Statistical Analysis Plan

The INVIGORATE 2 Trial: A Single-Center, Randomized, Double-Masked, Crossover Design, Vehicle-Controlled, Phase 3 Clinical Trial to Assess the Efficacy and Safety of Reproxalap Ophthalmic Solution (0.25%) Compared to Vehicle in Subjects with Seasonal Allergic Conjunctivitis Using the Environmental Exposure Chamber (EEC)

Protocol Number:	ADX-102-AC-026
Protocol Version and Date:	Version 3.0, 05DEC2022
	[redacted]
Sponsor:	Aldeyra Therapeutics, Inc
Indication:	Allergic Conjunctivitis
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SPONSOR SIGNATURE PAGE

Protocol Title: The INVIGORATE 2 Trial: a single-center, randomized, double-masked, crossover design, vehicle-controlled, phase 3 clinical trial to assess the efficacy and safety of reproxalap ophthalmic solution (0.25%) compared to vehicle in subjects with seasonal allergic conjunctivitis using the environmental exposure chamber (EEC)

Protocol Number: ADX-102-AC-026

Sponsor: Aldeyra Therapeutics, Inc.
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By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol and all applicable regulatory guidance and guidelines.

Author:

[redacted]

Approver:

[redacted]

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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
AR1	First-order Autoregressive
ATC	Anatomical Therapeutic Chemical
CI	Confidence Interval
CRF	Case Report Form
CS	Compound Symmetric
EEC	Environmental Exposure Chamber
[redacted]	[redacted]
IOP	Intraocular Pressure
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
NCT	Non-Contact IOP Tonometry
PP	Per-Protocol
PT	Preferred Term
SD	Standard Deviation
SLE	Slit Lamp Examination
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TOSS	Total Ocular Symptom Score
VA	Visual Acuity
WHO	World Health Organization
WOCBP	Women of Childbearing Potential

1. INTRODUCTION

This statistical analysis plan is designed to outline the statistical methods for protocol ADX-102-AC-026:

"The INVIGORATE 2 Trial: A single-center, randomized, double-masked, crossover design, vehicle-controlled, Phase 3 clinical trial to assess the efficacy and safety of reproxalap ophthalmic solution (0.25%) compared to vehicle in subjects with seasonal allergic conjunctivitis using the environmental exposure chamber (EEC)"

This document has been prepared based on protocol version 3.0 dated 05 December 2022.

