A Double-Masked Comparison of Apraclonidine Hydrochloride Ophthalmic Solution to Vehicle for the Reduction of Ocular Redness

STUDY ID

DEW422-C001

STATISTICAL ANALYSIS PLAN

NCT06444529

Alcon

Statistical Analysis Plan for DEW422-C001
Title: A Double-Masked Comparison of Apraclonidine Hydrochloride Ophthalmic Solution to Vehicle for the Reduction of Ocular Redness.

Author: [redacted]
Principal Statistician

This version of the Statistical Analysis Plan is based on Version 3.0 of the study protocol.

The purpose of this Statistical Analysis Plan (SAP) is to describe in detail the planned analyses and reporting for protocol DEW422-C001. The statistical analysis methods outlined in this document will supersede those described in the clinical protocol. If additional analyses are required to supplement the planned analyses in this SAP, they may be conducted and will be documented in the clinical study report.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonization (ICH) E9 Guideline, *Guidance for Industry: Statistical Principles for Clinical Trials*, ICH E9 (R1) *Addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials*, and the ICH E3 Guideline, *Guidance for Industry: Structure and Content of Clinical Study Reports.*

Executive Summary:

Primary Objective:

The primary objective of this study is to demonstrate superiority of Apraclonidine Hydrochloride (HCl) 0.125% Ophthalmic Solution, when compared to vehicle, at 15-minutes and 10-hours (600-minutes) postinstillation, in relieving redness of the eye due to minor eye irritations.

Secondary Objective(s):

The secondary objective of this study is to demonstrate superiority of Apraclonidine HCl 0.125% Ophthalmic Solution, when compared to vehicle, at 1-minute, 8-hours (480-minutes) and 12-hours (720-minutes) postinstillation timepoints.

Decision Criteria for Study Success:

The success of this study will be based on demonstrating the superiority of Apraclonidine HCl 0.125% Ophthalmic Solution in relieving ocular redness compared to vehicle at 15-minutes and 10-hours (600-minutes) postinstillation at the 5% (two-sided) significance level in the Site Compliance Full Analysis Set (cFAS) where, cFAS will include all randomized subjects except for the site with systemic Good Clinical Practice (GCP) and Good Documentation Practice (GDP) noncompliance issues.

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List of Acronyms and Abbreviations

Abbreviation	Definition
ADR	Adverse drug reaction
AE	Adverse event
AESI	Adverse event of special interest
AR	Auto-regressive
ATC	Anatomical Therapeutic Chemical
BCVA	Best corrected visual acuity
BID	Twice a day
bpm	Beats per minute
cFAS	Site Compliance Full Analysis Set
cPPS	Site Compliance Per Protocol Analysis Set
CI	Confidence interval
CRF	Case report form
eCRF	Electronic case report form
EDC	Electronic data capture
FAS	Full analysis set
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GDP	Good Documentation Practice
GEE	Generalized Estimating Equation
HCl	Hydrochloride
ICE	Intercurrent event
ICH	International Conference on Harmonization
IOP	Intraocular pressure
IP	Investigational product
LoE	Lack of efficacy
LS	Least Squares
logMAR	Logarithm of the minimum angle of resolution
mmHg	Millimeters of mercury
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
MMRM	Mixed model repeated measure
MNAR	Missing not at random
MAR	Missing at random

N/A	Not applicable
OTC	Over the counter
PD	Protocol deviation
PP	Per protocol
PT	Preferred term
REML	Restricted maximum likelihood
RTSM	Randomization and trial supply management
SAE	Serious adverse events
SAP	Statistical Analysis Plan
SAR	Serious adverse reaction
SE	Standard error
SLE	Slit lamp examination
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
VA	Visual acuity
WHO	World Health Organization

1 STUDY OBJECTIVES AND DESIGN

1.1 Study Objectives and Endpoints

Table 1-1 Primary Objectives and Endpoints

Objective	Co-Primary Endpoint(s)
To demonstrate superiority of Apraclonidine HCl 0.125% Ophthalmic Solution when compared to vehicle at 15-minutes and 10-hours (600-minutes) postinstillation, in relieving redness of the eye due to minor eye irritations.	• Mean change from baseline in investigator-assessed ocular redness at 15-minutes postinstillation on Day 1. • Mean change from baseline in investigator-assessed ocular redness at 10-hours (600-minutes) postinstillation on Day 1.

Table 1-2 Secondary Objectives and Endpoints

Objective	Endpoint(s)
To demonstrate superiority of Apraclonidine HCl 0.125% Ophthalmic Solution when compared to vehicle at 1-minute, 8-hours (480-minutes) and 12-hours (720-minutes) post instillation timepoints.	• Mean change from baseline in investigator-assessed ocular redness at 1-minute postinstillation on Day 1. • Mean change from baseline in investigator-assessed ocular redness at 8-hours (480-minutes) postinstillation on Day 1. • Mean change from baseline in investigator-assessed ocular redness at 12-hours (720-minutes) postinstillation on Day 1.

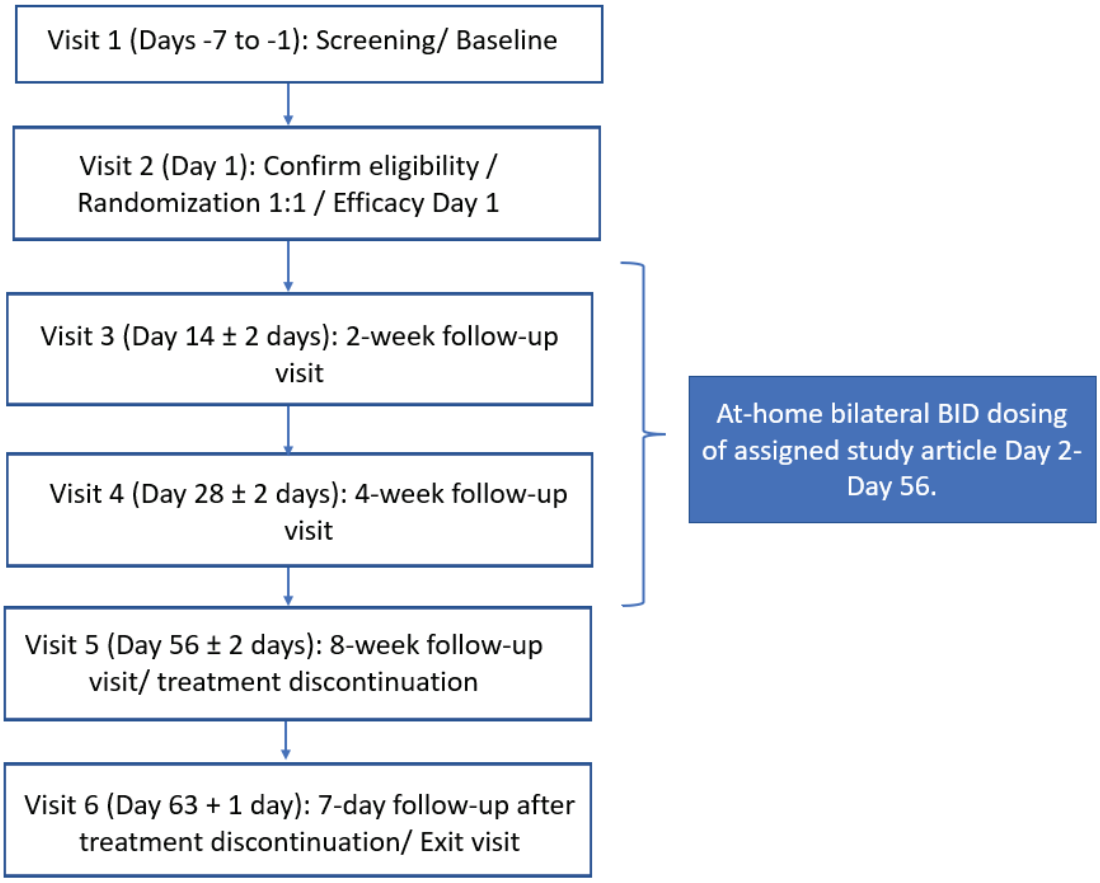


1.2 Study Description

This is a phase 3, multicenter, double-masked, randomized, vehicle-controlled, parallel-group study in healthy adults who have a history of using redness relief drops within the last 6 months, or have an interest to use OTC vasoconstrictor eye drops for redness relief, or would benefit from the use of redness relief drops as per the Investigator's opinion. Subjects must show a baseline redness of >1 unit in both eyes on Day 1 (Visit 2) to qualify. Approximately 120 subjects will be randomized 1:1 to receive Apraclonidine HCl 0.125% Ophthalmic Solution or vehicle.

There will be 6 visits over a period of approximately 10 weeks. The study design is illustrated in Figure 1-1. The schedule of visits is outlined in Appendix 1 (Section 11.1).

Figure 1-1 Study Design



1.3 Randomization

Qualified subjects will be randomized in a 1:1 ratio to the Apraclonidine HCl 0.125% Ophthalmic Solution or vehicle, using block randomization, stratified by clinical site to ensure a balanced treatment allocation across clinical sites. The randomization schedule for study treatment assignment will be generated [redacted]

[redacted]

1.4 Masking

This is a double-masked study. [redacted]

This level of masking will be maintained throughout the conduct of the study. Unmasking will occur only after all planned study data have been validated and the database locked.

Study personnel must avoid seeking information that may compromise masking.

1.5 Interim Analysis

There are no plans to conduct an interim analysis and no criteria by which the study would be terminated early based upon statistical determination.

2 ANALYSIS SETS

2.1 Efficacy Analysis Sets

The following analysis populations will be considered:

- The Site Compliance Full Analysis Set (cFAS) will include all randomized subjects except for the site with systemic GCP and GDP noncompliance issues. Subjects will be analyzed according to the treatment to which they were randomized.
- The Full Analysis Set (FAS) will include all randomized subjects. Subjects will be analyzed according to the treatment to which they were randomized.



[redacted]

The efficacy analysis will be based on the Site Compliance Full Analysis Set (cFAS), and the success of this study will be determined by demonstrating superiority in this population. [redacted]

2.2 Safety Analysis Set

The safety analysis set will include all randomized subjects who have received at least one instillation of the study treatments. Subjects in the safety analysis set will be analyzed according to the treatment they received. All safety analyses will be based on the safety population.

3 SUBJECT CHARACTERISTICS AND STUDY CONDUCT SUMMARIES

3.1 Disposition of Subjects

Subject disposition will be presented in terms of the numbers and percentages of subjects who were enrolled (i.e., signed informed consent), [redacted] Additionally, the number and percentage of subjects who completed the study and those who discontinued the study will be summarized. Subjects who do not discontinue from the study will be considered study completers. Disposition will be summarized by treatment group and overall, for all subjects.

The total number of screened subjects, along with the number of screen failures and study discontinuation prior to randomization, will be presented. The reasons for premature study discontinuation will be summarized by treatment group for all discontinued subjects after randomization, with percentages calculated using the total number of randomized subjects. The summarized reasons for study discontinuation after randomization will include adverse events (AEs), protocol violations, lack of efficacy (LoE), lost to follow-up, Sponsor termination of the study, technical problems, voluntary withdrawal, pregnancy (if applicable), death and other reasons. A subject listing will be provided, detailing the date and reason for premature study discontinuation.

The number and percentage of subjects with any PD, major PD, and minor PD per DEP will be summarized by treatment group for all randomized subjects. A subject listing will be provided for major deviations and separately for minor deviations that includes the visit if applicable, deviation category, and summary.

In addition, subject listings will be provided that include the informed consent date, any inclusion and exclusion criteria violations, and subject inclusion in analysis populations, along with reasons for exclusion, where applicable. Details of the study randomization, randomized treatment arm, and actual treatment arm, will also be included within a subject listing.

3.2 Demographics Variables

Demographic variables will be summarized [redacted] by treatment group using continuous descriptive statistics (mean, standard deviation, median, minimum, and maximum). Additionally, age will be categorized as follows: <65 years and ≥65 years. Age will be reported in full years, calculated using the formula:

Age = (Informed Consent Date – Date of Birth) / 365.25, truncated as an integer

The number and percentage of subjects will be presented, both overall and by treatment group, for the following categories: age category (<65 years and ≥65 years), sex at birth, race, ethnicity, [redacted]. Subjects who record more than one race will be grouped into a single category denoted as Multiple. [redacted]

3.3 Pretreatment Variables

Ocular redness will be summarized at the eye level and alertness will be summarized at the subject level by treatment group and overall. Continuous variables will be summarized using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Categorical variables will be summarized by counts and percentages. [redacted]

3.4 Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 27.0.

Ocular and non-ocular medical history will be summarized separately using discrete summary statistics and presented by treatment group and overall, for all subjects at the subject level by System Organ Class (SOC) and Preferred Term (PT) for the Safety Population. If a subject reports the same PT multiple times within the same SOC, that PT will only be reported once within that SOC. As with the PT, if a subject reports multiple

conditions within the same SOC, that SOC will only be reported once. SOCs and PTs within an SOC are listed in order of descending frequency across all subjects.

Listings of medical history will be generated separately for ocular and non-ocular data.

3.5 Concomitant Medications and Procedures

Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO Drug) Global, B3, March 2024 and summarized to the therapeutic drug class (Anatomical Therapeutic Chemical [ATC] 2 classification) and preferred name. If the ATC 2 classification is not provided, then the next lowest classification that is provided in the coding dictionary will be used. The preferred name will be defined as the active ingredient; if the active ingredient is not provided or includes more than two ingredients (e.g., multivitamins), then the drug name will be summarized as the preferred name.

Concomitant medications are defined as those medications listed as having been taken (1) prior to initiation of study treatment administration and continuing for any period following the first administration of study treatment or (2) at any time following the first administration of study treatment.

Ocular and non-ocular concomitant medications will be summarized separately using discrete summary statistics and presented by treatment group and overall, for the Safety Population. Medications will be tabulated for each treatment group using frequencies and percentages. Subjects may have more than one medication per ATC text. At each level of subject summarization, a subject will be counted once if s/he reports one or more medications. Percentages will be based on the number of subjects in each treatment group. ATC Classes and preferred names within ATC Classes are ordered by descending frequency based on all subjects.

