[redacted]

# STATISTICAL ANALYSIS PLAN

## Randomized, Controlled, Multicenter, Double-Masked, Parallel, Phase 3 Trial to Evaluate the Safety and Efficacy of TP-03 for the Treatment of *Demodex* Blepharitis (Saturn-2)

Sponsor: Tarsus Pharmaceuticals, Inc.

Protocol Number: TRS-010

[redacted]

Date: 22MAR2022

[redacted]

Registration No: NCT04784091

[redacted]

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**List of Abbreviations**

| Abbreviation | Definition |
|---|---|
| ADaM | Analysis Data Model |
| AE | Adverse Event |
| ANCOVA | Analysis of Covariance |
| ATC | Anatomical Therapeutic Chemical |
| BID | *Bis in die* (Two Times a Day) |
| CDVA | Corrected Distance Visual Acuity |
| CI | Confidence Interval |
| COVID-19 | Coronavirus Disease 2019 |
| CS | Clinically Significant |
| eCRF | Electronic Case Report Form |
| EDC | Electronic Data Capture |
| ETDRS | Early Treatment Diabetic Retinopathy Study |
| FAS | Full Analysis Set |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| IOP | Intraocular Pressure |
| IRB | Institutional Review Board |
| IRT | Interactive Response Technology |
| logMAR | Logarithm of the Minimum Angle of Resolution |
| LS | Least Squares |
| MCMC | Markov Chain Monte Carlo |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MI | Multiple Imputation |
| NCS | Not Clinically Significant |
| OD | *Oculus dexter* (Right Eye) |
| OS | *Oculus sinister* (Left Eye) |
| PDF | Portable Document Format |
| PP | Per Protocol |
| PT | Preferred Term |
| RDC | Remote Data Capture |
| RTF | Rich Text Format |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SD | Standard Deviation |
| SDC | Statistics & Data Corporation |
| SDTM | Study Data Tabulation Model |
| SE | Standard Error |
| SOC | System Organ Class |
| TEAE | Treatment-Emergent Adverse Event |
| TP-03 | Lotilaner Ophthalmic Solution, 0.25% |
| WHODrug | World Health Organization Drug Dictionary |

## 1. Introduction

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and reporting for protocol TRS-010, Version 4.0 dated 09DEC2021.

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.

This SAP describes the data that will be analyzed and the subject characteristics, efficacy, and safety assessments that will be evaluated. This SAP provides details of the specific statistical methods that will be used. 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.

## 2. Study Objectives

The overarching objective of this study is to demonstrate the safety and efficacy of TP-03, 0.25%, for the treatment of Demodex blepharitis.

The primary objective of this study is to demonstrate the safety and efficacy of lotilaner ophthalmic solution (TP-03), 0.25%, compared to its vehicle as a cure of mild to severe *Demodex* blepharitis.

The secondary objectives of this study are:

- To demonstrate the efficacy of TP-03, 0.25%, to eradicate *Demodex* mites from the eyelid margin;
- To demonstrate the efficacy of TP-03, 0.25%, to eliminate collarettes and erythema from the eyelid margin; and
- To demonstrate the efficacy of TP-03, 0.25%, to eliminate erythema from the eyelid margin.

[redacted]

### 2.1 Primary Endpoint

The primary efficacy endpoint is cure based on a collarette score of 0 for the upper eyelid of the analysis eye at Day 43. The primary safety endpoint is treatment-related adverse effects.

### 2.2 Secondary Endpoints

The secondary efficacy endpoints are:

- *Demodex* mite eradication (mite density of 0 mites/lash) from the analysis eye, at Day 43

- Composite cure based on collarette score (0) and erythema score (0) for the upper eyelid of the analysis eye at Day 43
- Erythema cure based on erythema score (0) for the upper eyelid of the analysis eye at Day 43

[redacted]

### 2.4 Safety and Other Assessments

The safety and other assessments to be performed include the following:

- Adverse events (AEs)
- Corrected distance visual acuity (CDVA)
- Slit lamp biomicroscopy assessment
- Corneal staining
- Intraocular pressure (IOP) measurement

[redacted]

### 2.5 Statistical Hypotheses

The null and alternative hypotheses, based on the primary efficacy endpoint, are:

$H_{01}$: The proportion of subjects cured at Day 43 based on collarette score of 0 for the upper eyelid of the analysis eye, by treatment with TP-03, 0.25%, is ≤ the proportion cured by treatment with vehicle.