2. STUDY DESIGN

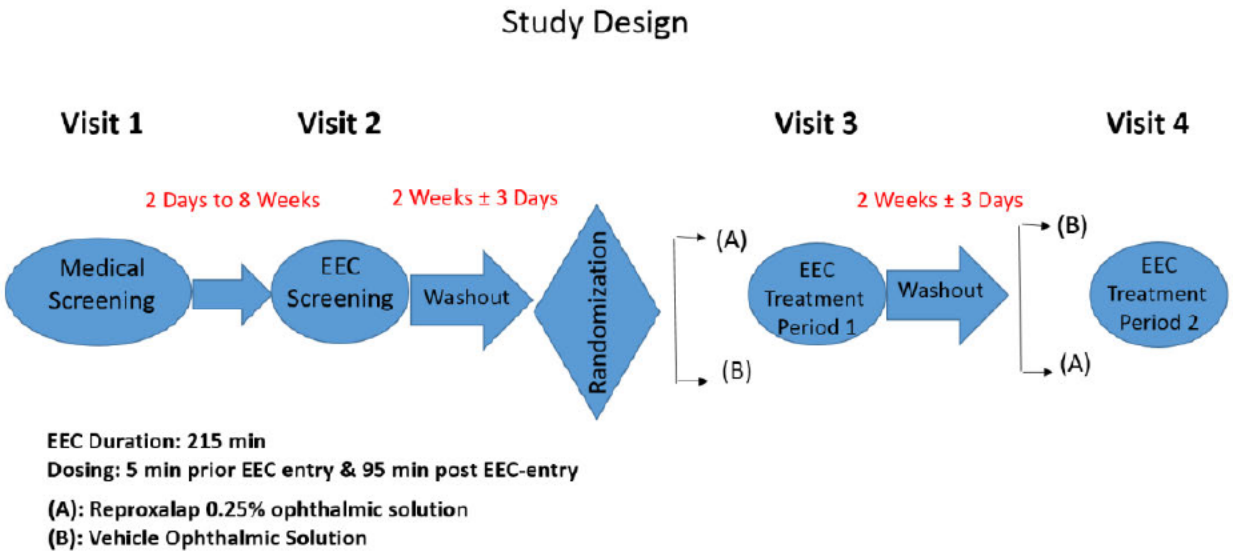
2.1 OVERVIEW OF STUDY DESIGN

The double-masked, vehicle-controlled, randomized, two-way crossover INVIGORATE-2 clinical trial allows for the testing of Reproxalap Ophthalmic Solution (0.25%, reproxalap) versus Vehicle Ophthalmic Solution (vehicle) in subjects with ragweed-induced allergic conjunctivitis in the EEC.

The clinical trial consists of four visits to the clinic (one Medical Screening visit, one Screening EEC visit, and two EEC treatment visits). The EEC sessions will be separated by an approximate two-week washout period to ensure adequate elimination of responses caused by allergen exposure in the EEC and allow therecuperation of mast cells.

The EEC clinical research facility is a room designed with the capacity and control mechanisms to expose participants to airborne ragweed pollen grains and maintains target temperature and relative humidity throughout.

Figure 1: Study Design



Eligible subjects will be randomized 1:1 to one of two treatment sequences of AB or BA where:

- Treatment A: Reproxalap Ophthalmic Solution (0.25%)
- Treatment B: Vehicle Ophthalmic Solution (phosphate buffered saline)

Approximately 138 subjects (approximately 69 per treatment sequence) will be enrolled and will follow the schedule for assessments (Table 1) .

Table 1: Schedule of Events and Assessments

Procedures	Visit 1 Medical Screening	Visit 2 Screening EEC	Visit 3 Treatment EEC Session One	Visit 4 Treatment EEC Session Two
Visit Window		Within 2 days to 8 weeks from Visit 1	2 weeks ± 3 days from Visit 2	2 weeks ± 3 days from Visit 3

Procedures	Visit 1 Medical Screening	Visit 2 Screening EEC	Visit 3 Treatment EEC Session One	Visit 4 Treatment EEC Session Two
Visit Window		Within 2 days to 8 weeks from Visit 1	2 weeks ± 3 days from Visit 2	2 weeks ± 3 days from Visit 3

At the **Medical Screening Visit (Visit 1),** subjects will undergo the informed consent process, and information about demographics, baseline characteristics, medical history, social history, physical examination will be performed, and concomitant medication will be collected. Vital signs and samples for standard clinical safety laboratory tests will be obtained. A urine pregnancy test will be administered to women of childbearing potential (WOCBP).

Subjects will undergo ophthalmic examinations (Snellen VA, SLE, NCT, and a dilated fundus examination) to ensure initial eligibility criteria are met. A skin prick test for a panel of test allergens will be conducted; results must be positive (i.e., a wheal that is 3 mm greater than the negative control) for at least one test allergen and must include ragweed in order to proceed to Visit 2.

At all EEC sessions, assessments of ophthalmic evaluations will be collected using an [redacted] In the event of an [redacted] failure, paper diary cards will be used as a backup to collect symptom scores. For subject-reported symptoms of ocular itching, a standard 9 point (i.e., 0-4 with 0.5 unit increments) scale will be employed. For staff-assessed grading of conjunctival redness, a standard 9-point (i.e., 0-4 with 0.5 unit increments) will be employed. [redacted]

[redacted]

At the **EEC Screening Visit (Visit 2),** qualified site staff will update concomitant medications and collect AEs, as applicable and perform tests for vital signs. A urine pregnancy test will be administered to WOCBP. Fundus exam, Snellen VA, SLE, subject rating of symptoms, and staff grading of conjunctival redness will be performed to ensure the anterior segment of the eye is healthy. Designated site staff will administer saline solution in each eye just prior to EEC entry.