Listings of prior and concomitant medications and procedures will be generated separately for ocular and non-ocular data.

Incomplete or missing concomitant medications start and end date will be handled as defined in Appendix 2.

3.6 Dosing Compliance

Dosing compliance (% compliance) will be assessed by calculating the percentage of number of doses taken over the number of expected doses. [redacted]

[redacted] If a subject discontinues the study, the date of their last visit will be used in place of Date of Visit 5.

A categorical dosing compliance variable will also be defined as non-compliant (<80%), over compliant (>120%), and compliant (≥80% and ≤120%). Average, median, minimum, and maximum dosing compliance (%) and compliance category frequency will be summarized for each treatment group and overall using the safety analysis set. A subject listing of study treatment accountability will be generated.

4 ANALYSES SUPPORTING PRIMARY EFFICACY OBJECTIVES

4.1 Primary Efficacy Endpoint(s)

Co-Primary Endpoints:

- Mean change from baseline in investigator-assessed ocular redness at 15-minutes postinstillation on Day 1.
- Mean change from baseline in investigator-assessed ocular redness at 10-hours (600-minutes) postinstillation on Day 1.

Subjects must have a baseline ocular redness of >1 unit in both eyes to qualify. Investigator-assessed ocular redness scores are used to assess the severity of ocular redness. The score is graded using the Ora Calibra™ Ocular Hyperemia Scale, ranging from 0 to 4 (0 = none; 4 = extremely severe), with half-point increments (0.5) are allowed. Baseline (preinstillation) score can range from 1.5 to 4, while the postinstillation (post-baseline) score can range from 0 to 4. The change from baseline, calculated as postinstillation minus baseline scores, can range between -4 and +2.5. Positive values indicate increased ocular redness (worsening), zero indicates no change, and negative values indicate improvement (decrease in severity).

4.2 Hypothesis and Statistical Methods for Analysis of Primary Endpoint(s)

The following hypotheses will be tested by comparing Apraclonidine HCl 0.125% Ophthalmic Solution to vehicle. Both co-primary null hypotheses (H_0) below must be rejected in favor of Apraclonidine HCl 0.125% Ophthalmic Solution at the 0.05 (two-sided) significance level to claim efficacy.

- H_{01}: $\mu_1(v) = \mu_1(t)$ vs. H_{a1}: $\mu_1(v) \neq \mu_1(t)$

where $\mu_1(v)$ and $\mu_1(t)$ denote the mean change from baseline in investigator-assessed redness score at 15-minutes postinstillation on Day 1 with vehicle and Apraclonidine HCl 0.125%, respectively.

- H_{02}: $\mu_2(v) = \mu_2(t)$ vs. H_{a2}: $\mu_2(v) \neq \mu_2(t)$

where $\mu_2(v)$ and $\mu_2(t)$ denote the mean change from baseline in investigator-assessed redness score at 600-minutes postinstillation on Day 1 with vehicle and Apraclonidine HCl 0.125%, respectively.

To evaluate the hypothesis that the mean change from baseline in investigator-assessed ocular redness with Apraclonidine HCl 0.125% Ophthalmic Solution is different than the mean change from baseline in the ocular redness with vehicle at 15-minutes and 600-minutes postinstillation on Day 1 (Visit 2), a mixed model repeated measures (MMRM) analysis will be employed. The mixed model will include fixed effect terms for baseline (preinstillation) ocular redness score, treatment group, clinical site, timepoints (1-minute, 5-minutes, 15-minute, 60-minutes, 240-minutes, 480-minutes, 600-minutes, and 720-minutes postinstillation), and treatment-by-time interaction (see model below), with restricted maximum likelihood (REML) and Kenwood-Roger approximation for the degrees of freedom. Within-subject correlation due to bilateral ocular redness assessment across eyes as well as correlation due to repeated measures over time will be accounted for using UN@UN Kronecker product of variance-covariance structure (Ying et al, 2021). Statistical and programming specifications for MMRM analysis of efficacy on Day 1 redness score is provided in Appendix 3.

In case the MMRM using UN@UN variance-covariance structure fails to converge, initial estimates of the covariance parameters will be obtained by Fisher scoring algorithm (via the SCORING option in PROC MIXED, Longford 1987). If convergence is still not achieved, the structures UN@AR1 and UN@CS will be considered sequentially.

After addressing the intercurrent events (ICEs) as outlined in Section 4.3, any missing data on Day 1 will be handled using the MMRM approach. The model assumes that the data are missing at random (MAR), meaning that, conditional on the observed data, the missingness mechanism is independent of the unobserved scores. Under these assumptions, valid inferences can be obtained through MMRM analysis without the need for explicit modeling of the dropout process, provided the missingness is considered ignorable.

Descriptive statistics, without imputation, will be provided for the change from baseline in investigator-assessed ocular redness on Day 1, by treatment group and timepoints. These statistics will include the mean, standard deviation, median, minimum, maximum, and the total number of observations for each eye, as well as the average of both eyes. MMRM results, including Least squares (LS) means, standard errors (SEs) and 95% confidence intervals (CIs) for the mean change from baseline in investigator-assessed ocular redness will be reported by treatment group at 15-minutes and 600-minutes on Day 1. Additionally, the differences in LS means, SEs, 95% CIs and two-sided p-values for the mean change of the co-primary endpoints between treatment groups will also be presented. These results will be presented for all analysis sets as defined in Section 2.1.

To demonstrate the superiority of Apraclonidine HCl 0.125% Ophthalmic Solution over vehicle in relieving eye redness caused by minor irritations, ocular redness will be measured at 15-minutes and 600-minutes postinstillation on Day 1. The primary estimand will assess the treatment effect in the population of subjects with ocular redness, as defined by protocol's eligibility criteria, based on their randomized assignment to study treatment. This estimand accounts for the use of treatment indicated to relieve ocular redness, study discontinuation due to AEs or LoE considered as markers of treatment failure. Table 4-1 presents all estimand attributes, particularly regarding ICEs. Details for supplementary estimand are provided in Section 4.6.1.

Table 4-1 Primary Estimand for co-primary endpoints

Treatment	Apraclonidine HCl Ophthalmic Solution 0.125% vs. vehicle.
Population	Healthy adult subjects (aged ≥ 18) at the time of informed consent, with history of using (or expressed a desire to use) redness relief drops within the last 6 months, and who show a baseline redness of > 1 unit in both eyes at Visit 2 (Day 1)
Variable	Change from baseline in investigator-assessed ocular redness scores at 15-minutes and 10-hours (600-minutes) postinstillation on Day 1. The change from baseline ranges from -4 to +2.5, with positive values indicating increased redness, zero indicating no change, and negative values indicating improvement.
Population-level summary	Difference between treatments (Apraclonidine HCl 0.125% - vehicle) in adjusted LS means for change from baseline in investigator-assessed ocular redness at 15-minutes and 600-minutes postinstillation, along with the associated 95% Cl. Negative values indicate superiority of Apraclonidine HCl 0.125% compared to the vehicle.

Handling of ICEs	**Use of prohibited medication indicated for the treatment of ocular redness.** This event will be considered as missing post-ICE and will be handled using vehicle-based multiple imputation. **Use of prohibited medication not indicated for the treatment of ocular redness**. This event will be considered as missing post-ICE and will be handled using the MMRM [hypothetical strategy]. **Study discontinuation due to AE or LoE**. This event will be considered as missing post-ICE and will be handled using the vehicle-based multiple imputation. **Study discontinuation due to reasons other than AE or LoE**. This event will be considered as missing post-ICE and will be handled using the MMRM [hypothetical strategy]. **Treatment not received**. This event will be handled using multiple imputation under the assumption of MAR [hypothetical strategy].

4.3 Handling of Intercurrent Events of Primary Estimand(s)

ICEs are defined as events that occur after study treatment initiation and may influence either the interpretation or existence of measurements relevant to the clinical question. In this study, the following ICEs have been identified, and strategies have been developed to address them in alignment with the primary estimand(s):

- **Use of prohibited medication indicated for the treatment of ocular redness (e.g., eye whiteners, ocular decongestants)**:
    - **Strategy**: Post-ICE data will be excluded from analysis and the resulting missing data handled using vehicle-based multiple imputation.
    - **Rationale**: Since this category of medication is directly used to treat the clinical condition under investigation, it could confound the assessment of the treatment effects. Data following such events will be excluded and treated as missing. Moreover, the use of these medications might suggest a LoE from the assigned treatment. Therefore, missing data will be imputed using a vehicle-based multiple imputation strategy, where such events in the active treatment arm are penalized to reflect lack of benefit, while occurrences in the vehicle arm are handled to reflect the vehicle's effect.
- **Use of prohibited medication not indicated for the treatment of ocular redness**:
    - **Strategy**: Post-ICE data will be excluded from analysis and the resulting missing data handled using a MMRM based on a hypothetical strategy.

  - **Rationale**: This category includes medications that could affect ocular redness, potentially confounding the assessment of the treatment effects. However, these medications are not intended for treating ocular redness but may cause it as a secondary effect. Since the use of these medications is assumed to be unrelated to the assigned treatment and equally probable in both treatment arms, the MAR assumption is considered valid when post-event assessments are treated as missing. This approach addresses the question of what would have happened if the prohibited medication had not been used, thereby excluding its impact on the outcome.

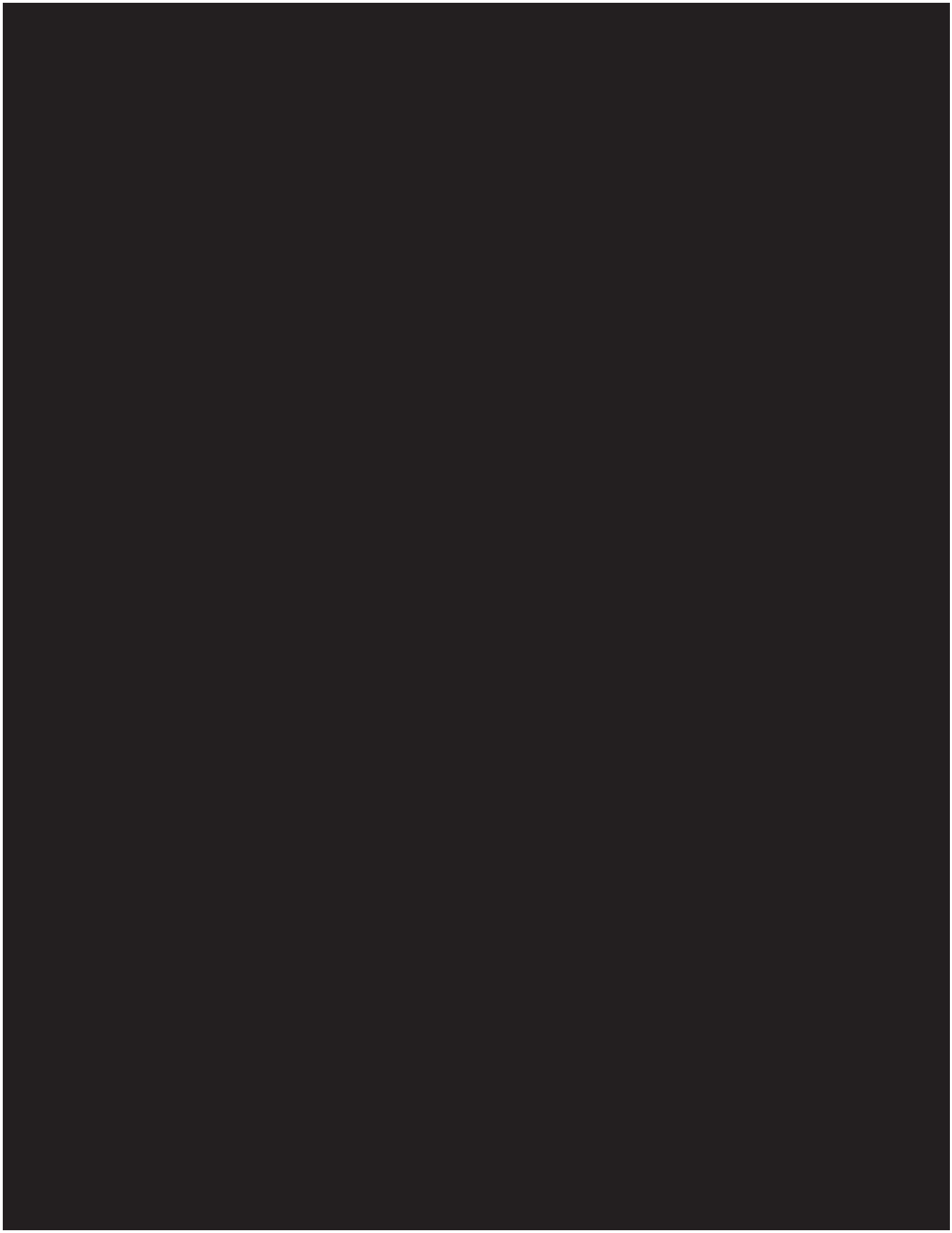
- **Study discontinuation due to an AE or LoE**:
  - **Strategy**: Missing data following these ICEs will be imputed using vehicle-based multiple imputation.
  - **Rationale**: Discontinuation due to AE or LoE is viewed as a negative outcome of the assigned treatment. Data collected before discontinuation will reflect the outcome while on treatment, no further benefit from the treatment is assumed post-discontinuation. The vehicle-based multiple imputation method used to handle the resulting missing data will reflect the lack of continuing benefit after discontinuation due to AE or LoE.
- **Study discontinuation for reasons other than AE or LoE**:
  - **Strategy**: Data will be handled using MMRM based on a hypothetical strategy.
  - **Rationale**: Discontinuations for reasons unrelated to AE or LoE are considered unrelated to the assigned treatment. Since these discontinuations are expected to occur equally across both treatment arms, the MAR assumption is considered valid. MMRM will be used to account for these cases, preventing bias in treatment comparisons.
- **Treatment not received**:
  - **Strategy**: Missing data due to non-receipt of assigned treatment will be handled using multiple imputations under the MAR assumption, based on a hypothetical strategy.
  - **Rationale**: Since the treatment was never administered, imputation under the MAR assumption will model what would have been observed if the treatment was received. The hypothetical strategy ensures that the estimation remains aligned with the clinical question of interest.