$H_{11}$: The proportion of subjects cured at Day 43 based on collarette score of 0 for the upper eyelid of the analysis eye, by treatment with TP-03, 0.25%, is > the proportion cured by treatment with vehicle.

The secondary efficacy hypotheses for *Demodex* eradication are:

$H_{02}$: The proportion of subjects with *Demodex* eradicated at Day 43 (mite density of 0 mites/lash) in the analysis eye by treatment with TP-03, 0.25%, is ≤ the proportion with *Demodex* eradicated by treatment with vehicle.

$H_{12}$: The proportion of subjects with *Demodex* eradicated at Day 43 (mite density of 0 mites/lash) in the analysis eye by treatment with TP-03, 0.25%, is > the proportion with *Demodex* eradicated by treatment with vehicle.

The secondary efficacy hypotheses for composite of collarette and erythema cure are:

$H_{03}$: The proportion of subjects with a composite cure, collarette score of 0 and erythema score of 0, for the upper eyelid of the analysis eye at Day 43 by treatment with TP-03, 0.25%, is ≤ the proportion with a composite cure by treatment with vehicle.

$H_{13}$: The proportion of subjects with a composite cure, collarette score of 0 and erythema score of 0, for the upper eyelid of the analysis eye at Day 43 by treatment with TP-03, 0.25%, is > the proportion with a composite cure by treatment with vehicle.

The secondary efficacy hypotheses for erythema cure are:

$H_{04}$: The proportion of subjects with an erythema cure, score of 0, for the upper eyelid of the analysis eye at Day 43 by treatment with TP-03, 0.25%, is ≤ the proportion with an erythema cure by treatment with vehicle.

$H_{14}$: The proportion of subjects with an erythema cure, score of 0, for the upper eyelid of the analysis eye at Day 43 by treatment with TP-03, 0.25%, is > the proportion with an erythema cure by treatment with vehicle.

### 2.6 Estimands

The primary comparisons in this trial will be between active drug versus vehicle at Day 43 in the Full Analysis Set (FAS, as outlined in Section 7.1) using the following estimands.

- Population: subjects with *Demodex* Blepharitis defined through enrollment criteria
- Endpoint:
  - Proportion of subjects cured, based on a collarette score of 0, for the upper eyelid of the analysis eye at Day 43
  - Proportion of subjects with their Demodex mites eradicated, mite density of 0 mites/lash, from the analysis eye at Day 43.
  - Proportion of subjects that achieved a composite cure, based on collarette and erythema scores of 0, for the upper eyelid of the analysis eye at Day 43.

- Proportion of subjects that achieved an erythema cure, based on erythema score 0, for the upper eyelid of the analysis eye at Day 43

- Intercurrent event:
  - Discontinuation of study medication and non-optimal compliance is ignored [treatment policy strategy].
  - Withdrawal due to lack of efficacy or AEs: missing data will be imputed as failure [hypothetical strategy].
  - Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs: missing data will be imputed employing Multiple Imputation (MI) using randomized treatment-based Markov Chain Monte Carlo (MCMC) methodology [hypothetical strategy].
- Population-level summary:
  - Difference in proportion of subjects cured, based on a collarette score of 0 for the upper eyelid of the analysis eye, at Day 43
  - Difference in proportion of subjects with their Demodex mites eradicated, mite density of 0 mites/lash, from the analysis eye at Day 43.
  - Difference in proportion of subjects that achieved a composite cure, based on collarette and erythema scores of 0 for the upper eyelid of the analysis eye, at Day 43.
  - Difference in proportion of subjects that achieved an erythema cure, based on erythema score 0 for the upper eyelid of the analysis eye, at Day 43

If the percentage of data missing at Day 43 due to discontinuation of study medication and non-optimal compliance exceeds 2%, these missing data will be imputed as failures and the primary efficacy analysis will be repeated in a separate analysis.

Sensitivity analyses on the primary efficacy endpoint and secondary efficacy endpoints are described in the Efficacy Analysis section (Section 13).