Visit 2 Pre-EEC entry (Baseline):

1) Subject assessment for ocular itching and tearing will be recorded at approximately -15 (+1) and 0 (-5) minute, and
2) Staff-assessed conjunctival redness will be recorded at -12 (+1) and -5 (+1) minutes just prior to EEC entry.

Visit 2 Post-EEC entry:

1) Subject assessment for ocular itching and tearing will occur at 10 (+1), 20 (+5), 30 (+5), 40 (+5), 50 (+5), 60 (+5), 70 (+5), 80 (+5), and 90 (+5) minutes.
2) Staff-assessed conjunctival redness will be recorded at 12 (+1), 22 (+5), 32 (+5), 42 (+5), 52 (+5), 62 (+5), 72 (+5), 82 (+5), and 92 (+5) minutes.

[redacted]

At the **Randomization/Treatment EEC Session One (Visit 3)**, qualified site staff will update concomitant medications, collect AEs, as applicable and collect vital signs. A urine pregnancy test will be administered to WOCBP. Ophthalmic evaluations will be conducted (Fundus exam, Snellen VA, NCT SLE, subject rating of symptoms, and staff grading of conjunctival redness).

[redacted]

Subjects will be randomized to receive the first dose of either reproxalap (Treatment A) or vehicle (Treatment B). Qualified site staff will instill one drop of the randomized treatment into each eye at approximately time zero (-5 minutes) prior to entry to the EEC after all the pre-EEC assessments are done.

Visit 3 Pre-EEC entry (Baseline):

1) Subject assessment for ocular itching and tearing will be recorded at approximately -15 (+1) and 0 (-5) minute, and
2) Staff-assessed conjunctival redness will be recorded at -12 (+1) and -5 (+1) minutes just prior to EEC entry.

Visit 3 Post-EEC entry:

1) Subject assessment for ocular itching and tearing will occur at 10 (+1), 20 (+5), 30 (+5), 40 (+5), 50 (+5), 60 (+5), 70 (+5), and 80 (+5) minutes.
2) Staff-assessed conjunctival redness will be recorded at 12 (+1), 22 (+5), 32 (+5), 42 (+5), 52 (+5), 62 (+5), 72 (+5), and 82 (+5) minutes.

At approximately 90-minutes post-EEC entry (and after subject assessed itching and tearing and staff assessed conjunctival redness for all time-points post first dose have been completed), a second dose (one drop in each eye) of the same randomized treatment will be administered in each eye by the qualified site staff. Subject will continue to assess symptoms of ocular itching and tearing after the second dose at approximately 100 (+1), 110 (+1), 120 (+5), 130 (+5), 140 (+5), 150 (+5), 160 (+5), 170 (+5), 180 (+5), 190 (+5), 200 (+5), and 210 (±5) minutes, and staff-assessed conjunctival redness will be recorded at approximately post second dose at 102 (+1), 112 (+1), 122 (+5), 132 (+5), 142 (+5), 152 (+5), 162 (+5), 172 (+5), 182 (+5), 192 (+5), 202 (+5), and 212 (±3) minutes.

Visit 3 Post-EEC exit (starting at approximately t = 215 minutes):

1) Subject assessed ocular itching and tearing will continue at 220 (+1), 230 (+5), 240 (+5), 250 (+5), and 260 (+5) minutes.
2) Staff assessed conjunctival redness will continue to be recorded post-EEC exit at approximately 222 (+1), 232 (+5), 242 (+5), 252 (+5), and 262 (+5) minutes.

Subjects will be asked to return in at least two weeks for Visit 4.

At the **Treatment EEC Session Two (Visit 4)**, all procedures performed at Visit 3 will be repeated except that each subject will be given a different treatment dependent on the treatment sequence the subject was randomized to [Reproxalap Ophthalmic Solution (0.25%) (Treatment A) or Vehicle Ophthalmic Solution (Treatment B)].

Before EEC entry, a urine pregnancy test will be administered to WOCBP.