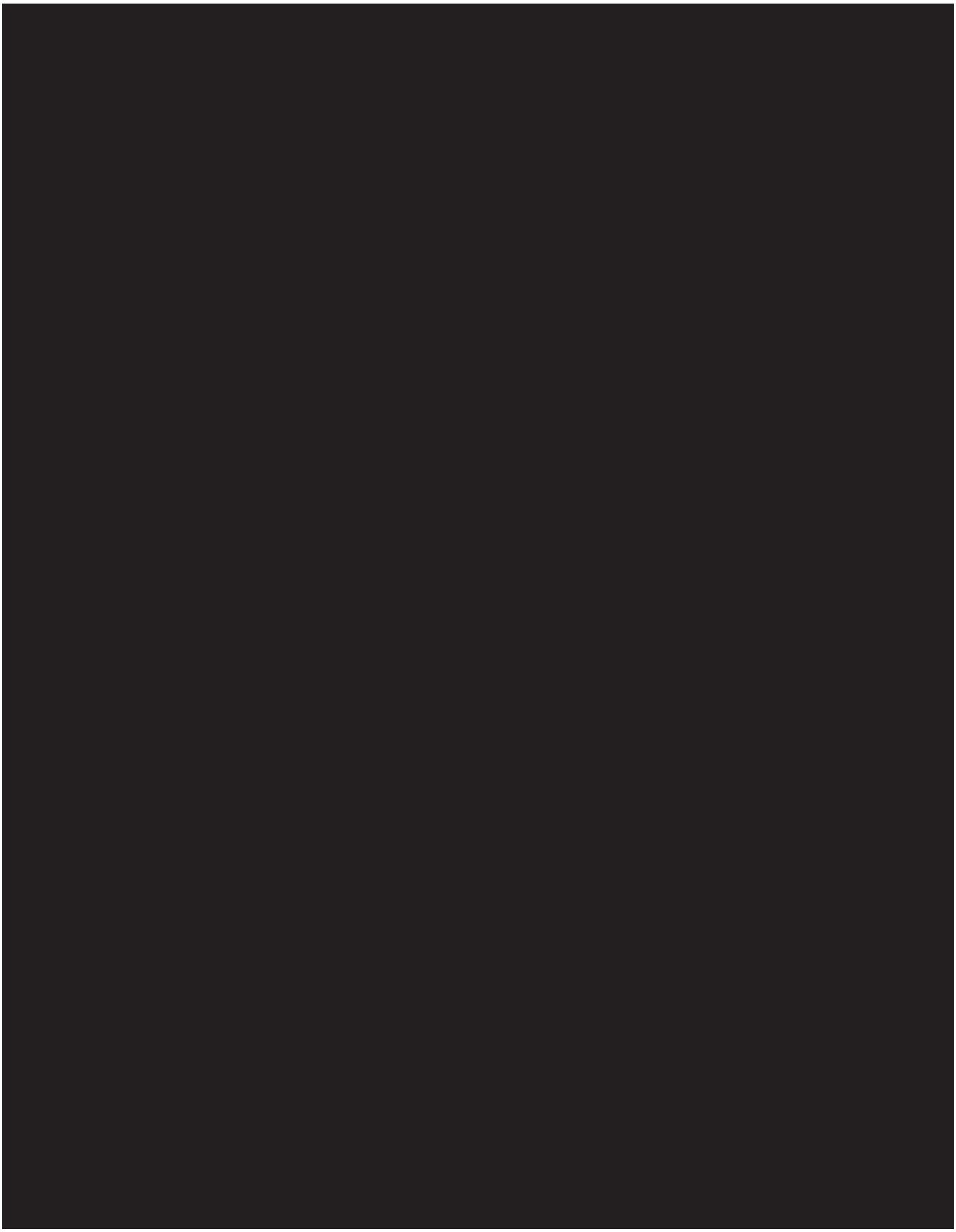
When multiple ICEs occur simultaneously, the handling strategy will prioritize the ICE with the most significant impact on the clinical interpretation. For example, if one ICE assumes a MAR mechanism and another assumes a missing not at random (MNAR) mechanism, the MNAR assumption will take precedence. In this case, vehicle-based multiple imputation will be used for the MNAR event to appropriately handle the missing data and ensure a conservative estimation of the treatment effect. In case if ICE that assumes MAR mechanism occurs earlier than ICE that assumes MNAR mechanism then vehicle-based multiple imputation will be used for the MNAR event from the occurrence of ICE that assumes MNAR mechanism onwards.

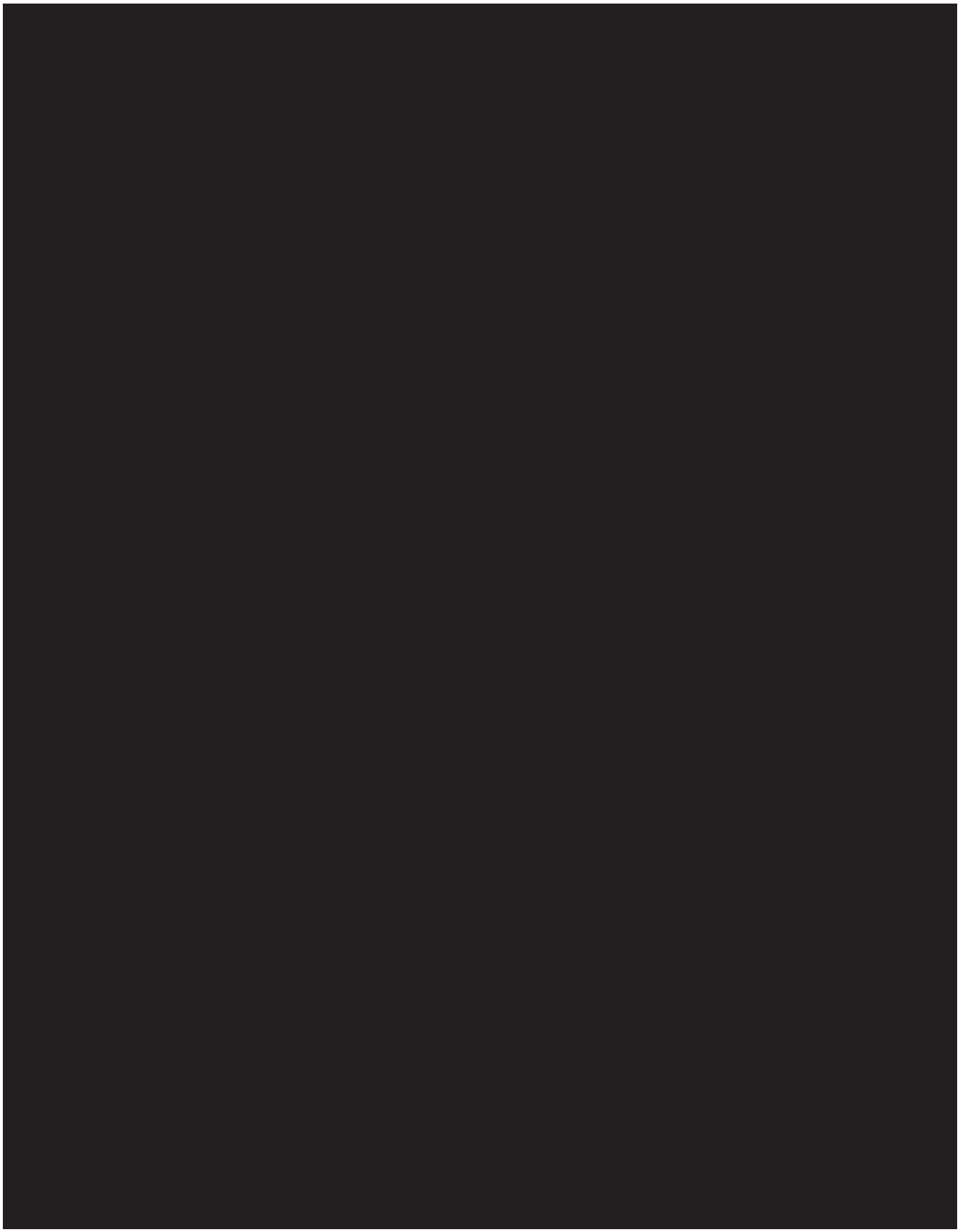
4.4 Handling of Missing Data Unrelated to ICEs

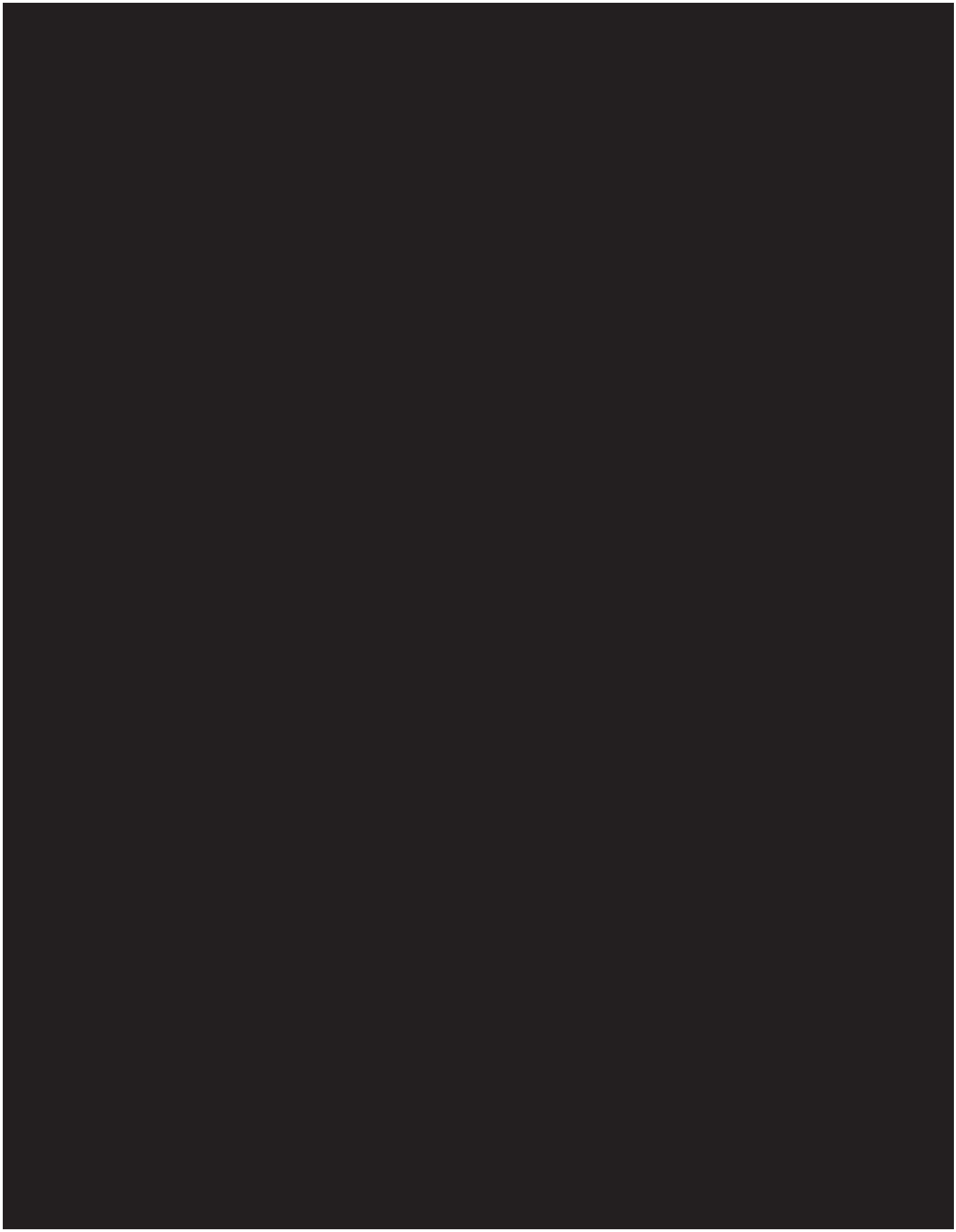
1. **Strategy**: All missing data unrelated to ICE will be handled using MMRM.
2. **Rationale**: Missing data unrelated to ICE may arise due to administrative or logistical reasons, such as intermittent missed visits. This type of missingness is assumed to be independent of the assigned treatment and is expected to be equally distributed across both treatment arms. MMRM efficiently handles incomplete data by utilizing all available information and modeling the repeated measures for each subject. As these missing data are not treatment-related, the MAR assumption is considered appropriate.

