NCT 05815758

STATISTICAL ANALYSIS PLAN

A Multi-Center, Double-Masked, Randomized, Vehicle-Controlled, Parallel-Group Study Evaluating the Safety and Pharmacokinetics of Brimonidine Tartrate 0.025%/Ketotifen Fumarate 0.035% Combination Ophthalmic Solution, Used Two Times Daily in Healthy Adult Subjects and in Pediatric Subjects with a History or Family History of Atopic Disease (including Allergic Conjunctivitis)

Sponsor: Bausch & Lomb Incorporated
400 Somerset Corporate Boulevard
Bridgewater, NJ 08807

Protocol Number: 913/22-100-0006

Author: [redacted]
Statistics & Data Corporation

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Statistical Analysis Plan Approval

Prepared by: [redacted]

Reviewed by: [redacted]

Approved by: [redacted]

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List of Abbreviations

Abbreviation	Definition
ADaM	Analysis Data Model
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
AUC_{0-t}	Area under the Plasma Drug Concentration-Time Curve from Time 0 to the last quantifiable Drug Concentration
AUC_{0-4h}	Area under the Plasma Drug Concentration-Time Curve from Time 0 through 4 h
BCVA	Best-Corrected Visual Acuity
BID	*Bis in die* (Twice Daily)
BLQ	Below the Limit of Quantitation
Combo	Brimonidine Tartrate 0.025%/Ketotifen Fumarate 0.035% Combination Ophthalmic Solution
CI	Confidence Interval
C_{max}	Maximum Observed Plasma Concentration
C_{min}	Minimum Observed Plasma Concentration
CS	Clinically Significant
CV	Coefficient of Variation
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ETDRS	Early Treatment of Diabetic Retinopathy Study
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IOP	Intraocular Pressure
logMAR	Logarithm of the Minimum Angle of Resolution
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not Clinically Significant
PDF	Portable Document Format
PK	Pharmacokinetic(s)
PT	Preferred Term
RTF	Rich Text Format
R_{AUC}	Accumulation Ratio of AUC_{0-t} at Steady-State to AUC_{0-t} after the First Dose
R_{Cmax}	Accumulation Ratio of C_{max} at Steady-State to C_{max} after the First Dose
RTSM	Randomization and Trial Supply Management
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDC	Statistics & Data Corporation
SDTM	Study Data Tabulation Model
SEM	Standard Error of the Mean
SOC	System Organ Class

Abbreviation	Definition
TEAE	Treatment-Emergent Adverse Event
T_{max}	Time of Maximum Observed Plasma Concentration
WHODrug	World Health Organization Drug Dictionary

1. Introduction

The purpose of this statistical analysis plan (SAP) is to describe in detail the planned analyses and reporting for protocol 913/22-100-0006, Amendment 1.0 dated 07JUL2023. The statistical analysis methods presented in this document will supersede the statistical analysis methods described in the clinical protocol. If additional analyses are required to supplement the planned analyses described in this SAP, they may be completed and will be identified in the clinical study report.

This SAP is being written with due consideration of the recommendations outlined in the most recent International Conference on Harmonisation (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports.

2. Study Objectives

The primary objective is to compare the safety and tolerability of brimonidine tartrate 0.025%/ketotifen fumarate 0.035% combination ophthalmic solution (Combo) versus its vehicle in healthy adult subjects and in pediatric subjects with a history or family history of atopic disease (including allergic conjunctivitis).

The secondary objective is to characterize the plasma PK of Combo following a single dose and 22-day twice daily (BID) topical ocular dosing in a subset of healthy adult subjects.

2.1 Study Variables

There are no efficacy variables or endpoints in this Phase 3 safety study.

2.2 Safety Variables

The variables to assess safety are the following:

- Adverse events (AEs; reported, elicited, and observed)
- Urine Pregnancy Test
- Best-Corrected Visual Acuity (BCVA) using an Early Treatment of Diabetic Retinopathy Study (ETDRS) chart
- Slit Lamp Biomicroscopy
- Intraocular Pressure (IOP; to be performed on subjects ≥ 10 years age)
- Physical Examination
- Vital Signs
- Dilated Fundus Examination.

2.3 Tolerability Variables

Tolerability measures will be assessed by drop comfort assessment.

2.4 Pharmacokinetic Variables

The variables for PK analysis will be blood samples collected from the PK population at pre-dose, 0.25 (±3 min), 0.5 (±5 min), 1 (±10 min), 2 (±10 min), and 4 (±20 min) hours following the first topical ocular instillation of Combo or vehicle on Day 1 and also on Day 22 to measure the plasma concentrations of brimonidine and ketotifen.

If the data allow, the following PK parameters will be estimated: C_{max}, C_{min}, T_{max}, AUC_{0-t}, AUC_{0-4h}, and accumulation ratio (R_{Cmax} and R_{AUC}).

3. Study Design and Procedures

3.1 General Study Design

This is a multi-center, double-masked, randomized, vehicle-controlled, parallel-group, safety study. Approximately 600 subjects will be screened in order to enroll approximately 501 subjects from approximately 6 sites. Of the subject total, approximately 100 pediatric subjects are anticipated to be enrolled into this study.