Subjects will undergo ophthalmic examinations (Snellen VA, SLE, NCT, and a dilated fundus examination), clinical safety lab tests and vital signs will be collected to ensure safety prior to exit of the clinical trial.

2.2 Sample Size

Approximately 345 subjects with seasonal allergic conjunctivitis to ragweed will be screened in order to randomize approximately 138 subjects, with approximately 124 subjects completing the

trial. This assumes a 60% of screening failure rate and 10% of the drop-out rate for the randomized subjects.

Assuming at each single time-point between 110 - 210 minutes, the true treatment difference from the vehicle is 0.5 units in change from baseline assessed by 9-point scale (0-4) itching score with an estimated standard deviation of 1.075 units, by adopting a two-way crossover design, a total sample size of 124 subjects (i.e., 62 completers for each treatment sequence) will provide a power of 95% at a 2-sided significance level of 0.05.

2.3 Randomization and Masking

2.3.1 Randomization

Each subject screened for the clinical trial will be assigned a unique subject number that will be used to identify the subject throughout subject participation in the clinical trial. If a subject fails to be randomized, the reason should be documented in the source documents and case report form (CRF). The subject will be considered a screen failure.

Eligible subjects will be randomized 1:1 to one of two treatment sequences of AB or BA where:

- Treatment A: Reproxalap Ophthalmic Solution (0.25%)
- Treatment B: Vehicle Ophthalmic Solution (phosphate buffered saline)

Approximately 138 subjects (approximately 69 per treatment sequence) will be enrolled.

2.3.2 Masking

Investigators, qualified site personnel, and subjects will be masked to the investigational product (IP) administered. The Sponsor will also be masked to the IP administered until database lock.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

- To evaluate the efficacy and safety of Reproxalp Ophthalmic Solution (0.25%) compared to Vehicle Ophthalmic Solution for the treatment of allergic conjunctivitis using the EEC clinical trial design

3.2 Endpoints Assessment

3.2.1 Clinical Trial Assessments and Endpoints

- Ocular itching rated by the subject on the [redacted] using a 9-point, discrete, 0-4 scale with 0.5 increments
- Tearing rated by the subject on the [redacted] using a 4-point discrete 0-3 scale

- Ocular redness in the nasal and temporal conjunctiva separately in both eyes, based on a [redacted] descriptive and photographic scale from 0-4 with 0.5 steps
- Total Ocular Symptom Score (TOSS): composite score of ocular itching (0 – 4), ocular tearing (0 – 3) and ocular redness (0 – 4) with a maximum score of 11 units

3.2.2 Efficacy Endpoints

3.2.2.1 Primary Efficacy Endpoint

- Change from baseline in ocular itching score on a 9-point scale (0-4) from 110-210 minutes

3.2.2.2 Key Secondary Endpoint

- Change from baseline in conjunctival redness on a 9-point scale (0-4) over duration of the chamber (approximately 12 to 212 minutes)

3.2.2.3 Secondary Endpoints

- Change from baseline in tearing on a 4-point scale (0-3) over the duration of the chamber (approximately 10 to 210 minutes)
- Change from baseline in TOSS on an 11-point composite score (sum of maximum score of itching, tearing and redness) over the duration of the chamber (approximately 10 to 212 minutes)

3.2.3 Safety Endpoints

- Adverse events (reported, elicited, and observed)
- Ophthalmic examinations
  - Snellen visual acuity (VA)
  - Slit lamp examination (SLE)
  - Non-contact intraocular pressure tonometry (NCT)
  - Dilated fundus examination at screening Visit 1 and at Visit 4
  - Undilated fundus examination at Visits 2 and 3
- Vital signs
- Clinical laboratory tests

4. DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

4.1 Definition of Baseline

For safety evaluations, baseline is defined as the last non-missing value measured prior to initiation of study drug.

For efficacy analysis, baseline is defined as average measures prior to entry EEC treatment session in each period (visit 3 pre-EEC, visit 4 pre-EEC). In the case only one measurement prior to entry EEC treatment session is available, it will be used as baseline.

4.2 Handling of Missing Data

Generally, imputation of missing data will not be performed.