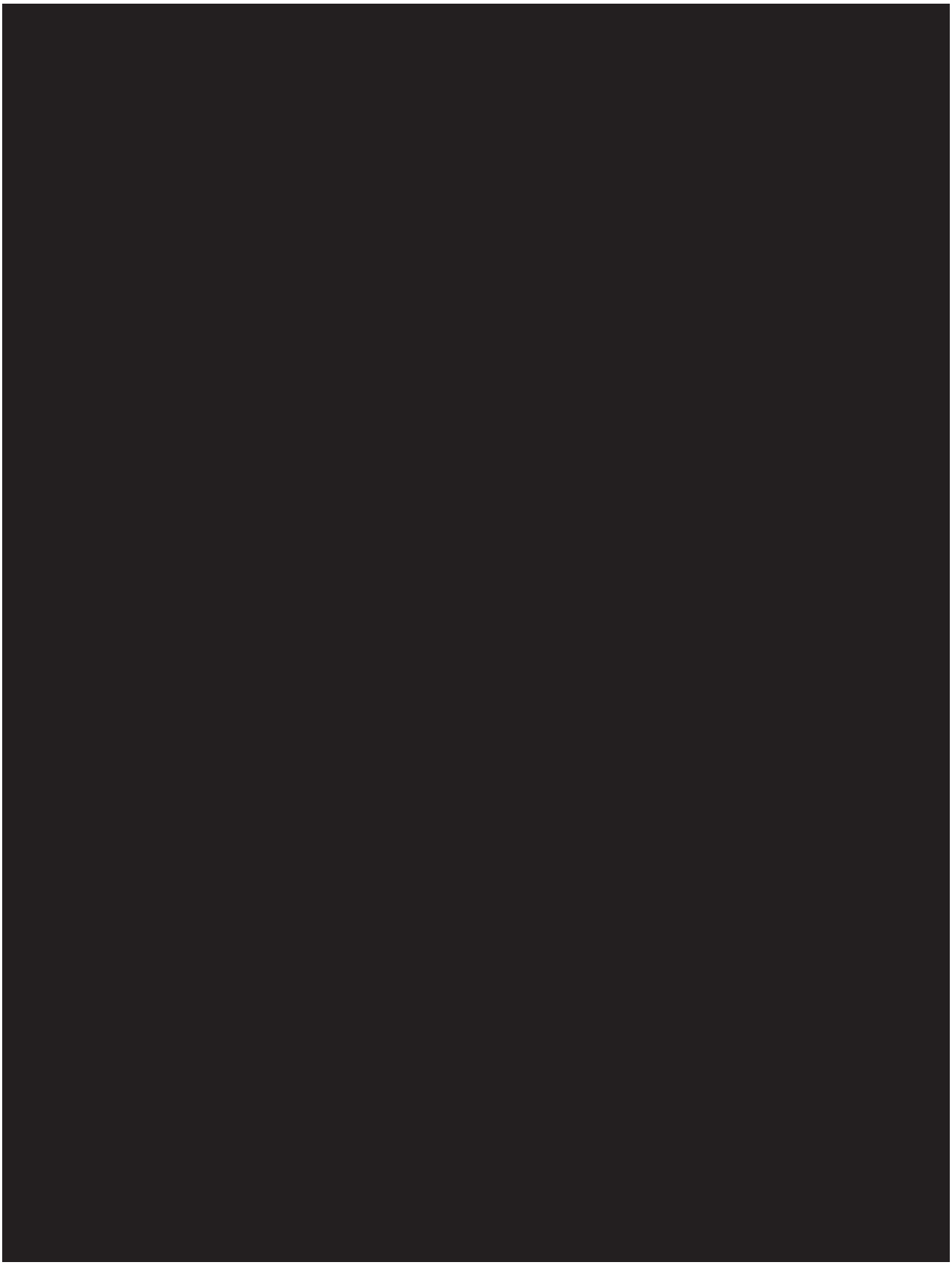












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5 ANALYSES SUPPORTING SECONDARY EFFICACY OBJECTIVES

The secondary objectives are to demonstrate superiority of Apraclonidine HCl 0.125% Ophthalmic Solution to vehicle on relieving redness of the eye due to minor eye irritations at 1-minute, 480-minutes (8-hours) and 720-minutes (12-hours) postinstillation on Day 1.

5.1 Secondary Efficacy Endpoint(s)

Secondary Endpoints:

- Mean change from baseline in investigator-assessed ocular redness at 1-minute postinstillation on Day 1
- Mean change from baseline in investigator-assessed ocular redness at 480-minutes postinstillation on Day 1
- Mean change from baseline in investigator-assessed ocular redness at 720-minutes postinstillation on Day 1

Change from baseline (preinstillation), defined as postinstillation (post-baseline) minus baseline, can range from -4 to 2.5 in 0.5 increments, where positive values indicate an increase in ocular redness (worsening), zero indicates no change, and negative numbers indicate improvement (decrease in severity).

5.2 Hypothesis and Statistical Methods for Analysis of Secondary Endpoint(s)

The change from baseline in investigator-assessed ocular redness for these secondary endpoints will be analyzed using the same statistical model employed for the co-primary endpoints, as detailed in Section 4.2. A single model will be used for all timepoints on Day 1. LS means, SEs and 95% CI for the mean change from baseline in ocular redness at 1-minute, 480-minutes, and 720-minutes on Day 1 will be reported by treatment group. LS means differences, SEs, 95% CIs and two-sided p-values for mean change between treatment groups

for these timepoints will also be presented. Descriptive statistics, without imputation, will be provided for the change from baseline in investigator-assessed ocular redness on Day 1, by treatment group and timepoints. These statistics will include the mean, standard deviation, median, minimum, maximum, and the total number of observations for each eye, as well as the average of both eyes.

5.3 Handling of Intercurrent Events of Estimand(s)

ICEs for the secondary endpoints will be conducted in a manner similar to the approach used for the primary estimand.

5.4 Handling of Missing Data Unrelated to Intercurrent Events

Similarly, intermittent missingness, which is assumed to be MAR under the treatment policy strategy, will be addressed using a MMRM analysis, consistent with the approach applied to the co-primary endpoints.



6 ANALYSES SUPPORTING SAFETY

There are no safety hypotheses planned in this study. All safety endpoints will be summarized descriptively based on the type of variable (e.g., continuous or categorical). The safety of Apraclonidine HCl 0.125% Ophthalmic Solution compared to vehicle will be assessed through a review of all safety parameters. All safety analyses will be conducted using the Safety population.

6.1 Safety Endpoints

The safety endpoints are:

- Extent of exposure
- Adverse events (AEs)
- Intraocular pressure (IOP)
- Biomicroscopy findings/Slit lamp examinations
- Dilated ophthalmoscopy
- Best-corrected visual acuity (BCVA) at distance
- Vital signs (blood pressure and pulse rate)
- Alertness (6 pt scale: fully alert, alert, lethargy,obtunded, stupor, coma)

[redacted]

6.2 Safety Hypotheses

There are no formal safety hypotheses in this study. The focus of the safety analysis will be a comprehensive descriptive assessment of safety endpoints listed in Section 6.1.

6.3 Statistical Methods for Safety Analyses

Except otherwise stated, the analysis set for all safety analyses is the safety analysis set as defined in Section 2.2. Baseline will be defined as the last measurement prior to exposure to study treatment, except otherwise stated.

6.3.1 Extent of Exposure

Extent of exposure to study treatment is calculated as duration of exposure to study treatment. Duration of exposure to study treatment is defined as the last day of exposure to

study treatment minus the first day of exposure to study treatment, plus 1 day. If the last day of exposure is unknown, the date of last contact with subject will be used instead. Extent of exposure will be summarized as a continuous measure (N, mean, median, standard deviation, minimum and maximum) overall and by treatment group using the Safety analysis set.

6.3.2 Adverse Events

All Adverse events will be coded using MedDRA dictionary, Version 27.0.

AEs, SAEs, and related definitions are in the study protocol. All AEs occurring from when a subject sign informed consent to when a subject exits the study will be accounted for in the reporting. A TEAE is an event not present prior to exposure to study treatment or any pre-existing event that worsens following exposure to study treatment. AEs recorded in the eCRF which began prior to treatment will not be included in the summary tables but will be included in the AE data listings.

An overall summary will be presented that includes the number of events and the number and percentage of subjects who experienced at least one TEAE by treatment group. This summary will also include breakdowns of TEAEs further categorized as adverse drug reactions, serious adverse drug reactions, TEAEs leading to treatment discontinuation, and TEAEs leading to death. The number of subjects with any TEAEs by maximum severity will be displayed.

Additional summaries of TEAEs will be provided showing the number and percentage of subjects who experienced at least one TEAE by treatment group and overall, for all subjects. TEAEs will be summarized using counts and percentages for the number of subjects with at least one event and counts for the number of events withing a category. In the summary, SOCs will be listed in ascending alphabetical order; PTs will be listed in order of descending frequency for all subjects within each SOC.

Separate summaries will be provided for the following categories of AEs:

- Ocular TEAEs
- Treatment-related ocular TEAEs
- Nonocular TEAEs
- Treatment-related nonocular TEAEs
- SAEs
- Treatment-related SAEs

- TEAEs leading to study treatment discontinuation
- TEAEs leading to death

All TEAEs will be presented in a subject listing. All pre-treatment AEs and AEs from screen failure subjects will be provided in a separate listing. Study duration is defined as the onset date minus the date of the first dose of the study treatment, plus one day.

Incomplete or missing AE start and end date will be handled as defined in Appendix 2.

6.3.3 Intraocular Pressure

Subjects' IOP will be assessed in each eye at Visits 1 (Day -7 to -1), 4 (Day 28), 5 (Day 56) and 6 (Day 63). Results will be taken from a single measurement and will be recorded in mmHg. The IOP values and changes from Visit 1 will be summarized using continuous descriptive statistics by visit and eye for each treatment group. A subject listing of IOP will also be produced.

6.3.4 Biomicroscopy Findings/Slit Lamp Examination

A slit-lamp examination will be performed at all visits to evaluate the anterior segment of each eye. Ocular signs will be captured according to the following grading criteria:

Assessment*	**Grading Scale**
Lids Erythema	0 (None), +0.5, +1 (Mild), +1.5, +2 (Moderate), +2.5, +3 (Severe), +3.5, +4 (Very Severe), Not Done
Lids Edema	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done
Conjunctiva Erythema/Hyperemia	0 (None), +0.5, +1 (Mild), +1.5, +2 (Moderate), +2.5, +3 (Severe), +3.5, +4 (Very Severe), Not Done
Chemosis	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done
Corneal Edema	0 (None), +0.5 (Mild), +1 (Moderate), +2 (Severe), +3 (Very Severe), Not Done
Corneal Erosion	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), Not Done
Endothelial Condition	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done
Lens Pathology	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done
Anterior Chamber Cells	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done

Assessment*	Grading Scale
Anterior Chamber Flare	0 (None), +1 (Mild), +2 (Moderate), +3 (Severe), +4 (Very Severe), Not Done

*: Assessments are same as CRF.

The results will be presented by the relevant grading scale pertinent to each biomicroscopy assessment as described above. The results will be summarized using counts and percentages for each treatment group and overall, for all subjects in the safety analysis set at each visit for each eye. Percentages will be based on the number of subjects in each treatment group.

For each assessment, a shift table showing slit lamp/biomicroscope grade at baseline relative to each post-baseline visit, relative to the last (exit) visit and relative to the visit representing worst change across all post-baseline visits will be presented by treatment group.

For each slit-lamp assessment, counts and percentages of subjects/eyes who/that experience an increase of ≥ 2 grades (e.g., from 0 to 2 or 1.5 to 3.5) from baseline to any post-baseline visit will be presented.

A listing will be provided which presents all subjects/eyes with an increase of ≥ 2 grades in any slit-lamp assessment at any visit compared to the grade of the same eye at baseline. The listing will include all slit-lamp data from all visits with the following variables: treatment, subject, age, sex, visit, eye, assessment, observed value, and change from baseline the visit.

6.3.5 Dilated Ophthalmoscopy

Dilated ophthalmoscopy will be performed by the investigator at Visit 1 (Day -7 to -1) and Visit 5 (Day 56) to evaluate the vitreous, retina, macula, choroid, optic nerve, and other. Eye regions will be graded as Normal or Abnormal. The results will be summarized using counts and percentages for each treatment group in the safety analysis set at each visit for each eye. Percentages will be based on the number of eyes at each applicable visit in each treatment group.

Shifts from normal to abnormal, abnormal to normal, or no change will also be summarized from baseline to Visit 5 (Day 56) using counts and percentages. A subject listing will be provided for abnormal dilated ophthalmoscopy.

6.3.6 Best Corrected Visual Acuity

Monocular visual acuity at distance is assessed by Snellen chart at each visit with manifest refraction.

The observed and change from baseline logarithm of the minimum angle of resolution (logMAR) BCVA will be summarized for each eye using continuous descriptive statistics by visit for each treatment group. A subject listing of manifest refraction including Sphere, Cylinder, and Axis will be produced.

Counts and percentages of subjects who experience pre-specified category of worst change from baseline (preinstillation on Day 1) across all post-baseline (any timepoints at any subsequent visits) assessments and category of change from baseline to last on-treatment BCVA assessment (logMAR) will be presented according to the following categories: ≤ 0, >0 to ≤ 0.09, ≥ 0.1 to ≤ 0.19, ≥ 0.2 to ≤ 0.29 and ≥ 0.3. For change to any visit, a subject will be counted only in the category that represents their worst change from baseline across all post-baseline assessments.

A listing will be provided which presents all subjects with a ≥ 0.2 logMAR increase in BCVA from baseline to any visit [Visits 3 (Day 14) - 6 (Day 63)]. The listing will include the following variables: treatment, subject ID, age, sex, visit, eye, observed value, change from baseline value and maximum change from baseline.