The age classification for the pediatric population will be approximately 50 subjects in each of the following groups:

- 5-12 years old
- 13-17 years old

Subjects will not be stratified by age group when assigned to investigational product.

A subset of up to 25 healthy adult subjects (~16 subjects for the investigational product and ~9 subjects for the vehicle) will undergo PK blood draws to assess the systemic exposure of brimonidine and ketotifen.

The subject, the subject's caregiver, or the subject's parent/legal guardian (if applicable, for subjects less than 18 years of age) will instill 1 drop of the assigned investigational drug in each eye BID approximately 7.5 hours apart for up to 6 consecutive weeks.

Subjects will have visits on Day 1 (Visit 1), Day 8 (Visit 2), Day 22 (Visit 3) and Day 43 (Visit 4) for safety evaluations

3.2 Schedule of Visits and Assessments

The schedule of visits and assessments is provided in Table 1.

Table 1. Schedule of Visits and Assessments

Assessments Performed	Screening	Visit 1 (Baseline)	Visit 2	Visit 3	Visit 4
	Day -28 to Day 1	Day 1	Day 8 ± 2	Day 22 ± 3	Day 43 + 3
Informed Consent/Assent/HIPAA[1]	X				
Demographics	X				
Medical/Medication/Ocular/Non-Ocular History	X				
Medical and Medication History Update		X	X	X	X
Review of Inclusion/Exclusion Criteria	X	X			
Urine Pregnancy Test[2]		X	X	X	X
Visual Acuity[5]		X	X	X	X
Slit Lamp Biomicroscopy [6]		X	X	X	X
Intraocular Pressure[7]		X			X
Vital Signs (Resting Blood Pressure and Pulse)		X			X
Physical Exam[4]		X			X
Body Weight Determination (subjects ≤12 years of age)[3]		X			
Dilated Fundoscopy		X			X
Enrollment/Randomization		X			
In-Office Investigational Drug Instillation		X[8]	X	X	
Drop Comfort Assessment[9]		X	X	X	
PK blood draw[10]		X		X	
Dispense Investigational Drug		X	X[11]	X	
Dispense Dosing Diary		X	X	X	
Collection of Returned Investigational Drug & Dosing Diary[12]Assessment of Adverse Events		X	X	X	X
Assessment of Adverse Events	X	X	X	X	X
Exit					X

1. Assent is to be taken from subjects who are at least 7 years of age and less than 18 years of age. Informed consent and/or Assent must be signed before any study-related procedure can be performed. If washout of certain medications is necessary, informed consent must be obtained at Screening Visit.
2. To be conducted on females of childbearing potential. At Visits 2 and 3, a urine pregnancy test will be conducted for females who were premenarchal at the previous visit and became menarchal thereafter.
3. The Investigator will refer to Protocol Appendix 4 for subjects <12 years of age only.
4. Physical Examination includes general health, head, eyes, ears, nose, throat (HEENT), and any other comments.
5. If a subject used correction at the baseline visit then they should use the same correction throughout all subsequent VA assessments. For subjects under 10 years of age who are developmentally unable to use ETDRS chart, a best attempt at obtaining

VA will be made by using a LEA symbols VA chart (measured as Snellen equivalent units). If subject requires Visual Behavior, they must have a passing score.

6. Evaluated prior to and 15 minutes (+ 3 minutes) post IP instillation at Visits 1-3, and once at Visit 4.
7. Age > 10 years old.
8. Subjects and/or subject's parent/legal guardian will instill IP at Visit 1 and will be observed by a trained study technician. The subjects enrolled to the PK study will receive 1 drop of the assigned IP into each eye from a trained technician.
9. Subjects ≥ 10 years old will assess comfort immediately upon instillation, at 30 seconds, and at 1-minute post-instillation using a [redacted] for each eye. Subjects < 10 years old will not assess drop comfort.
10. (For subjects agreeing to undergo PK blood draws) Pre-dose (within 1 hour prior to dosing) and at 0.25 (±3 min) hour, 0.5 (±5 min) hour, 1 (±10 min) hour, 2 (±10 min) hours, and 4 (±20 min) hours after the first study drug administration in the morning on Day 1 and on Day 22.
11. A new kit is dispensed at Visit 1 and Visit 3. At Visit 2, the kit that was dispensed at Visit 1 is re-dispensed for dosing until Visit 3.
12. A new dosing diary will be distributed at Visits 1, 2, and 3.

3.3 Study Treatments

The treatments for this study are:

- Brimonidine tartrate 0.025% / ketotifen fumarate 0.035% combination ophthalmic solution (Combo) (n=334)
- Vehicle ophthalmic solution (n=167)

All subjects screened for the study who sign an informed consent form (ICF) will be assigned a subject number that will be entered in the Screening and Enrollment Log. The subject number will consist of the three-(3)-digit site number, followed by the three-(3)-digit unique subject identifier, starting with 001.

Once a subject meets all qualification criteria at Visit 1, they will be randomized to Combo or vehicle in a 2:1 ratio. Each subject who is randomized will be assigned a unique randomization number in the Randomization and Trial Supply Management (RTSM) system. Randomization numbers will be assigned in a sequential order starting at the lowest number available. No numbers will be skipped or omitted. Randomization numbers will be 5 digits and will be created in the RTSM system. Randomization will be used to avoid bias in the assignment of subjects to treatment, to increase the likelihood that known and unknown subject attributes (e.g., demographics and baseline characteristics) are evenly balanced across the RTSM treatment groups, and to enhance the validity of statistical comparisons between treatment groups. Masked treatment will be used to reduce potential bias during data collection and evaluation of clinical endpoints.