4.3 Unscheduled Visits for Safety Analysis

All unscheduled visit values will be excluded from summary tables but will be included on data listing.

4.4 Handling of Partial Dates for Medications

When determining prior or concomitant medications, partial start dates of prior and concomitant medications will be assumed to be the earliest possible date consistent with the partial date.

For the purposes of analysis, incomplete medication start dates and stop dates will be imputed.

- If a medication start date is incomplete, January will be imputed for missing month and/or the first day of the month will be imputed for missing day.
- If a medication stop date is incomplete, December will be imputed for missing month and the last day of the month will be imputed for missing day.
- If the imputed medication stop date is after the date of study completion, the date of study completion will be used instead.

4.5 Handling Partial Dates for Adverse Events

When determining the treatment emergent AE, partial dates will be handled as follows.

- If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as first dose date. In this case, the onset date will be assumed to be the first date of treatment.
- If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the day of treatment to conservatively report the event as treatment-emergent.
- A missing onset date will be coded as the day of treatment. If the resulting onset date is after a reported date of resolution, the onset date will be set equal to the date of resolution.
- Imputation of partial dates is used only to determine whether an event is treatment-emergent; data listings will present the partial date as recorded in the eCRF.

5. ANALYSIS POPULATIONS

5.1 Enrolled Population

Enrolled Population includes all subjects with a signed informed consent form.

5.2 Safety Population

Safety Population includes all randomized subjects who use at least one dose of investigational drug (Reproxalap Ophthalmic Solution or Vehicle Ophthalmic Solution), regardless of whether clinical trial assessments were performed.

5.3 Intent-to-Treat (ITT) Population

ITT Population includes all randomized subjects who use at least one dose of investigational drug and have any post-dose assessments. Subjects are evaluated according to the investigational drug treatment of the visit as per the randomized treatment sequence.

5.4 Per-Protocol (PP) Population

Per-Protocol Population includes all subjects in ITT Population who do not have a major deviation from the protocol.

5.5 Application of Analysis Population

Unless otherwise noted, the analysis populations that will be used for creating the summary tables of each type is provided in Table 2.

Table 2. Application of Populations on Tables

Type	Enrolled	Safety	ITT	PP
Disposition	X			
Demographics		X	X	X
Medical and Social history		X		
Protocol Deviations		X		
Safety Evaluations Endpoints		X		
Primary Efficacy Endpoints			X	X
Key Secondary Efficacy Endpoints			X	
Secondary Efficacy Endpoints			X	

6. STATISTICAL CONSIDERATIONS

All analyses described in this plan are considered a priori analyses in that they have been defined prior to locking the database. All other analyses, if any, designed subsequently to locking the database, will be considered post hoc analyses and will be described as exploratory analyses in the Clinical Study Report.

All summaries and statistical analysis will be performed using SAS v9.4 or later.

6.1 General Statistical Procedures

Frequency distributions for categorical variables will be provided as number of patients in the category and the percentages of the total number of patients in the given population as noted. Percentages will be reported to one decimal place.

The descriptive statistics for continuous variables will be number of subjects (n), mean, standard deviation (SD), median, minimum and maximum. Mean and median will be reported to 1 more decimal place than the raw data, while the SD will be reported to 2 more decimal places than the raw data. Minimum and maximum will be reported the same as the original data.

All statistical tests will be two-sided with a significance level of 0.05 ($\alpha = 0.05$) unless otherwise specified. Confidence interval (CI) for differences between treatment will be two-sided at 95% confidence interval.

P-value will be rounded to at most 3 decimal places and will be reported as < 0.001 if it is smaller than 0.001.

Unless otherwise specified, subjects' characteristics at baseline will be summarized by treatment sequences (Reproxalap 0.25%/Vehicle, Vehicle/Reproxalap 0.25%). Safety and efficacy will be summarized by treatment (Reproxalap 0.25%, Vehicle) where appropriate.

In general, all listings will be ordered by subject number and visit for available data unless otherwise specified in the text.