6.3.7 Full Physical Examination

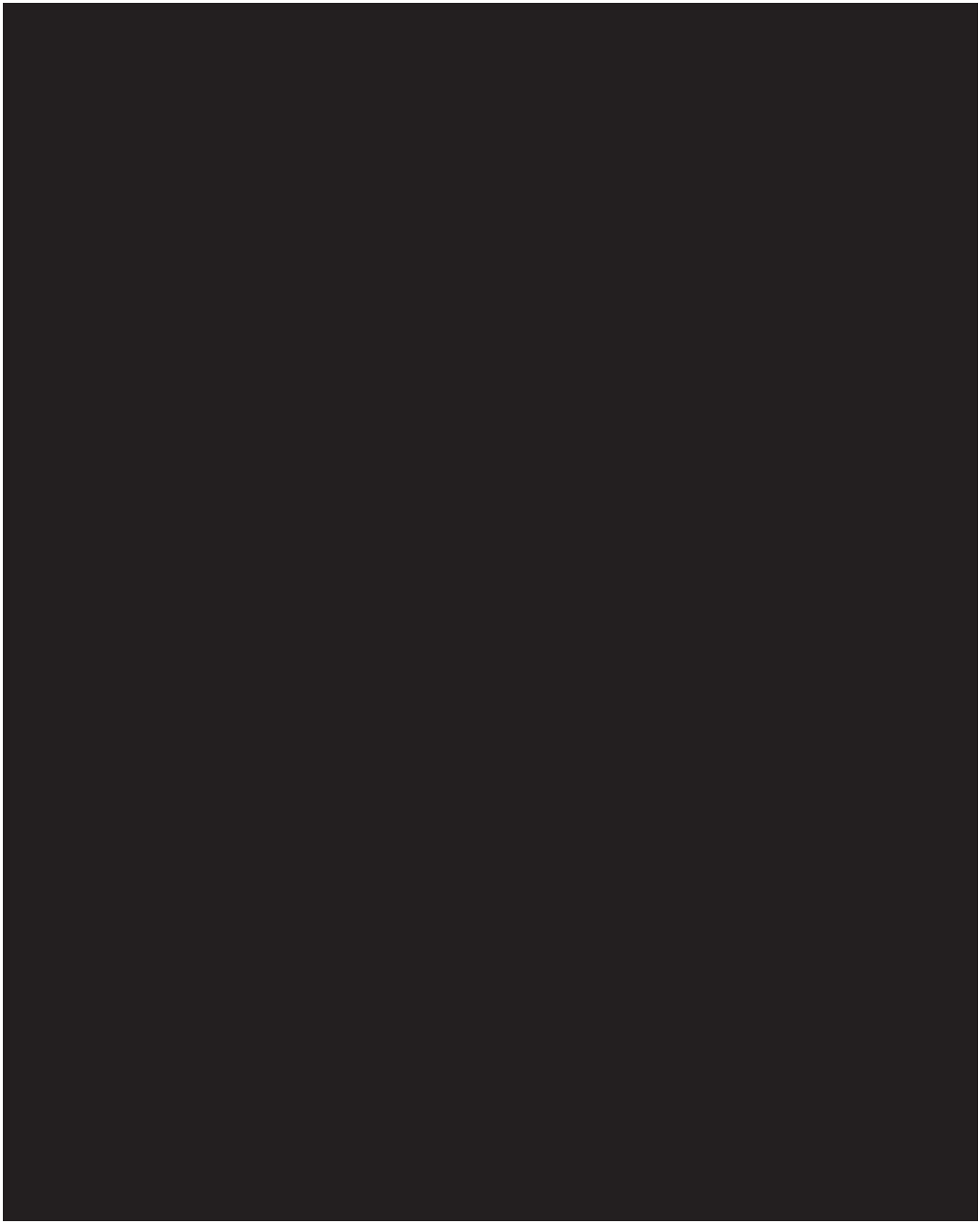
No specific parameters from the physical examination will be collected. Medical history will be recorded at the baseline visit, and new findings will be reported as adverse events. All data will be summarized in the report.

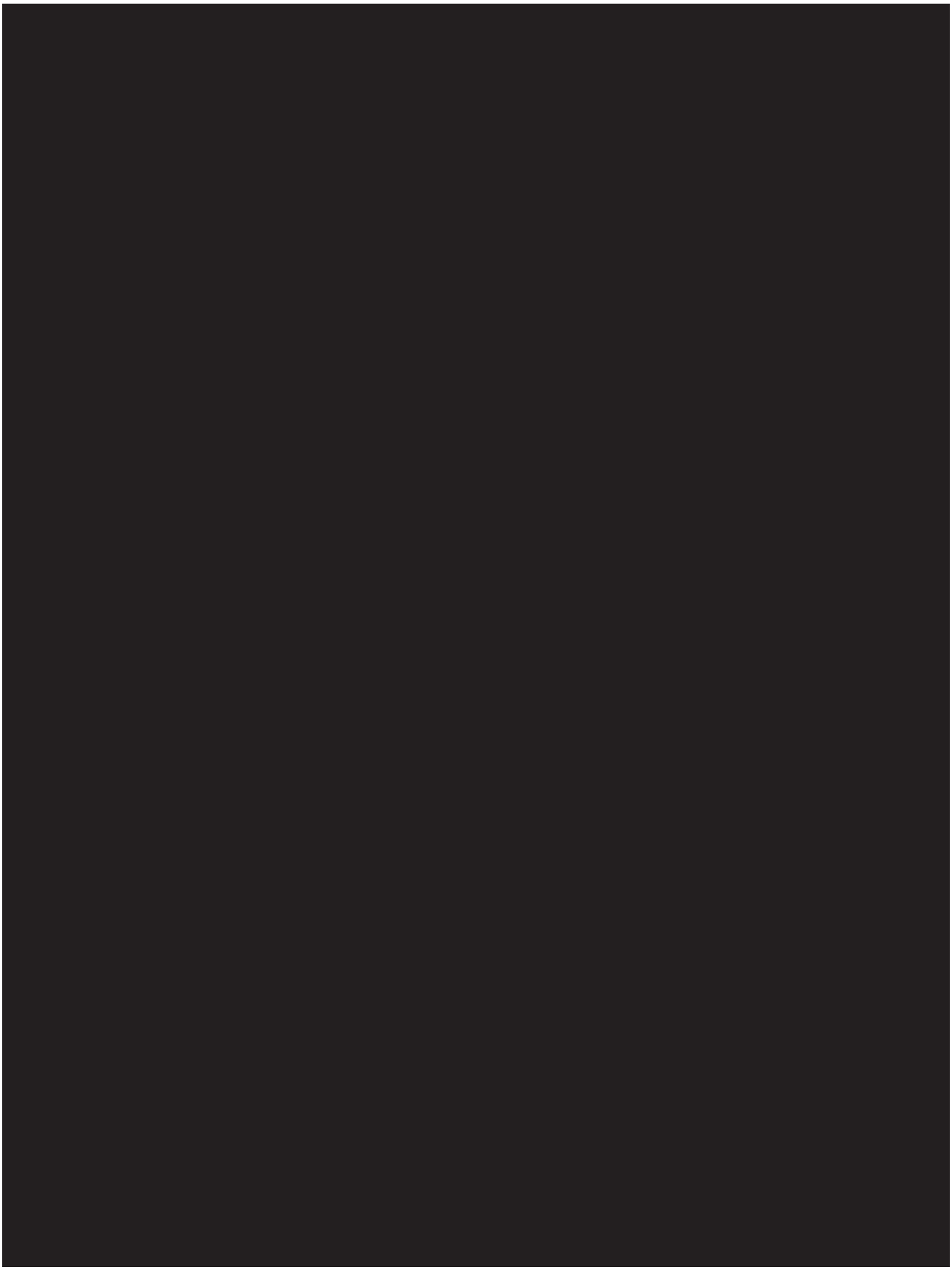
6.3.8 Vital Signs

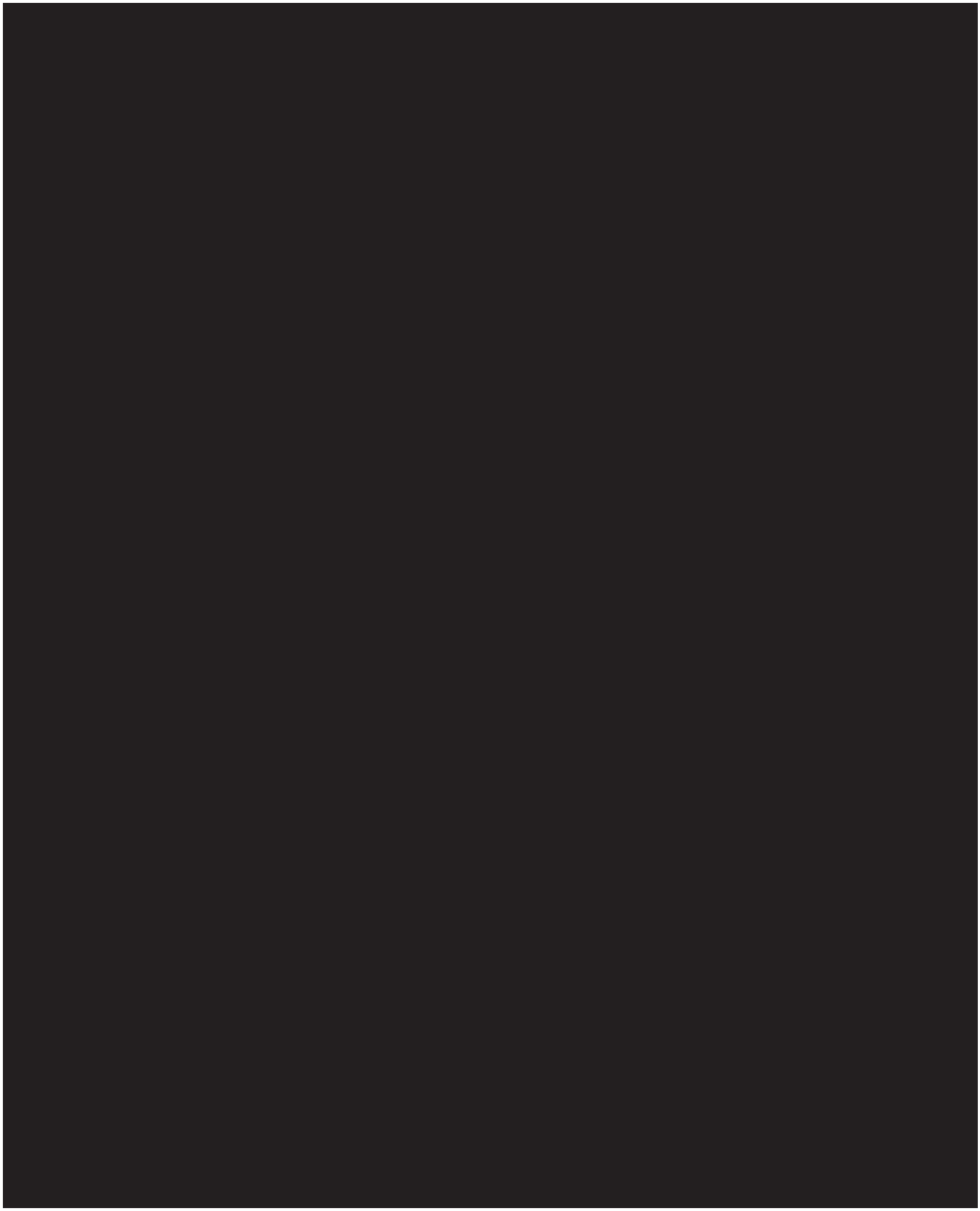
Resting blood pressure and pulse rate will be measured at all visits except for exit visit (Day 63, Visit 6). Descriptive summaries (N, mean, median, standard deviation, minimum and maximum) of observed values and change from baseline (preinstillation on Day 1) at each visit will be presented. Additionally, for each measure, a worst-case low and worst-case high post-baseline value and change from baseline value will be presented. Vital signs outside of the protocol-defined range will be displayed in a subject data listing. Blood pressure outside of the protocol-defined range is defined as ≤ 90 or ≥ 160 mmHg (systolic) or ≤ 60 or ≥ 100 mmHg (diastolic). Pulse rate at rest outside of the protocol-defined range is defined as < 60 or > 90 bpm.