### 2.7 Study Milestones for Analyses

Two study milestones for analysis purposes are defined based on subject completion of key visits:

Milestone 1 will be met after all subjects have completed efficacy assessment visits: The last efficacy measures are scheduled at Day 57 for Cohort 1 subjects and Day 43 for Cohort 2 subjects. Milestone 1 will be used to assess efficacy and safety during the on-treatment interval, which is defined as screening through Day43/57. After this milestone is met, the database will be cleaned, locked, unmasked and all tables, listings, and figures will be produced with data for the on-treatment interval (excluding Day 90). However, all available data for adverse events and concomitant medications and procedures with start dates within the on-treatment interval will be included in the tables and listings, and thus AEs or concomitant medications or procedures with start dates during

the on-treatment interval and end dates after Day 43/57 will be shown in Milestone 1 tables and listings.

Milestone 2 will be met after all eligible subjects have completed safety and other assessments at Day 90. Milestone 2 will be used to assess safety during the post-treatment interval, which is defined as after Day 43/57 through Day 90. After this milestone is met listings and tables for subject disposition, protocol deviations, analysis population inclusion, subjects effected by COVID-19 and the assessments performed at Day 90 (slit-lamp biomicroscopy, endothelial cell density, CDVA) will be re-run to include the Day 90 assessments. Adverse event and concomitant medication and procedure listings will be re-run to include the Day 90/post-treatment interval data, as will listings for any safety or efficacy endpoints collected during an unscheduled visit during the post-treatment interval. The tables for the AEs and concomitant medications and procedures with start dates during on-treatment interval will also be re-run and updated to include the Day 90/post-treatment interval data. Separate tables for AEs and concomitant medications and procedures with start dates after milestone 1, during the post-treatment period, will be generated for Milestone 2.

## 3. Study Design and Procedures

### 3.1 General Study Design

This Phase 3 study is a randomized, controlled, multicenter, double-masked, parallel trial to evaluate the safety and efficacy of TP-03, 0.25%, for the treatment of *Demodex* blepharitis.

A subject's participation is to start with screening at Day -14 to 1. At the Screening visit, potential subjects will be evaluated for eligibility. Prior to performing any study specific procedures, potential subjects must provide informed consent using the current Institutional Review Board (IRB) approved informed consent form (ICF). Potential subjects who meet all eligibility criteria can proceed directly to the Day 1 procedures or the Day 1 visit can be scheduled within the next 14 days. If a subject does not come in for the Day 1 visit within 14 days, the Screening visit procedures must be repeated. If the subject is considered eligible for the study at the end of the Screening visit, the subject will be randomized at Day 1 to receive one of the following treatments administered bilaterally two times a day (BID) for approximately 43 days: TP-03, 0.25%, or the TP-03 vehicle. Subjects in Cohort 1 (signed informed consent on or before 21 October 2021) will complete an observational study assessment visit (Day 57) while Cohort 2 subjects (signed informed consent on 22 October 2021 or later) will not complete a Day 57 visit. Subjects from both cohorts at sites using specular microscopy will also attend a safety assessment including endothelial cell counts and end of study visit (Day 90).

For subjects reporting for an unscheduled visit, assessments may be performed at the discretion of the investigator. At a minimum, CDVA and the occurrence of any AEs should be assessed.

In this study, subjects will be instructed to administer a single drop of the study product BID (morning and evening) in each eye. The treatment period will be for approximately 43 days.

At Day 1, site staff will supervise the subject's first dose of the study medication. The remaining doses of the study medication will be administered by the subjects at home.

During waking hours, if a dose is missed in the morning, subjects will be instructed to administer the drop if it is at least an hour prior to the second dose of the day. If it is less than an hour, the subject should simply dose the second drop of the day.

Study day will be referred to in all tables and listings. Table 1 shows the planned study day and the acceptable window per protocol for each study visit:

**Table 1. Study Visit Windows**

| Planned Study Day | Day Window Per Protocol |
|---|---|
| Day -14 to Day 1 (Screening) | N/A |
| Day 1 (Randomization) | N/A |
| Day 8 | ± 3 Days |
| Day 15 | ± 3 Days |
| Day 22 | -3 / +4 Days |
| Day 43 | -3 / +7 Days |
| Day 57 (Cohort 1 subjects only) | -6 / +14 Days |
| Day 90 (selected sites only) | ± 14 Days |

[redacted]

## 3.2 Schedule of Activities

The Schedule of Activities is provided in Table 2.