4. Sample Size

A total of 501 subjects will be randomized yielding approximately 334 subjects randomized to Combo and 167 subjects randomized to vehicle. Three-hundred thirty-four (334) actively treated subjects yield at least 95% probability of detecting AEs that occur at a rate of 1% or greater.

Of the subject total, approximately 100 pediatric subjects are anticipated to be enrolled into this study.

5. Data Preparation

5.1 Input Data

Study data will primarily be recorded on the electronic Case Report Forms (eCRFs) supplied by Statistics & Data Corporation (SDC) using iMedNet™ Electronic Data Capture (EDC) system. In addition, the following study data which are not captured directly within the system but are obtained from external vendors will also be included for analysis. These data sources are described in detail in data transfer agreements developed between data management and the respective external laboratory:

- PK concentration data

When all prerequisites for database lock have been met, including availability of all masked concentration data, the database will be locked. Following database lock, approval will be obtained from the Sponsor to unmask the study. Any changes to the database after data have been locked can only be made with the approval of the Sponsor in consultation with SDC.

Final analysis will be carried out after the following have occurred:

- Database lock has occurred, including receipt of all final versions of external vendor data, with written authorization provided by appropriate SDC and Sponsor personnel
- Analysis populations have been determined
- Randomized treatment codes have been unmasked

5.2 Output Data

Data from EDC and external data will be transferred to Biostatistics and incorporated into standard formats following the Study Data Tabulation Model (SDTM). Data will then be mapped to analysis datasets using the Analysis Data Model (ADaM). Both SDTM- and ADaM-formatted data will be used to create the subject listings, while all tables and figures will be based on the ADaM-formatted data.

The SDTM and ADaM versions, implementation guide versions, and Pinnacle 21 version will be documented in the respective reviewer's guides in the final CDISC package.

6. Analysis Populations

6.1 Safety

The Safety population includes all subjects who have received at least one dose of study drug. All safety and tolerability parameters will be analyzed using the Safety population and using the treatment that subjects actually received.

6.2 Pharmacokinetic

The PK population includes all enrolled subjects who provide at least one blood sample drawn post-dose.

7. General Statistical Considerations

7.1 Unit of Analysis

For tolerability and non-ocular safety analyses, the unit of analysis will be the subject. For drop comfort assessments that are recorded for each eye, the average of the eyes will be used.

7.2 Missing or Inconclusive Data Handling

In general, there will be no imputation of missing data other than for partial or missing dates where complete dates are required to flag data as treatment-emergent or concomitant with treatment. 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.
- 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.
- 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.

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).

Values of 0 will be considered missing when calculating the geometric means.

7.3 Definition of Baseline

Baseline is defined as the last measurement prior to the first dose of study medication. Change from baseline will be calculated as, Follow-up Visit – Baseline Visit.

7.4 Data Analysis Conventions

All data analysis will be performed by SDC. Statistical programming and analyses will be performed using SAS® Version 9.4 or higher. Output will be provided in rich text format (RTF) for tables and portable document format (PDF) for tables, listings, and figures using landscape orientation.

Summaries for continuous and ordinal variables will include the number of observations (n), arithmetic mean, standard deviation (SD), median, minimum, and maximum. Additionally, 25th and 75th percentiles, geometric mean, and CV% will be provided for PK concentrations and parameters. Minima and maxima will be reported with the same precision as the raw values; means and medians will be presented to one additional decimal place than reported in the raw values. Standard deviations will be presented to two additional decimal places than reported in the raw values. All summary statistics, except n for PK concentrations and parameters, will be presented with the same number of significant digits as raw PK concentrations (3 significant figures). Summaries for discrete variables will include counts and percentages. All percentages will be rounded to one decimal place (i.e., XX.X%). Differences between active treatment group and vehicle will be calculated as, Active minus Vehicle.

All statistical tests will be two-sided with a significance level of 0.05 ($\alpha = 0.05$) unless otherwise specified. Confidence intervals (CIs) for differences between treatment groups will be two-sided at 95% confidence. All p-values will be rounded to 4 decimal places; p-values less than 0.0001 will be presented as "<0.0001"; p-values greater than 0.9999 will be presented as ">0.9999."

Unless otherwise specified, summaries will be presented by treatment group and, where appropriate, visit. Listings will be based on all randomized subjects unless otherwise specified and sorted by subject number, randomized treatment, visit/time point, and parameter as applicable.

Visits in the outputs will be labelled as Day 1 (Visit 1), Day 8 (Visit 2), Day 22 (Visit 3), Day 43 (Visit 4).

7.5 Adjustments for Multiplicity

There are no formal hypotheses for this study, therefore adjustments for multiplicity do not need to be taken into consideration.

8. Disposition of Subjects

Subject disposition will be presented in terms of the numbers and percentages of subjects who are included in each population, who completed the study, and who discontinued from the study. Subjects who are not discontinued from the study will be considered study completers. Disposition will be summarized by

treatment group and overall for all subjects. The number of subjects enrolled, screen failed, and randomized will be presented.