6.2 Subject Disposition

Frequency and percentage of subject disposition will be summarized by treatment sequence and all subjects for Enrolled Population including:

- Number of subjects randomized
- Number of subjects in each analysis population
- Number of subjects who completed each treatment
  - Reproxalap Ophthalmic Solution 0.25%
  - Vehicle Ophthalmic Solution
- Number of subjects only completed first treatment session

Percentage will be based on number of subjects randomized.

Study discontinuation will be summarized by treatment for Safety Population including:

- Number of subjects discontinued from study
- Reasons for study discontinuation
  - Subject request/withdrawal
  - Adverse event

- Pregnancy
- Protocol violations
- Administrative reasons
- Sponsor termination
- Any sound medical reason
- Other

Percentage is based on number of subjects in Safety Population.

Subjects' disposition will be listed for Enrolled Population. In addition, subjects excluded from study will be listed for Enrolled Population.

6.3 Extent of Study Drug Exposure

Exposure of study drug will be listed for Safety Population.

6.4 Protocol Deviations

Protocol deviations will be reviewed, assessed and documented by sponsor personnel before database lock.

Number and percentage of subjects with protocol deviation will be tabulated by treatment for Safety Population. The protocol deviations will be grouped into different categories which may include, but are not limited to:

- Missed visit
- Eligibility violation
- Missed procedures/assessments
- Informed consent
- Documentation
- Study drug administration
- Safety
- Lack of compliance

All protocol deviations will be listed for Enrolled Population.

6.5 Demographic Characteristics

Subjects' demographic characteristics will be summarized by treatment sequence and for all subjects including:

- Age at screening (years). If not reported, calculated as integer of (date of informed consent signed – date of birth)/365.25 and rounded to integer.
- Sex

- Race
- Ethnicity

Demographics and baseline characteristics will be listed for ITT Population.

6.6 Medical and Social History

Medical and social history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.1 or higher.

The frequency count and percentage of patients experiencing any medical conditions will be tabulated by system organ classifications (SOC) and preferred term (PT) for each treatment. If a preferred term or system organ class was reported more than once for a subject, the subject would only be counted once in the incidence for that preferred term or system organ class.

Medical history data will be listed for ITT Population.

6.7 Prior and Concomitant Medications

All medications will be coded according to the WHO drug dictionary which includes the WHO Drug Preferred Name and the ATC Classification Level 2 and 4.

Prior medications are medications used 30 days prior to consent to treat any medical conditions and will be documented at medical screening visit.

Concomitant medications are medications being taken on or after first dose the study drug. Medications taken 30 days prior to informed consent date and were ongoing on the date of the first dose will be considered concomitant medications. Medications with missing end date are assumed to be concomitant medications.

Prior and concomitant medications will be listed ONLY for ITT Population.

7. Safety Analysis

7.1 Ophthalmic Examination Findings

Ophthalmic examination results will be summarized by anatomical location (eye) and side (left, right and both) at pre and post-EEC entry treatment sessions for Safety Population for following tests:

- Visual acuity measured using a Snellen eye chart
- Slit lamp examination (SLE)
- Non-contact intraocular pressure tonometry (NCT), measured using non-contact tonometry
- Fundoscopy including:
  - Dilated fundus examination at Visit 4
  - Undilated fundus examination at Visits 3

7.2 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary (Version 20.0 or higher) preferred term (PT) and system organ classification (SOC).

An AE will be considered treatment emergent (TEAE) if it begins or worsens in severity after the first dose of the study drug through 30 days after the last dose of study drug, or the date of initiation of another investigational agent or surgical intervention.

Drug-related TEAEs are defined as definitely related, possibly related, or probably related to study drug. Any AEs with missing relationship to study drug will be considered as related to study drug.

All TEAEs with start dates prior to first dose date in Visit 4 EEC Treatment Session will be classified as AEs occurred in Visit 3 EEC Treatment Session.

An overall summary for number of subjects with events in each treatment will be provided including:

- Number of subjects with at least one TEAE
- Number of subjects with at least one Ocular TEAE
- Number of subjects with at least one drug-related TEAE
- Number of subjects with at least one drug-related Ocular TEAE
- Number of subjects with at least one serious TEAE
- Number of subjects with at least one serious Ocular TEAE
- Number of subjects with at least one TEAE leading to study discontinuation
- Number of subjects with at least one Ocular TEAE leading to study discontinuation

An AE is classified as an ocular AE if system organ class is coded as eye disorders.

