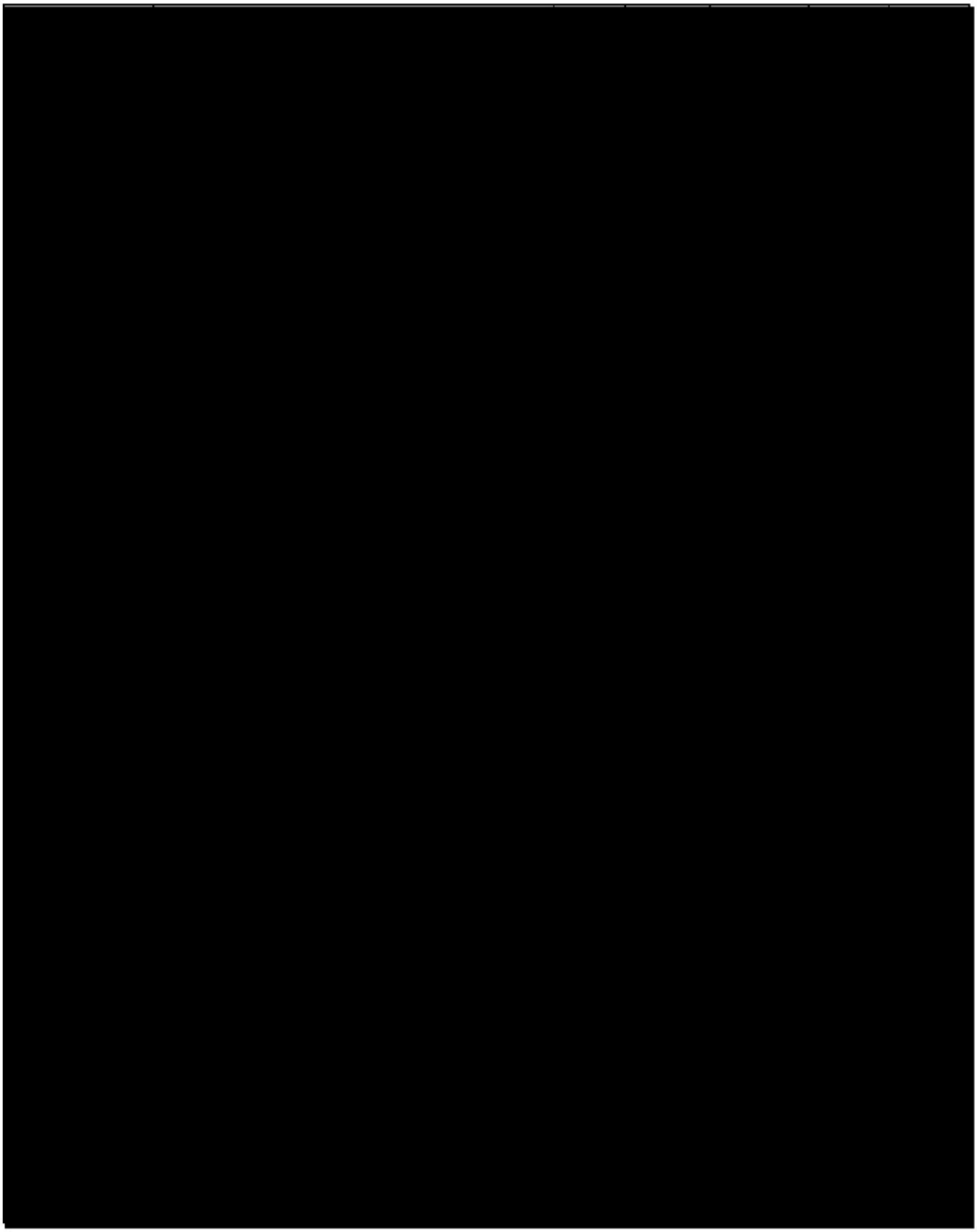












[redacted]

13.2. List of Planned Listings

[redacted]

13.3. Hierarchy of Sequential Testing