The number and percentage of subjects prematurely discontinued from the study and the reasons for study discontinuation will be summarized by treatment group and overall for all subjects. The reasons for study discontinuation will be based on the total number of discontinuations for that treatment group and overall for all subjects, and the reasons that will be summarized include: AE, lost to follow-up, study terminated by sponsor, physician decision, withdrawal by subject, protocol violation, and other. The subject disposition summary will also include the number and percentage of subjects with any protocol deviations, any minor deviations, or any major deviations by treatment group and overall for all subjects. A subject listing will be provided that includes the informed consent/assent or reconsent date (if applicable), if the subject is part of the PK sample subgroup, the date of study completion or discontinuation, and reason for premature study discontinuation.

The number and percentage of subjects with any major protocol deviations (major status decided by the study team at the conclusion of the study prior to database lock and unmasking) will be summarized by treatment group and overall for all subjects. The protocol deviation categories (codes) that will be summarized include: informed consent, inclusion/exclusion and randomization, test article/study drug instillation and assignment at site, improper protocol procedures at site (missed, repeated, not per protocol), site's failure to report SAE/AE, visit out of window (missed, early, late), subject's non-compliance with test article, subject's use of prohibited concomitant medication, subject's failure to follow instructions, and other. A subject listing will be provided that includes the date of the deviation, the visit at which the deviation occurred, the deviation code, deviation description, action taken, whether the deviation was COVID-19 related, and the classification of whether the deviation was judged to be major or minor.

Listings will also be provided for randomization schedule and inclusion/exclusion criteria.

9. Demographic and Baseline Disease Characteristics

9.1 Demographic Variables

The demographic variables collected in this study include age, sex, childbearing potential, race, and ethnicity. Subjects who record more than one race will be grouped into a single category denoted as Multiple in the summary table and will be presented as collected in the by-subject listing. Iris color will also be collected by eye. Demographic variables will be summarized for the Safety and PK populations.

Age (years) will be summarized by treatment group and overall for all subjects using continuous descriptive statistics.

The number and percentage of subjects will be presented by treatment group and overall for all subjects, for age categories (5-12 ,13-17, 18-64, and >64 years), iris color of right eye and left eye, sex, childbearing potential for female subjects, race, and ethnicity.

A subject listing that includes all demographic variables will be provided.

9.2 Baseline Disease Characteristics

At the screening visit, subjects will sign the informed consent form, and screening visit evaluations will be performed. The screening visit evaluations will be summarized along with post-treatment visits in their respective sections.

10. Medical History and Concomitant Medications

Listings of medical history and concomitant medications will be generated separately, as well as separately for ocular and non-ocular data for medical history.

10.1 Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 26.0.

Non-ocular medical history will be summarized using discrete summary statistics and presented by treatment group at the subject level by System Organ Class (SOC) and Preferred Term (PT) using the Safety population. Ocular medical history will be similarly summarized at the subject level. 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. In the summaries, SOCs and PTs within an SOC will be listed in alphabetical order.

10.2 Prior and Concomitant Medications

Prior and Concomitant medications will be coded using World Health Organization Drug Dictionary (WHODrug) Global B3, March 2023 and summarized to the therapeutic drug class (Anatomical Therapeutic Chemical [ATC] 4 classification) and preferred name. If the ATC 4 classification is not provided, then the next highest classification that is provided in the coding dictionary will be used.

Prior medications are defined as those medications listed as having been taken up to 30 days prior to initiation of study drug administration and ended before the initiation of study drug administration. Concomitant medications are defined as those medications listed as having been taken (1) prior to initiation of study drug administration and continuing for any period of time following the first administration of study drug or (2) at any time following the first administration of study drug.

Prior and concomitant medications will be summarized using 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 they report one or more medications. Percentages will be based on the number of subjects in each treatment group. In the summaries, ATC classes and preferred names within an ATC class will be listed in an alphabetical order.

11. Dosing Compliance and Treatment Exposure

Subject listings of study drug instillation for subject dosing diary entries, study drug assignment, and study drug instillation for in-office visits will be produced.

11.1 Dosing Compliance

Subjects will be dosed in office during Day 1 (Visit 1), Day 8 (Visit 2), and Day 22 (Visit 3). They will also be provided with a dosing diary to document BID dosing after Day 1 (Visit 1). Dosing compliance (% compliance) will be assessed by calculating the number of actual doses received and comparing that to the number of expected doses as follows:

$$\text{Compliance (\%)} = \frac{\text{Number of Actual Doses Received} \times 100\%}{\text{Number of Expected Doses}}$$

The number of actual doses received will be calculated by counting the number of dosed records in the subject diary and the in-office instillation CRF pages. From Day 1 (Visit 1) to Day 43 (Visit 4), subjects are expected to be dosed 2 times day, including in-office doses. However, on Day 1 (Visit 1), they get only 1 dose on site and are instructed to take no further doses at home. The at-home dosing starts on the day after Day 1 (Visit 1). The final dose of study drug will be the dose administered at home the evening prior to Day 43 (Visit 4).