The following types of summaries for each treatment will be provided. Summaries will be sorted by decreasing frequency of PT within SOC which will be sorted alphabetically.

- TEAEs by SOC and PT
- Ocular TEAEs by SOC and PT
- Non-Ocular TEAEs by SOC and PT
- TEAEs by Casualty, SOC and PT
- Ocular TEAEs by Casualty, SOC and PT
- Non-Ocular TEAEs by Casualty, SOC and PT
- Serious TEAE by SOC and PT
- Ocular Serious TEAE by SOC and PT
- Non-Ocular Serious TEAE by SOC and PT
- TEAE by SOC, PT and maximum Severity

- Ocular TEAE by SOC, PT and maximum Severity
- Non-Ocular TEAE by SOC, PT and maximum Severity
- TEAEs leading to study discontinuation by SOC and PT
- Ocular TEAEs leading to study discontinuation by SOC and PT
- Non-Ocular TEAEs leading to study discontinuation by SOC and PT

If a preferred term or system organ class was reported more than once for a subject, the subject would only be counted once in the incidence for that preferred term or system organ class.

- In the tabulation of TEAE by severity, only the most severe PT or SOC for each subject will be included. Missing severity will be counted as Severe.
- In the summary of drug-related TEAEs, the strongest relationship will in included.

Listing will be provided including for Safety Population:

- All AEs
- Serious TEAEs
- TEAEs leading to study discontinuation

7.3 Clinical Laboratory Tests

Standard clinical safety laboratory (chemistry and hematology) collected at the Medical Screening Visit (Visit 1) and the second Treatment EEC Session (Visit 4) as part of study exit procedures include following parameters:

- Hematology profile: hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count, and absolute platelet count
- Serum chemistry profile: albumin, alkaline phosphatase (ALP), ALT (SGPT), AST (SGOT), BUN, calcium, carbon dioxide (CO2), chloride, total cholesterol, creatinine, creatinine phosphokinase (CPK), glucose, phosphate, potassium, sodium, total protein, and uric acid.

All laboratory tests including pregnancy testing will be provided. Separate listing will also be provided for clinically significant abnormal laboratory values.

7.4 Vital Signs

Vital sign assessments will be taken with the subject in the sitting or supine position after 5 minutes at rest and will include body temperature, pulse rate, blood pressure (BP), and respiratory rate (RR) prior to EEC entry and at end of Visit 4 prior to study exit.

Observed and changes from baseline for vital signs will be summarized by visit. Baseline is defined as the last non-missing measurement taken prior to the first dose.

Listing will be provided for ITT Population.

8. Efficacy Analysis

The efficacy evaluation will be performed to assess the activity of Reproxalap Ophthalmic Solution based on the subjects reported ocular itching using a standard 9-point scale (0-4 scale with 0.5-unit increments) and tearing on a standard 4-point (0-3 scale) and trained staff assessed conjunctival redness using a standard 9-point scale (0-4 scale with 0.5 unit increments).

For all staff assessed and subject reported symptoms where values from both eyes are obtained at the same timepoint, the average score from both eyes will be used for analysis.

The following analysis will be performed for all efficacy endpoints using ITT Population:

- Observed and changes from baseline by visit and treatment
- Analysis using Mixed Model Repeated Measures (MMRM) using all available data collected during the EEC
- Plots for mean score over time, and mean changes of score from baseline over time

Baseline for efficacy endpoints is defined as average assessment prior to ECC entry treatment session in each period. In case only one assessment prior to EEC entry treatment session is available, it will be used as baseline.

The onset of action time point and the duration of action will be reported for each efficacy endpoint whenever applicable. Duration of action will be the longest (in minutes) series of time points over which majority is statistically significant in favor of test drug, including the first and last time points in the series. Onset of action will be the first time point in the duration series. If two or more duration series are equivalent in length (in minutes), then the onset will be the first time point in the series that occurs earliest.