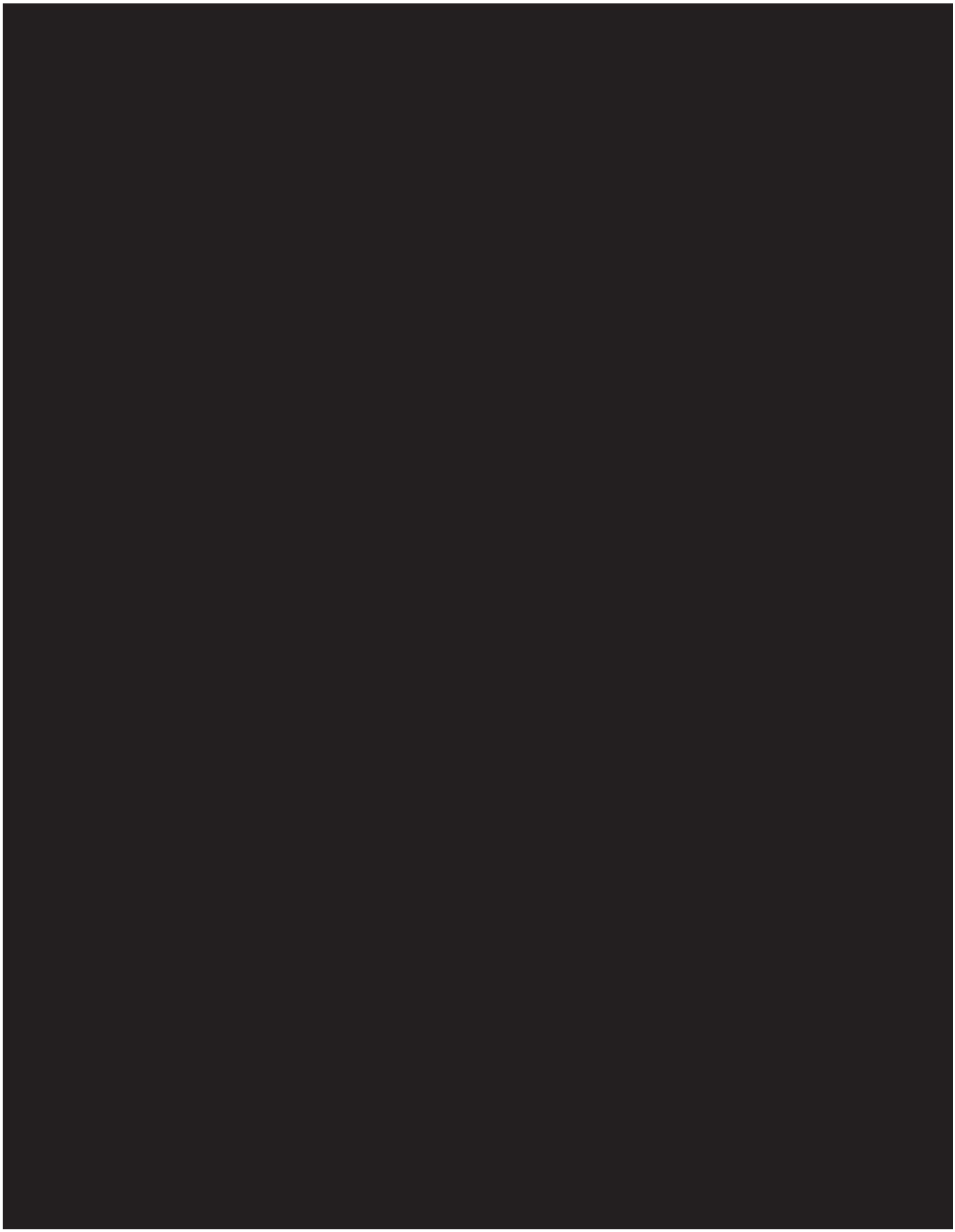
6.3.9 Alertness

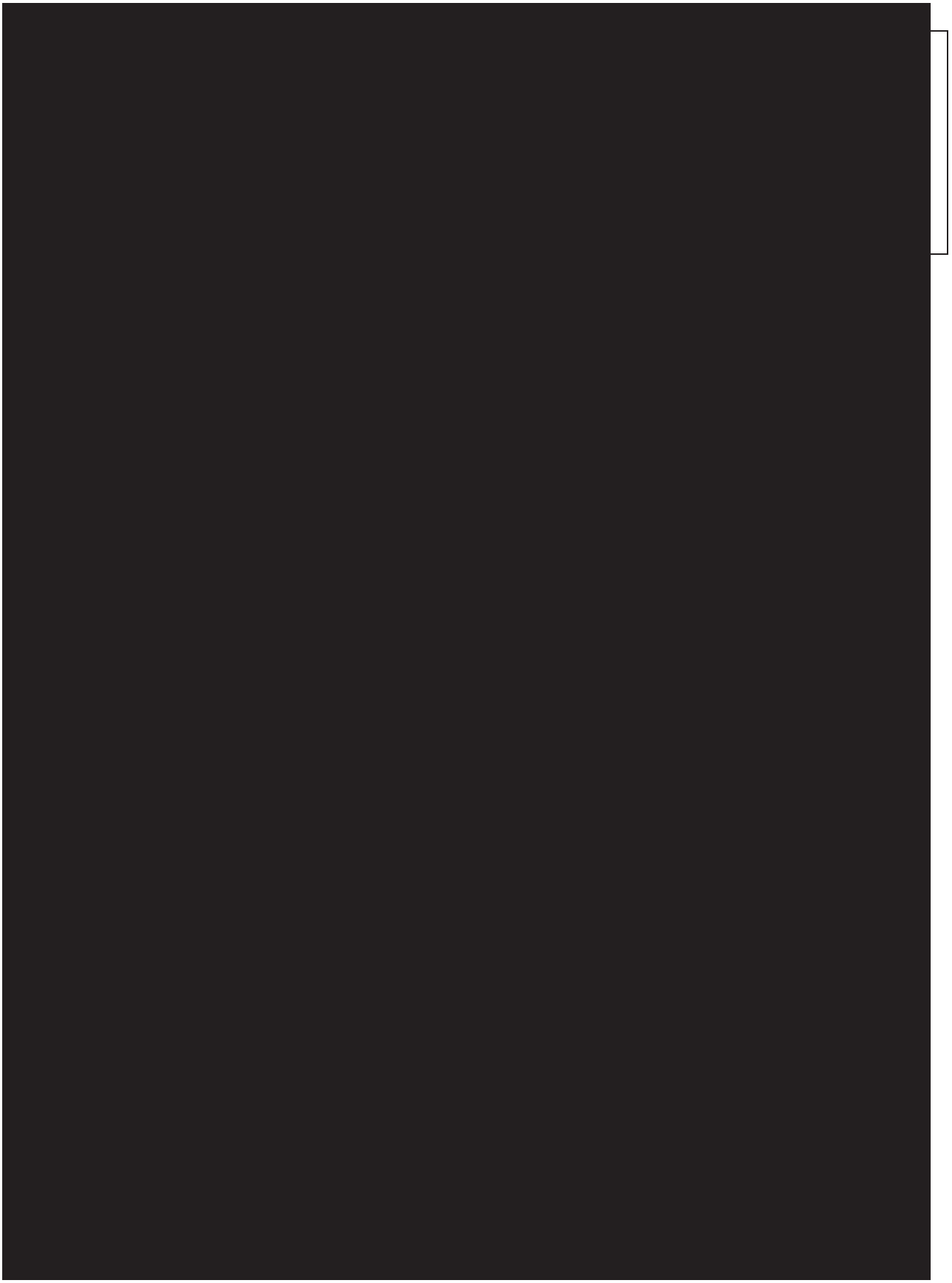
In-office alertness assessments will be summarized with counts and percentages by visit/day and treatment group. Subject listings of the alertness assessments will be produced.

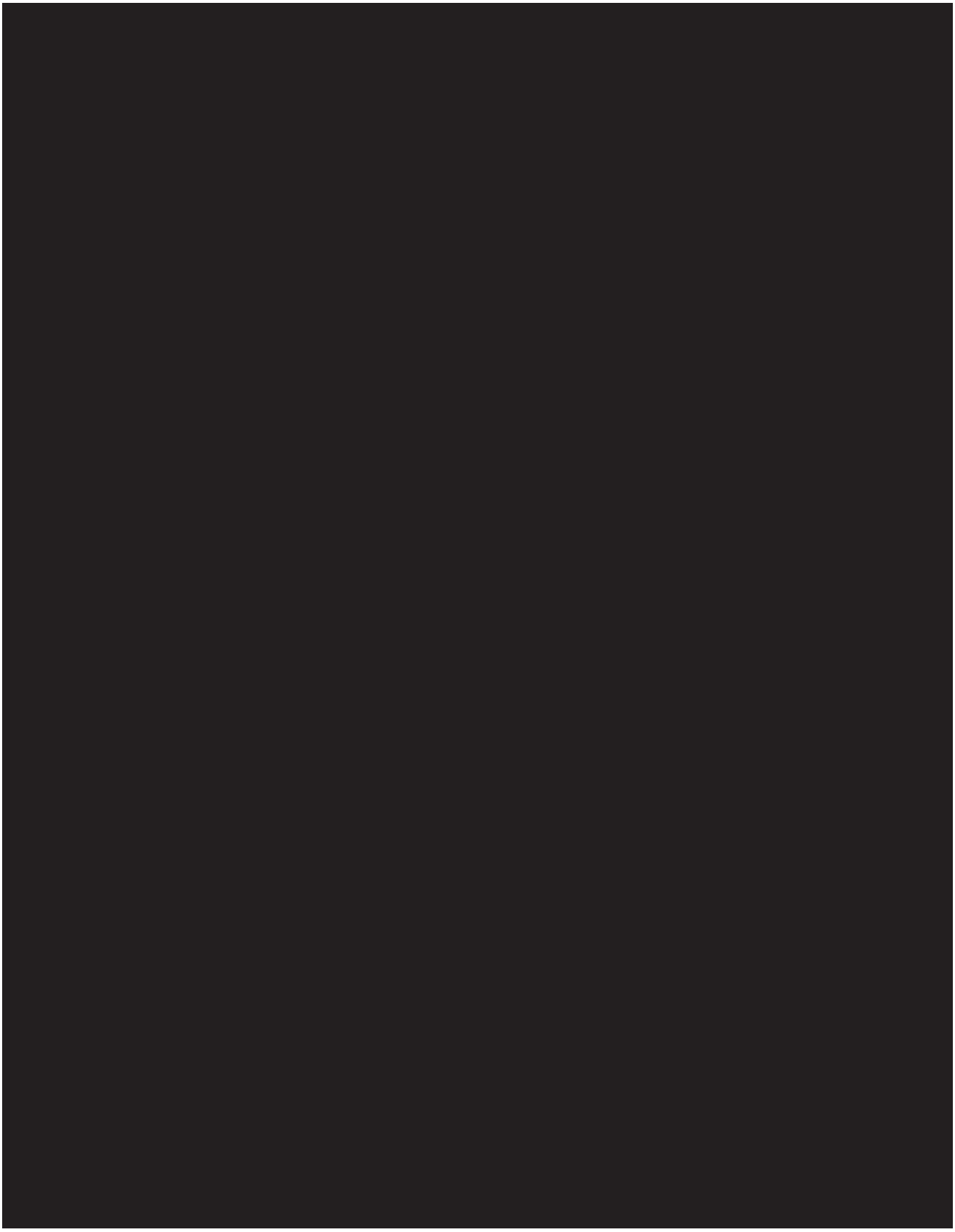


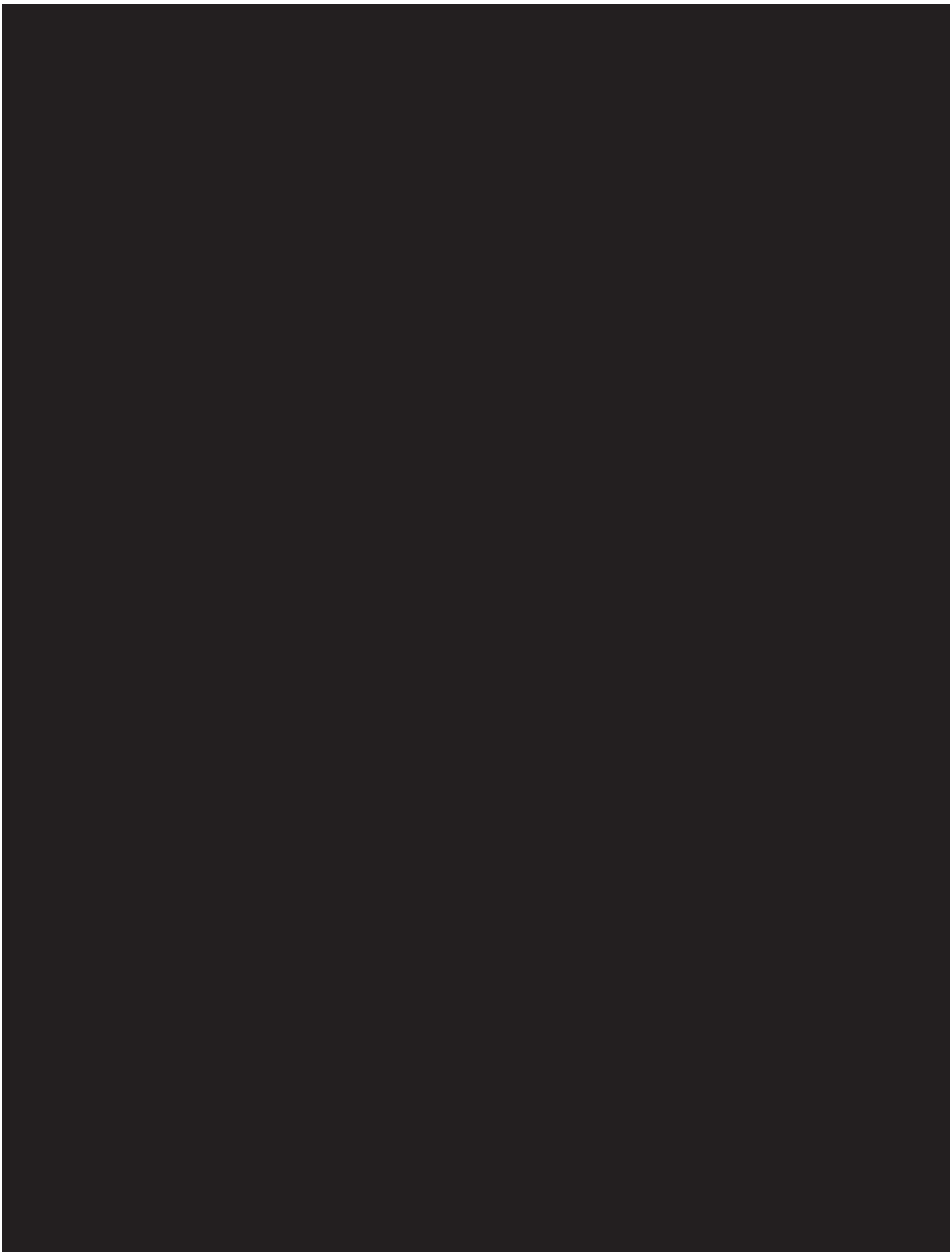










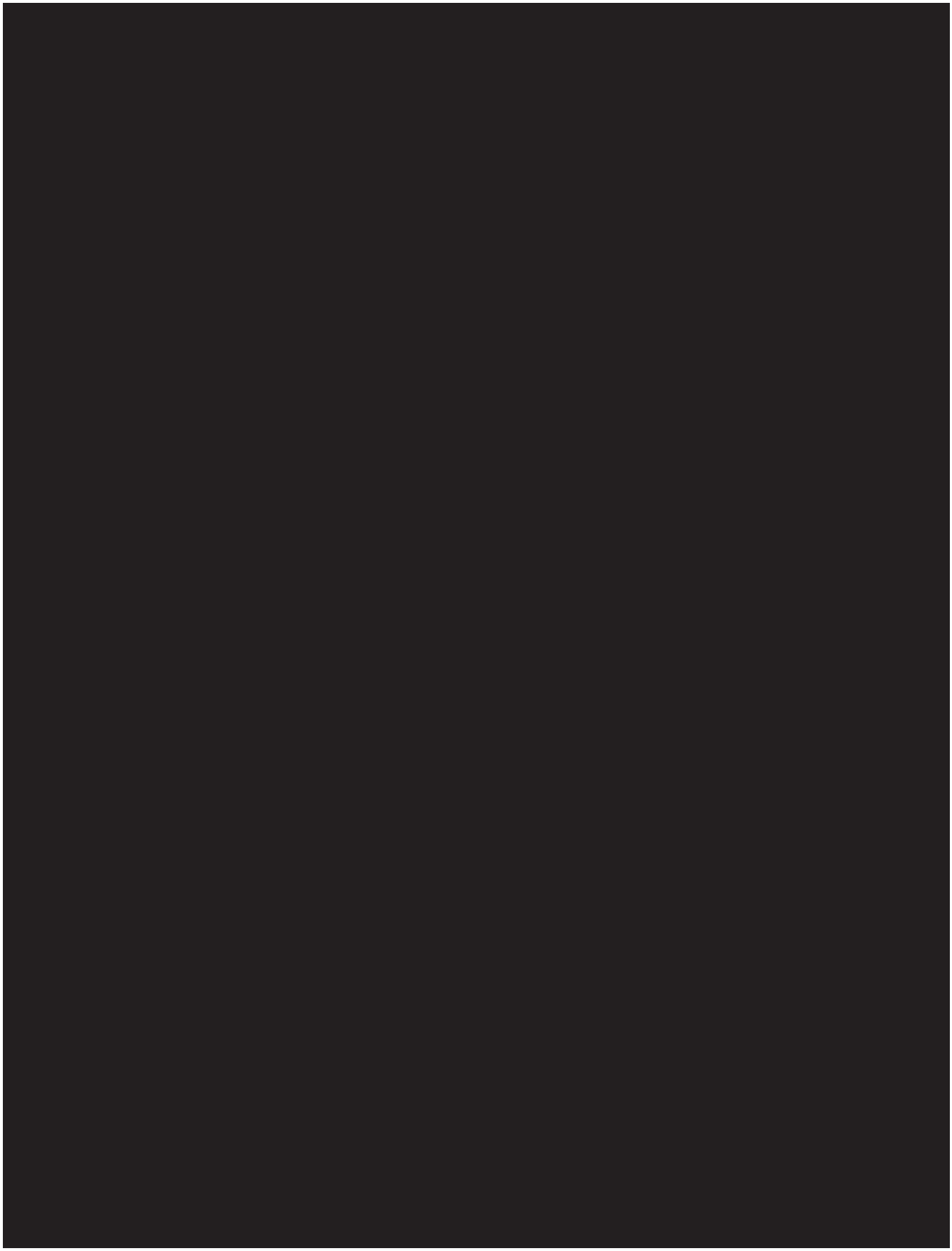




8.2 Interim Analysis

There is no interim analysis planned for this study.