The expected number of doses will be calculated as:

For subjects who complete Day 43 (Visit 4):

$$[\ 2 \times \{\ (\text{Date Day 43 (Visit 4)} - 1) - \text{Date of First Dose}\}\ ] + 1$$

For subjects who discontinue before Day 43 (Visit 4):

$$[\ 2 \times (\text{Date of Study Last Dose from the Dosing Diary or In-office Instillation Page} - \text{Date of First Dose})] + 1$$

Subjects that discontinue without completing Day 43 (Visit 4) will be expected to take 2 doses on their last dose date. Extent of exposure, defined as number of days each subject was on the study drug will be summarized with descriptive statistics. Number of days each subject was on the study drug will be calculated using subject diary and the in-office instillations.

A categorical dosing compliance variable will also be derived as non-compliant (<80%), compliant (≥80% and ≤120%), and over compliant (>120%).

Dosing compliance (%) will be summarized with continuous descriptive statistics for each treatment group using the Safety population. The compliance categories defined above will be summarized with discrete summary statistics.

11.2 Treatment Exposure

Extent of treatment exposure for completed or discontinued subjects will be calculated in days using the following:

For subjects that complete Day 43 (Visit 4):

Extent of Exposure (days) = (Date of Visit 4 – Date of First Dose)

For subjects who do not complete Day 43 (Visit 4):

Extent of Exposure (days) = (Date of Last Dose – Date of First Dose) + 1

Extent of treatment exposure for each subject exposed to study drug will be summarized with continuous descriptive statistics for each treatment group using the Safety population.

12. Pharmacokinetic Analyses

Blood samples will be collected from a subset of up to 25 healthy adult subjects at pre-dose, 0.25 (±3 min), 0.5 (±5 min), 1 (±10 min), 2 (±10 min), and 4 (±20 min) hours following the first topical ocular instillation of brimonidine tartrate 0.025%/ketotifen fumarate 0.035% combination ophthalmic solution or vehicle on Day 1 and also on Day 22 to measure the plasma concentrations of brimonidine and ketotifen.

PK analyses will be primarily performed on the PK population with observed data only. The PK endpoints will be summarized using descriptive statistics. The PK endpoints are maximum observed plasma drug concentration (C_{max}), minimum observed plasma concentration (C_{min}), time of maximum observed plasma concentration (T_{max}), area under the plasma drug concentration-time curve from time 0 to the last quantifiable drug concentration (AUC_{0-t}), area under the plasma drug concentration-time curve from 0 through 4 hours, (AUC_{0-4}). Accumulation ratio of C_{max} at steady-state to C_{max} after the first dose (R_{CMax}) and accumulation ratio of AUC_{0-t} at steady-state to AUC_{0-t} after the first dose (R_{AUC}) will be determined by comparison of Day 1 to Day 22. Additionally, the study drug concentrations will be summarized using continuous descriptive statistics by nominal time.

The PK parameters described above for plasma concentrations of brimonidine and ketotifen will be computed from the individual plasma study drug concentrations by applying a non-compartmental approach using Phoenix WinNonlin® 6.2.1. Actual sampling times will be used for the calculation of PK parameters.

Concentration values reported as below the limit of quantitation (BLQ) before the first quantifiable concentration or after the last quantifiable concentration will be set to zero for concentration descriptive statistics. If a concentration that is BLQ is imbedded between 2 measurable concentrations, the BLQ value will be set to missing.

Values of 0 will be considered missing when calculating the geometric means.

If the data allow, mean plasma-concentration versus time figures will be presented for each analyte on a linear scale, with and without SD error bars, and on a semi-logarithmic scale without SD error bars. Plasma concentration versus time figures for the individual subjects will be presented on semi-log and linear scales.

13. Safety Analyses

All safety analyses will be conducted using the Safety population. All safety analyses described in this section will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.1 Adverse Events

An adverse event is any untoward medical occurrence in a subject participating in a clinical study, which does not necessarily have a causal relationship with the study product/procedure. Therefore, an adverse event includes:

- Any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease onset, that occurs at any time between the signing of the ICF and study exit, without any judgement about causality (i.e., whether or not it is considered to be related to the study product)
- Exacerbation, worsening, or progression of a pre-existing illness, including an increase in severity, frequency, and/or duration of a pre-existing episodic event or condition
- Events occurring from drug overdose (accidental or intentional), drug abuse or misuse, drug hypersensitivity, drug extravasation, drug interactions, drug dependency, events occurring from drug withdrawal and medication errors
- A condition detected or diagnosed after study product administration even though it may have been present prior to the start of the study

The AE reporting period ends upon study exit. Study drug includes the investigational drug under evaluation and vehicle given during the study. All AEs will be coded using the MedDRA version 26.0.

Treatment-emergent adverse events (TEAE) are defined as an AE with a start date on or after the first dose of study drug, or that worsened following administration of study drug. Adverse events 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.

Severity of an AE is defined as a qualitative assessment of the degree of intensity of an AE as determined by the Investigator or reported to him/her by the subject. The assessment of severity is made irrespective of relationship to study drug or seriousness of the event and should be evaluated according to the following scale:

- *Mild:* Awareness of a sign or symptom but is easily tolerated, requires no treatment, and does not interfere with subject's daily activities

- *Moderate:* Low level of concern to the subject and may interfere with daily activities but can be relieved by simple therapeutic care.
- *Severe*: Interrupts the subject's daily activity and requires systemic therapy or other treatment

The relationship of each AE to the study drug should be determined by the Investigator using these explanations:

- *Related*: There is at least a reasonable possibility that the AE/SAE is related to the study drug. Reasonable possibility means that there is evidence to suggest a causal relationship between the drug and the AE.
- *Not Related*: There is little or no reasonable possibility that the AE/SAE is related to the study drug. This assessment implies that the AE/SAE has little or no temporal relationship to the study drug and/or a more likely or certain alternative etiology exists.