Subjects' assessment at Screening EEC and Post-EEC exit time points will be listed ONLY. All efficacy data will be listed for ITT Population.

In addition, the primary estimand in the study is defined through the following 5 attributes:

- **Population:** ITT Population includes all randomized subjects who use at least one dose of investigational drug and have any post-dose assessments.
- **Treatment conditions:** The treatment regimen of interest in this study is Reproxalap Ophthalmic Solution (0.25%) and Vehicle Ophthalmic Solution (phosphate buffered saline).
- **Variable (or endpoint):** Change from baseline in ocular itching score on a 9-point scale (0-4) to 210 minutes.
- **Strategy for addressing intercurrent events:** See description of intercurrent events below.
- **Population-level summary:** Difference in mean change of ocular itching score from baseline to 210 minutes between Reproxalap Ophthalmic Solution (0.25%) and

Vehicle Ophthalmic Solution (phosphate buffered saline) according to MMRM analysis.

The intercurrent events that will be considered are:

- Treatment discontinuation
- Lost to follow up
- Withdrawal from the study

The intercurrent events will be handled with a treatment policy strategy whereby any measured value will be used as is. Missing data resulting from these intercurrent events will be handled implicitly within the MMRM analysis that assumes missing at random.

The key secondary and secondary estimands will be defined and analyzed in the similar fashion as the primary estimand. The estimands are summarized in **Table 3**.

Table 3: Estimands of Study

Estimand	Definition	Attributes			
		A: Population	B: Variable (or endpoint)	C: Strategy for addressing intercurrent event	D: Population-level summary
		Main Analyses			
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 3: Estimands of Study

Estimand	Definition	Attributes			
		A: Population	B: Variable (or endpoint)	C: Strategy for addressing intercurrent event	D: Population-level summary

8.1 Primary Efficacy Analysis and Sensitivity Analysis

The primary efficacy endpoint is the change from baseline (pre-dose) in ocular itching scores on a 9-point scale (0-4 scale with 0.5-unit increments) from 110-212 minutes between Reproxalap Ophthalmic Solution (0.25%) and vehicle.

Observed and changes from baseline duration treatment period will be summarized by visit and treatment for ITT populations.

The treatment comparison will be performed by using a Mixed Model Repeated Measures (MMRM) approach for a crossover study with 2 treatment periods (Visit 3 treatment EEC Session One and Visit 4 treatment EEC Session Two).

The difference between treatment mean ocular itching changes from baseline at each time point will be calculated along with the 2-sided 95% CI and the associated p-value from the MMRM model.

If the MMRM model does not converge with any correlation structure, then a within-subject t-test (baseline-adjusted, if useful) will be used to compare the score difference across treatment groups at each time point.

In addition, a sensitivity analysis will be performed to evaluate the robustness of the primary analysis results. Analyses will be performed based on the outcome of the primary analyses including all subjects in the PP population.

8.2 Key Secondary and Secondary Efficacy Analysis

The key secondary efficacy endpoint is

- Change from baseline in conjunctival redness on a 9-point scale (0-4) during the chamber (approximately 10 to 210 minutes)

The secondary efficacy endpoints are

- Change from baseline in tearing on a 4-point scale (0-3) during the chamber (approximately 10 to 210 minutes)
- Change from baseline in TOSS on an 11-point composite score (sum of itching, tearing, and redness) during the chamber (approximately 10 to 210 minutes)

TOSS will be derived as the sum of ocular itching, conjunctival redness and tearing symptoms at each time point.

Observed and changes from baseline duration treatment EEC session will be summarized by visit and treatment for ITT population.

All secondary efficacy endpoints will be analyzed using a similar approach as the primary efficacy variable for the ITT Population. Reproxalap Ophthalmic Solution will be claimed to be better than Vehicle on secondary efficacy endpoints if the estimated overall corresponding changes from baseline score from MMRM model in the Reproxalap group is statistically lower than that of the Vehicle group during the chamber (approximately 10 to 210 minutes).

9. Appendix