**Table 2. Schedule of Activities**

| Procedures | Screening Day -14 to 1 | Enrollment/Initiation of Study Treatment Day 1 | Day 8 ± 3 days | Day 15 ± 3 days Day 22 -3/+4 days | Day 43 -3/+7 days | Day 57 -6/+14 days (Cohort 1 only) | Day 90 ± 14 days (only at sites performing specular microscopy) |
|---|---|---|---|---|---|---|---|
| Informed consent | X | | | | | | |
| Demographics | X | | | | | | |
| Medical/ophthalmic history | X | | | | | | |
| Concomitant medication review | X | X[a] | X | X | X | X | X |
| [redacted] | | | | | | | |
| Corrected distance visual acuity (CDVA) | X | X[a] | X | X | X | X | X |
| Slit lamp biomicroscopy | X | X[a] | X | X | X | X | X |
| Collarette grading; erythema | X | | X | X | X | X | |
| Corneal staining | | X | X | X | X | X | |
| Intraocular pressure | | X | | | X | X | |
| [redacted] | | | | | | | |
| *Demodex* count | X | | | X | X | X | |
| [redacted] | | | | | | | |
| [redacted] | | | | | | | |
| [redacted] | | | | | | | |
| [redacted] | | | | | | | |
| Urine pregnancy test[b] | X | | | | X | | |
| Randomization | | X | | | | | |
| Dispense study drug; diary | | X | | | | | |
| Collection and review of participant diary | | | X | X | | | |
| Collection of study drug and return to participant | | | X | X | | | |
| Adverse event review and evaluation | | X | X | X | X | X | X |
| Collect study drug; diary | | | | | X | | |
| Study exit | | | | | X[c] | X[d] | X[e] |

a: If the Screening and Day 1 visits are completed on the same day, this test does not have to be repeated.
b: Women of childbearing potential
c: Study exit for Cohort 2 subjects if site is not performing specular microscopy
d: Study exit for Cohort 1 subjects if site is not performing specular microscopy
e: Study exit for subjects at sites performing specular microscopy

## 4. Study Treatments

### 4.1 Method of Assigning Subjects to Treatment Arms

All subjects, investigators, study personnel involved with the conduct of the study and Sponsor will be masked with regard to treatment assignments.

A balanced (1:1) randomization will be used for this study. A computer-generated blocked randomization schedule will be generated by [redacted]. [redacted] will use the randomization schedule to package the study drug. The labelled study drug will be shipped to the US distribution center for distribution to the clinical sites.

While undergoing Screening, potential study subjects will be identified by their initials and screening number in the screening and enrollment log. All subjects screened for the study who sign an ICF will be assigned a 3-digit screening number. Screening numbers will be assigned in a sequential order beginning with 301. A dash (-) will be used in place of the middle initial for potential subjects who have no middle name.

If a potential study subject has provided written informed consent and met all eligibility criteria, the subject is considered eligible to participate. If the potential subject elects to participate in the study, they can continue with the Day 1 procedure or be scheduled to return for Day 1. At Day 1, they will be dispensed the next available study drug bottle from the current site inventory by the Interactive Response Technology (IRT) system ([redacted]), which uses block stratification by site. The subject will be randomized and receive their assigned study drug at the completion of the Day 1 visit and the subject will be considered enrolled in the study. The subject will be assigned a unique identification number consisting of their site number followed by a hyphen and then the screening number.

### 4.2 Masking and Unmasking

Throughout the study, the subject, investigator, site personnel performing study assessments and Sponsor will remain masked to the study medication. The active and vehicle control ophthalmic solutions will be indistinguishable in appearance and will be distributed in bottles identical in appearance.

Treatment assignments should only be unmasked if needed for proper care of the subject. Sites will be provided instructions on the procedure to be followed to unmask the treatment assignment for a subject. Inadvertent unmasking will be reported to the sponsor and IRB as required.

The IRT system will also be used to dispense any replacement bottles needed by a subject if they lose their current bottle or it becomes damaged.

## 5. Sample Size and Power Considerations

A total sample size of 300 subjects (150 subjects per arm) yields approximately 99% power to establish superiority of TP-03 to vehicle in the subjects meeting the primary efficacy endpoint assuming a response rate of 80.0% in TP-03 and 15.8% for vehicle treatments, respectively, using Pearson chi-squared to test a one-sided hypothesis with a significance level of 0.025. Response rates for sample size calculations are

based upon a prior outside United States clinical study of TP-03, 0.25%, and vehicle for the treatment of *Demodex* blepharitis.