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 ocular and non-ocular TEAEs, treatment-emergent serious adverse events (TE-SAEs), treatment-related TEAEs, treatment-related TE-SAEs, TEAEs leading to early treatment discontinuation, TEAEs leading to death, and TEAEs by maximum severity.

Additional summaries of TEAEs will be provided showing the number and percentage of subjects who experienced at least one TEAE by treatment group. Ocular and non-ocular TEAEs will be summarized separately by treatment group at the subject level by SOC and PT. 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. In the summary, SOCs and PTs within a SOC will be ordered in descending frequency of Combo subjects.

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

- Ocular TEAEs
- Non-ocular TEAEs
- Treatment-related ocular TEAEs
- Treatment-related non-ocular TEAEs
- Ocular TE-SAEs
- Non-ocular TE-SAEs
- Ocular TEAEs by Maximum Severity
- Non-Ocular TEAEs by Maximum Severity

To count the number of subjects with any TEAEs, if a subject has multiple TEAEs coded to the same PT within the same SOC, the subject will be counted once under the maximum severity.

All AEs will be presented in a subject listing. The AEs leading to study drug discontinuation will be listed separately. In addition, all SAEs will be presented in a separate listing.

All AE summaries will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.2 Best-Corrected Visual Acuity

The logarithm of the minimum angle of resolution (logMAR) BCVA is assessed at each visit using an ETDRS chart. Subjects should use their most recent correction to attain their BCVA.

The observed and change from baseline BCVA will be summarized for each eye using continuous descriptive statistics by visit for each treatment group. A subject listing of BCVA will also be produced. This analysis will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.3 Slit-Lamp Biomicroscopy

A slit-lamp biomicroscopy examination of the lid and lid margin (erythema, swelling), conjunctiva (palpebral and bulbar [erythema/hyperemia, chemosis]), cornea (edema, erosion, endothelial condition, lens pathology), and anterior chamber (cells, flare) will be performed at each visit. The results will be graded on the scale presented in Table 2.

Table 2. Grading Scale for Slit Lamp Biomicroscopy

Region	Subcategory	Scale
Lid and Lid Margin	Erythema	0 (None; 0), 0.5 (+0.5), 1 (Mild; +1), 1.5 (+1.5), 2 (Moderate; +2), 2.5 (+2.5) 3 (Severe; +3), 3.5 (+3.5) 4 (Very Severe; +4)
	Swelling	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)
Conjunctiva (Palpebral and Bulbar)	Erythema/Hyperemia	0 (None; 0), 0.5 (+0.5), 1 (Mild; +1), 1.5 (+1.5), 2 (Moderate; +2), 2.5 (+2.5) 3 (Severe; +3), 3.5 (+3.5) 4 (Very Severe; +4)
	Chemosis	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)
Cornea	Edema	0 (None; 0), 0.5 (Mild; +0.5), 1 (Moderate; +1), 2 (Severe; +2), 3 (Very Severe; +3)
	Erosion	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3)
	Endothelial Condition	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)
	Lens Pathology	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)
Anterior Chamber	Cells	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)
	Flare	0 (None; 0), 1 (Mild; +1), 2 (Moderate; +2), 3 (Severe; +3), 4 (Very Severe; +4)

The results will be summarized using both continuous descriptive statistics and counts and percentages for each treatment at each visit for each eye. For the summary with counts, percentages will be based on the number of the respective eyes with non-missing assessment at baseline and at the respective post-baseline visit for the population being analyzed. Shift tables for the slit-lamp biomicroscopy parameters will also be provided comparing each follow-up visit to baseline. A subject listing of the slit-lamp biomicroscopy parameters will also be produced.

This analysis will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.4 Intraocular Pressure

Subjects' IOP will be assessed by non-contact tonometry in each eye at Day 1 (Visit 1) and Day 43 (Visit 4). Results will be taken from a single measurement and recorded in mmHg.

The IOP values and changes from baseline for each eye will be summarized using continuous descriptive statistics by visit and eye for each treatment group. A subject listing of IOP will also be produced.

This analysis will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.5 Dilated Fundoscopy Examination

A dilated fundoscopy examination of the vitreous, retina, macula, choroid, and optic nerve will be performed at Day 1 (Visit 1) and Day 43 (Visit 4). The results will be graded as Normal, Abnormal (Not Clinically Significant [NCS]), or Abnormal (Clinically Significant [CS]).

The results will be summarized using counts and percentages for each treatment group at each visit for each eye. Percentages will be based on the number of the respective eyes with non-missing assessment at baseline and at the respective post-baseline visit for the population being analyzed. A shift table for the dilated fundoscopy parameters will also be provided comparing the follow-up visit to baseline. A subject listing of the dilated fundoscopy parameters will also be produced.