[redacted]

8.4 Sample Size and Power Calculations

The study will randomize 120 subjects between Apraclonidine HCl Ophthalmic Solution 0.125% and vehicle in a 1:1 ratio, targeting 102 evaluable subjects. The total number of randomized subjects has been adjusted to account for dropout rate, early termination due to cause, and evaluability (15%).

[redacted]

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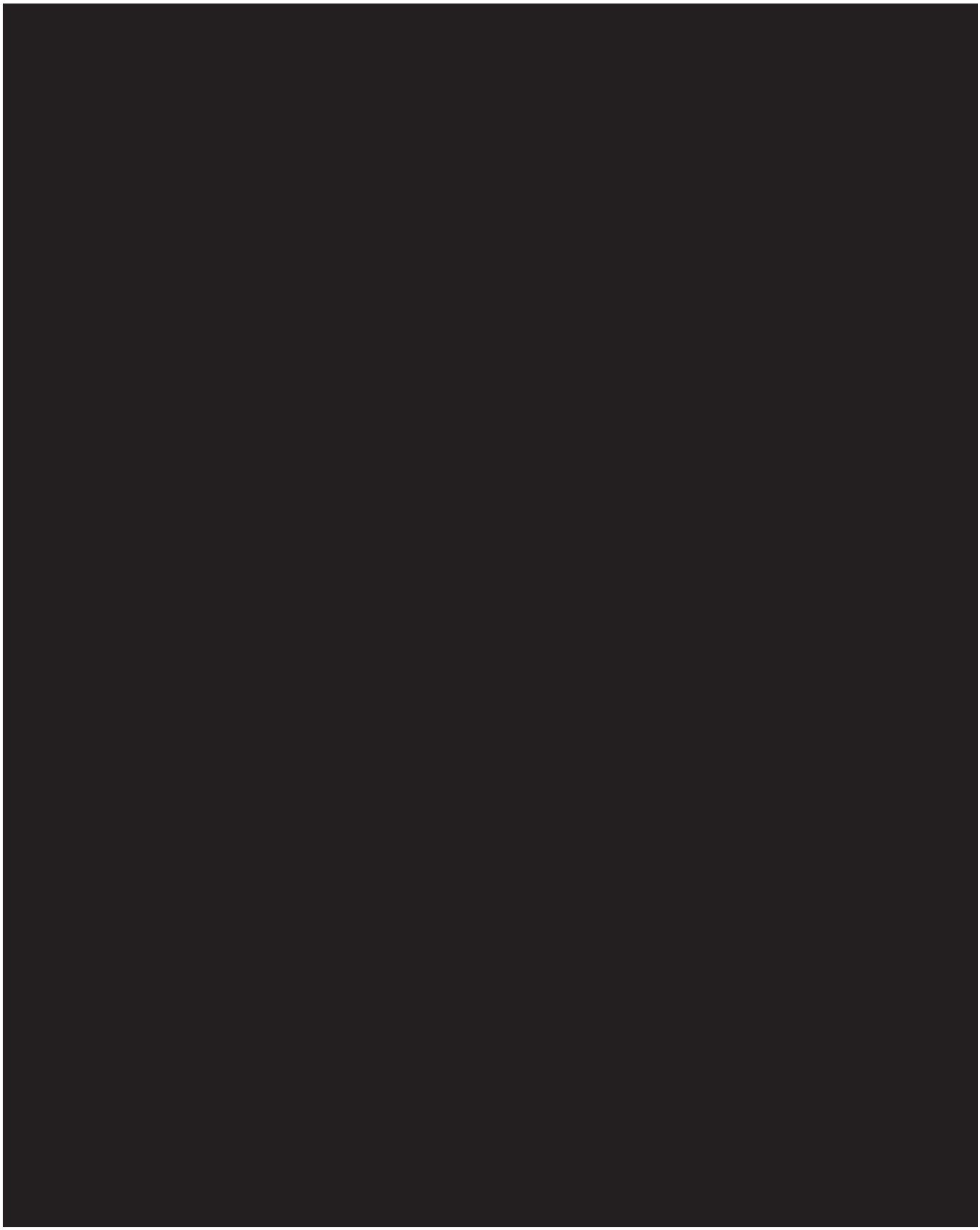
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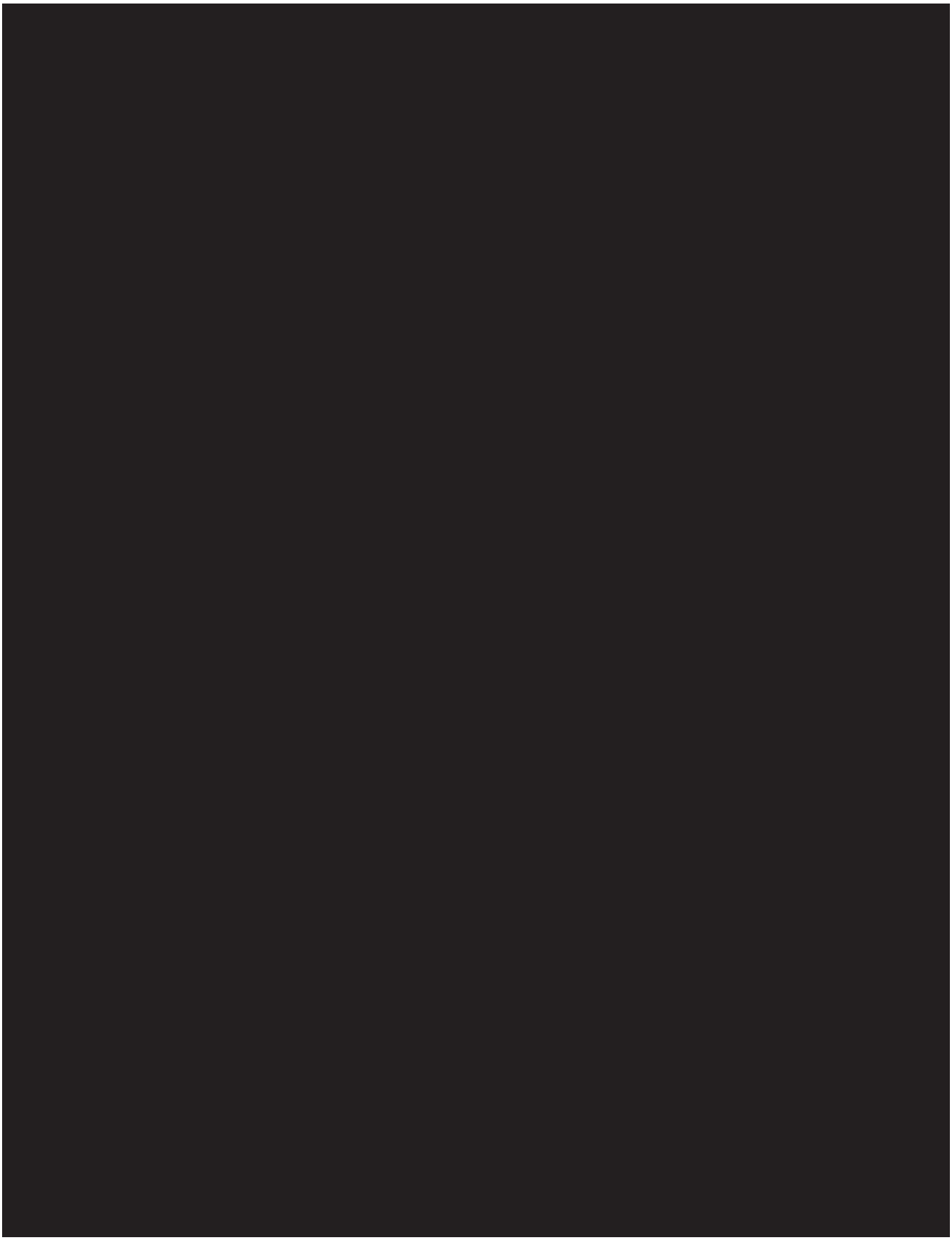
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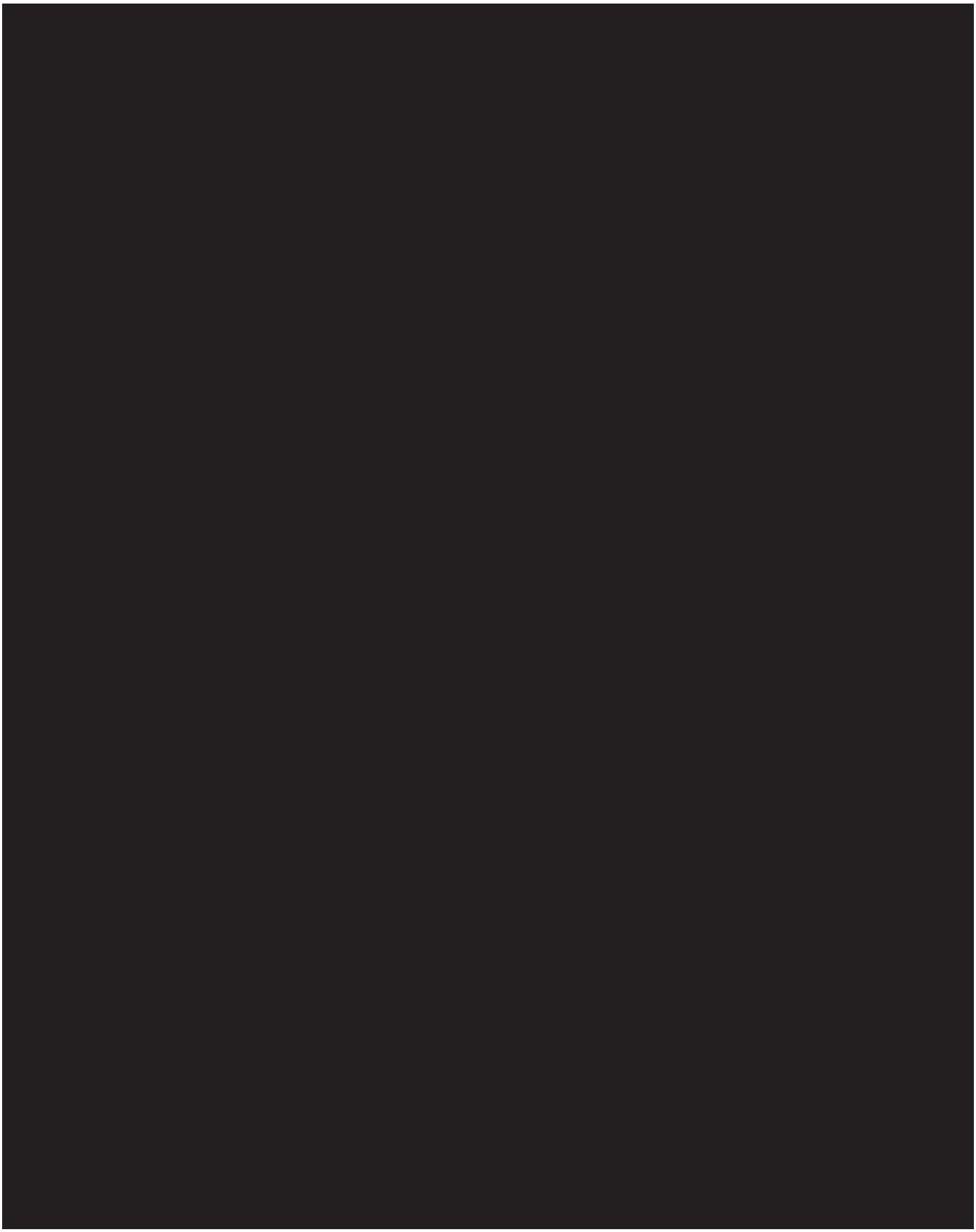
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11 Appendix

Appendix 1: Schedule of Study Procedures and Assessments

Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 / Early Exit	Visit 6 / Exit
Study Day and Visit Window	**Day -7 to -1**	**Day 1**	**Day 14 ± 2**	**Day 28 ± 2**	**Day 56 ± 2**	**Day 63 + 1**
Informed Consent/Enrollment	X					
Demographics	X					
Medical and Medication History	X					
Medical and Medication History Update		X	X	X	X	X
Resting Blood Pressure and Pulse	X	X[3]	X	X	X	
Full Physical Examination	X				X	
Urine Pregnancy Test[2]	X	X			X	
BCVA with Manifest Refraction utilizing Snellen Chart [≠]	X	X	X	X	X	X
Slit Lamp Biomicroscopy	X	X[3]	X	X	X	X
Intraocular Pressure	X			X	X	X
Dilated Ophthalmoscopy	X				X	
Review of Inclusion/Exclusion Criteria	X	X				
Randomization		X				
In-Office IP Instillation		X	X	X	X	
In-Office Alertness Evaluation[4]		X	X	X	X	
Adverse Events	X	X	X	X	X	X
Investigator-Assessed Ocular Redness		X[5]	X[6]	X[6]	X[6]	X

[redacted]

Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5 / Early Exit	Visit 6 / Exit
Study Day and Visit Window	**Day -7 to -1**	**Day 1**	**Day 14 ± 2**	**Day 28 ± 2**	**Day 56 ± 2**	**Day 63 + 1**
IP Dispensation for At-Home Dosing		X		X		
Dosing Compliance Review			X	X	X	
IP Collection (used and unused)				X	X	
[redacted]						
Study Exit						X

[2] For females of childbearing potential.