This analysis will be repeated on the following age subgroups: 5-12 years, 13-17 years, 18-64 years, and >64 years.

13.6 Physical Examination

The physical examination results, graded as normal or abnormal, will be summarized by treatment group and for all actively treated subjects using counts and percentages at Day 1 (Visit 1) and Day 43 (Visit 4). Shift table for the physical examination parameters Head, Eye, Ear, Nose, Throat and General Health will also be provided comparing the follow-up visit to baseline. A subject listing of the physical examination results will also be produced.

13.7 Vital Signs

Vital signs, including blood pressure (systolic and diastolic), pulse, height, weight, and body mass index, will be summarized with continuous descriptive statistics at Day 1 (Visit 1) and Day 43 (Visit 4) by treatment group and for all actively treated subjects. Change from baseline will also be summarized to each post-baseline visit.

A subject listing of the vital signs results will also be produced.

13.8 Drop Comfort Assessment

At Day 1 (Visit 1), Day 8 (Visit 2), and Day 22 (Visit 3), drop comfort will be assessed, on a [redacted] where [redacted], upon instillation, at 30 seconds post instillation, and 1 minute post instillation for both eyes. Drop comfort assessment scores will be averaged over both eyes and summarized with continuous descriptive statistics at each visit by treatment group. The mean difference, two-sided 95% CI, and p-value from a two-sample t-test comparing Combo and Vehicle will also be produced.

A subject listing of drop comfort assessment results will also be produced.

13.9 Pregnancy Test

Pregnancy test results will also be presented in a subject listing, if applicable for the subject.

14. Interim Analyses

There are no interim analyses planned for this study.

15. Changes from Protocol-Stated Analyses

There are no changes from the protocol-stated analyses.

16. References

1. *ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. 05 February 1998.
2. *ICH Harmonised Tripartite Guideline: Structure and Content of Clinical Study Reports E3*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. 30 November 1995.

17. Revision History

Documentation of revision to the SAP will commence after approval of the final version 1.0.

18. Tables

Tables that will be included in the topline delivery are shown in boldface font.

Table Number	Title	Population
Table 14.1.1	**Subject Disposition**	
Table 14.1.2	Major Protocol Deviations	Safety Population
Table 14.1.3.1	**Demographics**	**Safety Population**
Table 14.1.3.2	Demographics	Pharmacokinetic Population
Table 14.1.4.1	Ocular Medical History	Safety Population
Table 14.1.4.2	Non-Ocular Medical History	Safety Population
Table 14.1.5	Prior and Concomitant Medications	Safety Population
Table 14.1.6.1	Treatment Compliance	Safety Population
Table 14.1.6.2	Treatment Exposure to Study Drug	Safety Population

Table Number	Title	Population
Table 14.2.1.1.1	**Summary of Plasma Concentrations of Brimonidine**	**Pharmacokinetic Population**
Table 14.2.1.1.2	**Summary of Plasma Concentrations of Ketotifen**	**Pharmacokinetic Population**
Table 14.2.1.3	Summary of Plasma Pharmacokinetic Parameters of Brimonidine	Pharmacokinetic Population
Table 14.2.1.4	Summary of Plasma Pharmacokinetic Parameters of Ketotifen	Pharmacokinetic Population
Table 14.3.1.1.1	**Summary of Treatment-Emergent Adverse Events**	**Safety Population**
Table 14.3.1.1.2	Summary of Treatment-Emergent Adverse Events by Age Category	Safety Population
Table 14.3.1.2.1	**Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term**	**Safety Population**
Table 14.3.1.2.2	Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population
Table 14.3.1.3.1	Non-Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.1.3.2	Non-Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population
Table 14.3.1.4.1	Treatment-Related Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.1.4.2	Treatment-Related Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population
Table 14.3.1.5.1	Treatment-Related Non-Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.1.5.2	Treatment-Related Non-Ocular Treatment-Emergent Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population

Table Number	Title	Population
Table 14.3.1.6.1	Ocular Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.1.6.2	Ocular Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population
Table 14.3.1.7.1	Non-Ocular Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.1.7.2	Non-Ocular Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term by Age Category	Safety Population
Table 14.3.1.8.1	Ocular Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity	Safety Population
Table 14.3.1.8.2	Ocular Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity by Age Category	Safety Population
Table 14.3.1.9.1	Non-Ocular Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity	Safety Population
Table 14.3.1.9.2	Non-Ocular Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Severity by Age Category	Safety Population
Table 14.3.2.1.1	**Best Corrected Visual Acuity (logMAR)**	**Safety Population**
Table 14.3.2.1.2	Best Corrected Visual Acuity (logMAR) by Age Category	Safety Population
Table 14.3.2.2.1	Slit Lamp Biomicroscopy – Qualitative Summary	Safety Population
Table 14.3.2.2.2	Slit Lamp Biomicroscopy – Qualitative Summary by Age Category	Safety Population
Table 14.3.2.2.3	Slit Lamp Biomicroscopy – Quantitative Summary	Safety Population
Table 14.3.2.2.4	Slit Lamp Biomicroscopy – Quantitative Summary by Age Category	Safety Population
Table 14.3.2.2.5	Shifts in Slit Lamp Biomicroscopy from Baseline to Each Post-Baseline Visit	Safety Population

Table Number	Title	Population
Table 14.3.2.2.6	Shifts in Slit Lamp Biomicroscopy from Baseline to Each Post-Baseline Visit by Age Category	Safety Population
Table 14.3.2.3.1	**Intraocular Pressure (mmHg)**	**Safety Population**
Table 14.3.2.3.2	Intraocular Pressure (mmHg) by Age Category	Safety Population
Table 14.3.2.4.1	Dilated Fundoscopy Examination	Safety Population
Table 14.3.2.4.2	Dilated Fundoscopy Examination by Age Category	Safety Population
Table 14.3.2.5.1	Shifts in Dilated Fundus Exam Results from Baseline to Each Post-Baseline Visit	Safety Population
Table 14.3.2.5.2	Shifts in Dilated Fundus Exam Results from Baseline to Each Post-Baseline Visit by Age Category	Safety Population
Table 14.3.2.6.1	Physical Examination	Safety Population
Table 14.3.2.6.2	Physical Examination – Shift Table	Safety Population
Table 14.3.2.7	Vital Signs	Safety Population
Table 14.3.2.8.1	**Drop Comfort Assessment**	**Safety Population**
Table 14.3.2.8.2	Drop Comfort Assessment by Age Category	Safety Population

19. Listings

Listing Number	Title	Population
Listing 16.1.7	Randomization Schedule	All Randomized Subjects
Listing 16.2.1	Subject Disposition	All Randomized Subjects
Listing 16.2.2	Protocol Deviations	All Randomized Subjects
Listing 16.2.3	Inclusion Exclusion Criteria	All Enrolled Subjects
Listing 16.2.4.1	Demographics	All Randomized Subjects
Listing 16.2.4.2.1	Ocular Medical History	All Randomized Subjects
Listing 16.2.4.2.2	Non-Ocular Medical History	All Randomized Subjects
Listing 16.2.4.3	Prior and Concomitant Medications	All Randomized Subjects
Listing 16.2.5.1	Study Drug Instillation - Diary	All Randomized Subjects
Listing 16.2.5.2	In-Office Study Drug Instillation	All Randomized Subjects

Listing Number	Title	Population
Listing 16.2.5.3	Returned Study Kits	All Randomized Subjects
Listing 16.2.6.1	Pharmacokinetic Concentrations of Brimonidine (pg/mL)	All Randomized Subjects
Listing 16.2.6.2	Pharmacokinetic Concentrations of Ketotifen (pg/mL)	All Randomized Subjects
Listing 16.2.6.3	Pharmacokinetic Parameters for Brimonidine	All Randomized Subjects
Listing 16.2.6.4	Pharmacokinetic Parameters for Ketotifen	All Randomized Subjects
Listing 16.2.7.1	All Adverse Events	All Randomized Subjects
Listing 16.2.7.2	Serious Adverse Events	All Randomized Subjects
Listing 16.2.7.3	Adverse Events Leading to Study Drug Discontinuation	All Randomized Subjects
Listing 16.2.8.1	Best Corrected Visual Acuity	All Randomized Subjects
Listing 16.2.8.2	Slit Lamp Biomicroscopy	All Randomized Subjects
Listing 16.2.8.3	Intraocular Pressure	All Randomized Subjects
Listing 16.2.8.4	Physical Examination	All Randomized Subjects
Listing 16.2.8.5	Vital Signs	All Randomized Subjects
Listing 16.2.8.6	Dilated Ophthalmoscopy	All Randomized Subjects
Listing 16.2.8.7	Urine Pregnancy Test Results - Female Subjects Only	All Randomized Subjects
Listing 16.2.9	In-Office Study Drug Instillation [redacted] Comfort	All Randomized Subjects

20. Figures

If the data allow, the following figures will be produced. If the data do not allow, the following figures will not be produced.

Figure Number	Title	Population
Figure 14.2.1.1	Mean (+/-SD) Plasma-Concentration versus Time of Brimonidine - Linear Scale	Pharmacokinetic Population
Figure 14.2.1.2	Mean Plasma-Concentration versus Time of Brimonidine - Linear Scale	Pharmacokinetic Population

Figure Number	Title	Population
Figure 14.2.1.3	Mean Plasma-Concentration versus Time of Brimonidine - Semi-Log Scale	Pharmacokinetic Population
Figure 14.2.1.4	Mean Plasma-Concentration versus Time of Brimonidine by Subject - Linear Scale	Pharmacokinetic Population
Figure 14.2.1.5	Mean Plasma-Concentration versus Time of Brimonidine by Subject - Semi-Log Scale	Pharmacokinetic Population
Figure 14.2.2.1	Mean (+/-SD) Plasma-Concentration versus Time of Ketotifen - Linear Scale	Pharmacokinetic Population
Figure 14.2.2.2	Mean Plasma-Concentration versus Time of Ketotifen - Linear Scale	Pharmacokinetic Population
Figure 14.2.2.3	Mean Plasma-Concentration versus Time of Ketotifen - Semi-Log Scale	Pharmacokinetic Population
Figure 14.2.2.4	Mean Plasma-Concentration versus Time of Ketotifen by Subject - Linear Scale	Pharmacokinetic Population
Figure 14.2.2.5	Mean Plasma-Concentration versus Time of Ketotifen by Subject - Semi-Log Scale	Pharmacokinetic Population