[3] Assessments to be completed predose and following the 12 hours (720 minutes) redness assessment.

[4] In-Office Alertness Evaluation will be performed predose and postdose at Visit 2 (12 hours postinstillation), and will be performed postdose at Visits 3, 4, and 5.

[5] Investigator-Assessed Ocular Redness assessments to be completed predose, and 1 (+0.5), 5 (+1), 15 (+1), 60 (+10), 240 (+15), 480 (+15), 600 (+15), and 720 (+15) minutes postinstillation in-office.

[6] Investigator-Assessed Ocular Redness assessments to be completed pre-dose, and 1 (+0.5), 5 (+1), and 1 5(+1) minutes postinstillation in-office.

[redacted]

≠ Visits 2 to 5: BCVA to be performed pre- and postinstillation. BCVA will be assessed 12 hours postinstillation on Day 1 (Visit 2).

Appendix 2: Handling Incomplete or Missing Dates

Partial/missing start and end dates for AEs and concomitant medications will be imputed as follows:

Partial/missing start date:

- Dates with missing day only will be imputed as the 1st of the month unless the month and year are same as the month and year of first dose of study medication, in which case missing day will be imputed as the first dose day of study medication. Additionally, if month and year of start date, first dose date of study medication and end date are same then start day will be imputed as earliest of first dose date of study medication or end date.
- Dates with both day and month missing will be imputed as 1 Jan unless the year is same as the year of first dose of study medication, in which case missing day and month will be imputed as the first dose day and month of study medication only if end date is after first dose of study medication. Additionally, if year of start date is earlier than year of first dose date of study medication and end date is later than first dose date of study medication then day and month will be imputed as last day and month of the year.
- Completely missing dates will be imputed as the first dose date of study medication unless the end date is on or before the first dose date of study medication, in which case missing date will be imputed as 1 Jan of the same year as the end date.

Partial/missing end date:

- Dates with missing day only will be imputed as the last day of the month unless the month and year are the same as the month and year of the last dose of study medication, in which case missing day will be imputed as the last dose day of study medication.
- Dates with both day and month missing will be imputed as 31 Dec unless the year is same as the year of the last dose of study medication, in which case missing day and month will be imputed as the last dose day and month of study medication.
- If the ongoing flag is missing or “Yes” then the date will not be imputed unless death date is available, in which case the missing date will be imputed as the death date. If ongoing is “No” then the missing end date will be imputed as the last dose date.
- If the imputed date is after the date of death, then the end date will be set equal to the date of death.

If imputed AE/CM start date and end date are same, then duration will be set to 24 hours.

The original dates will be displayed in data listings and the imputed dates will be used in derivations only (study day, treatment-emergence status, etc.).

Appendix 3: Statistical and Programming Specifications

Statistical and Programming Specifications for MMRM Analysis of Apraclonidine HCl 0.125% Efficacy on Day 1 Redness Score

Model Overview

To evaluate the efficacy of Apraclonidine HCl 0.125% Ophthalmic Solution compared to vehicle, we will use the following mixed model repeated measures (MMRM) model. We are modeling the change in redness score (CHG_{ijk}) for each subject i, eye j, at time point k. The model incorporates fixed effects to control for baseline characteristics and site-specific factors, and it accounts for the repeated measurements within subjects over eyes and time points using a specific covariance structure.

Statistical Model Specification

$$\begin{aligned} CHG_{ijk} = \quad & \alpha + \beta_1 \cdot \text{BASE}_{ij} + \beta_2 \cdot \text{SITEID}_{102} + \beta_3 \cdot \text{SITEID}_{103} + \beta_4 \cdot \text{SITEID}_{104} + \beta_5 \cdot \text{SITEID}_{105} \\ & + \beta_6 \cdot \text{TRT}_{\text{HCl 0.125\%}} + \beta_7 \cdot \text{TIME}_5 + \beta_8 \cdot \text{TIME}_{15} + \beta_9 \cdot \text{TIME}_{60} + \beta_{10} \cdot \text{TIME}_{240} \\ & + \beta_{11} \cdot \text{TIME}_{480} + \beta_{12} \cdot \text{TIME}_{600} + \beta_{13} \cdot \text{TIME}_{720} \\ & + \beta_{14} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_5) + \beta_{15} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{15}) \\ & + \beta_{16} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{60}) + \beta_{17} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{240}) \\ & + \beta_{18} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{480}) + \beta_{19} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{600}) \\ & + \beta_{20} \cdot (\text{TRT}_{\text{HCl 0.125\%}} \times \text{TIME}_{720}) + \epsilon_{ijk} \end{aligned}$$

Where:

- CHG_{ijk}: Change from baseline (preinstallation) redness score for subject i, eye j, at time k.
- α: Overall intercept.
- β_1: Coefficient for baseline (preinstallation) redness score BASE_{ij}.
- β_2 to β_5: Coefficients for site indicators SITEID_{102} to SITEID_{105} (with SITEID_{101} as the reference).
- β_6: Coefficient for treatment indicator $\text{TRT}_{\text{HCl 0.125\%}}$ $\text{TRT}_i = \begin{cases} 0 & \text{if Vehicle} \\ 1 & \text{if Apraclonidine} \end{cases}$.
- β_7 to β_{13}: Coefficients for time point indicators TIME_5 to TIME_{720} (with TIME_1 as the reference).
- β_{14} to β_{20}: Coefficients for interaction terms between treatment and time.
- ϵ_{ijk}: Residual error term.

Variance-Covariance Structure

The residual errors ϵ_{ijk} are assumed to follow a multivariate normal distribution with a mean of zero and a covariance structure that accounts for repeated measures over eyes and time within each subject Specifically:

$$\epsilon_i = \begin{bmatrix} \epsilon_{i1,1} \\ \epsilon_{i1,2} \\ \\ \epsilon_{i1,K} \\ \epsilon_{i2,1} \\ \epsilon_{i2,2} \\ \\ \epsilon_{i2,K} \end{bmatrix} \sim MVN(0, \Sigma_{\text{EYE}} \otimes \Sigma_{\text{TIME}})$$

Where:

- ϵ_i: Vector of residuals for subject i across both eyes ($j = $ OD,OS) and all time points ($k \in \{1,5,15,60,240,480,600,720\}$), resulting in a 16×1 vector.
- $\mathbf{\Sigma}_{\text{EYE}}$: 2×2 unstructured covariance matrix between eyes.
- $\mathbf{\Sigma}_{\text{TIME}}$: 8×8 unstructured covariance matrix across time points.
- $\otimes$: Kronecker product (combines eye-level and time-level covariance structures).

Eye Covariance Matrix ($\mathbf{\Sigma}_{\text{EYE}}$):

$$\mathbf{\Sigma}_{\text{EYE}} = \begin{bmatrix} \sigma^2_{\text{OD}} & \sigma_{\text{OD,OS}} \\ \sigma_{\text{OD,OS}} & \sigma^2_{\text{OS}} \end{bmatrix}$$

- Explanation: Captures the variance (σ^2_{OD}, σ^2_{OS}) and covariance ($\sigma_{\text{OD,OS}}$) between the right eye (OD) and the left eye (OS) within the same subject.

Time Covariance Matrix ($\mathbf{\Sigma}_{\text{TIME}}$):

$$\mathbf{\Sigma}_{\text{TIME}} = \begin{bmatrix} \sigma^2_1 & \sigma_{1,5} & \sigma_{1,15} & \sigma_{1,60} & \sigma_{1,240} & \sigma_{1,480} & \sigma_{1,600} & \sigma_{1,720} \\ \sigma_{1,5} & \sigma^2_5 & \sigma_{5,15} & \sigma_{5,60} & \sigma_{5,240} & \sigma_{5,480} & \sigma_{5,600} & \sigma_{5,720} \\ \sigma_{1,15} & \sigma_{5,15} & \sigma^2_{15} & \sigma_{15,60} & \sigma_{15,240} & \sigma_{15,480} & \sigma_{15,600} & \sigma_{15,720} \\ \sigma_{1,60} & \sigma_{5,60} & \sigma_{15,60} & \sigma^2_{60} & \sigma_{60,240} & \sigma_{60,480} & \sigma_{60,600} & \sigma_{60,720} \\ \sigma_{1,240} & \sigma_{5,240} & \sigma_{15,240} & \sigma_{60,240} & \sigma^2_{240} & \sigma_{240,480} & \sigma_{240,600} & \sigma_{240,720} \\ \sigma_{1,480} & \sigma_{5,480} & \sigma_{15,480} & \sigma_{60,480} & \sigma_{240,480} & \sigma^2_{480} & \sigma_{480,600} & \sigma_{480,720} \\ \sigma_{1,600} & \sigma_{5,600} & \sigma_{15,600} & \sigma_{60,600} & \sigma_{240,600} & \sigma_{480,600} & \sigma^2_{600} & \sigma_{600,720} \\ \sigma_{1,720} & \sigma_{5,720} & \sigma_{15,720} & \sigma_{60,720} & \sigma_{240,720} & \sigma_{480,720} & \sigma_{600,720} & \sigma^2_{720} \end{bmatrix}$$

- Explanation: Captures the variance (σ^2_t) at each time point and the covariance ($\sigma_{t,t'}$) between different time points within the same eye.

Combined Covariance Structure:

The overall covariance matrix $\mathbf{\Sigma}$ for the residuals within each subject i is constructed using the Kronecker product:

$$\mathbf{\Sigma} = \mathbf{\Sigma}_{\text{EYE}} \otimes \mathbf{\Sigma}_{\text{TIME}}$$

- This results in a 16×16 covariance matrix that models the covariance between all pairs of observations for subject i, considering both eye and time correlations.

- Interpretation: The Kronecker product allows us to capture the interaction between the eye and time covariance structures, providing a comprehensive model of the residual variability.

Attributes of the UN@UN Covariance Structure:

The UN@UN covariance matrix offers a framework for modeling the complex correlation structures in bilateral eye data with repeated measures over time. It allows for different variances across time points and between eyes, meaning that variability can differ not only between the right and left eyes but also at each assessed time point.

This structure accommodates inter-eye correlation by allowing a unique covariance between the two eyes, assumed constant across all time points, reflecting the natural correlation between a subject's eyes. It models longitudinal correlations by permitting varying correlations between different pairs of time points, which are the same for both eyes, capturing changes in relationships over time.

Additionally, the UN@UN covariance matrix captures cross-correlations between measurements from different eyes at different time points, accurately reflecting the interconnectedness of the data arising from bilateral assessments and repeated measurements.

Covariance Between Residuals:

For any two observations within the same subject i, corresponding to eye j at time k and eye j' at time k', the covariance between the residual errors is given by:

$$\text{Cov}(\epsilon_{ijk}, \epsilon_{ij'k'}) = \Sigma_{\text{EYE}}(j, j') \times \Sigma_{\text{TIME}}(k, k')$$

- Where:
    - $\Sigma_{\text{EYE}}(j, j')$ is the element in the eye covariance matrix corresponding to eyes j and j'.
    - $\Sigma_{\text{TIME}}(k, k')$ is the element in the time covariance matrix corresponding to time points k and k'.

Assumptions:

The model assumes that the residuals ϵ_{ijk} are independent across different subjects i. Within each subject, the residuals are correlated according to the specified covariance structure. All residuals are assumed to be normally distributed with mean zero. The Kronecker product covariance structure effectively captures the correlations due to repeated measures over eyes and time points within subjects.

Statistical Methodology:

- **Estimation Method:** The model will be estimated using Restricted Maximum Likelihood (REML).
- **Degrees of Freedom:** Approximated using the Kenward-Roger method.
- **Covariance Structure Selection:**
    - **Primary Choice:** UN@UN Unstructured covariance for both eyes and time.
    - **Alternative Choices:** If convergence issues occur, alternative structures like If convergence is still not achieved, UN@AR1(unstructured for eyes and autoregressive

for time) and UN@CS (unstructured for eyes and compound symmetry for time) will be considered sequentially.

- **Hypothesis Testing:** Hypotheses for the co-primary endpoints at 15 minutes and 600 minutes post-instillation will be tested using the least squares means (LSMeans) of the treatment-by-time interaction, comparing Apraclonidine HCl 0.125% to vehicle.

The purpose of this model is to assess the effect of the treatment over time on the change in redness score, while adjusting for baseline redness and site-specific effects. By explicitly modeling the covariance structure over eyes and time points, the model appropriately accounts for the complex correlation inherent in bilateral assessments and repeated measures over time. This detailed formulation ensures valid statistical inference of the treatment effects and their interaction with time for the co-primary, secondary, and exploratory efficacy endpoints, thereby providing insights into the efficacy of Apraclonidine HCl 0.125% Ophthalmic Solution compared to vehicle across different time points.