Based on the results of a more recent Phase 2b/3 study, TRS-009 (Saturn-1), with a response rate of 44.0% for the TP-03 treatment group and 7.4% for the vehicle treatment group, a total sample size of 300 subjects still provides greater than 99% power.

Similarly, 300 subjects (150 subjects per arm) yields 99% power to establish superiority of TP-03 to vehicle treatment in the proportion of subjects with *Demodex* eradicated and 99% power for the proportion of subjects with a composite of collarette and erythema cure at Day 43 assuming a response rate of 73.3% in TP-03 and 21.1% in vehicle for *Demodex* eradication and 73.3% in TP-03 and 10.5% in vehicle for composite cure using Pearson chi-squared to test a one-sided hypothesis with a significance level of 0.025.

Based on the results from TRS-009 (Saturn-1) with response rates of 67.9% in the TP-03 treatment group and 17.6% in the vehicle treatment group for Demodex eradication and 13.9% in the TP-03 treatment group and 1.0% in the vehicle treatment group for composite cure, a sample size of 300 subjects still provides greater than 99% power to demonstrate superiority of TP-03 to vehicle for each endpoint.

For erythema cure, a sample size of 300 subjects will provide 88% power to demonstrate superiority of TP-03 to vehicle at a one-sided significance level of 0.025 based on the results from TRS-009 (Saturn-1), response rate of 19.1% for TP-03 and 7.4% for vehicle.

The study will be considered a success and TP-03, 0.25% will be considered superior to vehicle in clinical cure if $H_{01}$ is rejected in favor of $H_{11}$. With this sample size, the study will have > 95% power to claim success for clinical cure. A closed hierarchical testing structure will be used such that the analysis will first be performed for the primary efficacy endpoint. Only if the null hypothesis for the primary efficacy endpoint is rejected will analyses be performed for the three secondary efficacy endpoints using the Hochberg testing strategy. Additional details can be found in Section 8.5. If in addition to success for clinical cure, the largest p-value among the secondary endpoints allows for rejection of the null hypothesis in favor of the alternative hypothesis at a significance level of 0.025 then the study will also claim superiority of TP-03, 0.25%, to vehicle for all secondary endpoints. If the study cannot reject the null hypothesis for the secondary endpoint with the largest p-value, the next largest p-value among the remaining two endpoints will be evaluated at a significance level of 0.0125. The study will claim superiority of TP-03, 0.25%, to vehicle for both remaining endpoints if the null hypothesis is rejected in favor of the alternative hypothesis. If the study cannot reject the null hypothesis, the remaining secondary endpoint will be evaluated at a significance level of 0.0083. If the null hypothesis is rejected in favor of the alternative, the study will be able to claim success for the remaining secondary endpoint.

Accounting for a 30% discontinuation, approximately 209 subjects per arm (approximately 418 total) will be randomized. The planned enrollment is much greater than typically planned to achieve 300 completed

subjects due to concerns regarding the impact of COVID-19 and the possibility that up to two sites may be unable to complete the study.

## 6. Data Preparation

### 6.1 Input Data

Electronic Case Report Forms (eCRF) will be developed by Statistics & Data Corporation (SDC). Data from source documents will be entered into the eCRF by site personnel. All users will complete role-based system and study-specific eCRF training prior to receiving access to the study database. User access will be granted based on a user's role in the study and will be controlled through individual login credentials including a unique User ID and password.

The clinical study database will be developed and tested in iMedNet™ v1.204.0 or higher. iMedNet™ is delivered as a single-instance multi-tenant Software-as-a-Service (SaaS) electronic data capture (EDC) system and is developed, maintained, and hosted by MedNet Solutions located in Minnetonka, Minnesota. Over the duration of the study, MedNet Solutions may apply system updates to the EDC system as part of their continuous improvement efforts.

After data are entered into the clinical study database at each milestone, described in Section 2.7, electronic edit checks and data review will be performed. All data validation specifications and procedures are detailed in the Data Validation Manual as a separate document. When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after data have been locked can only be made with the approval of Tarsus Pharmaceuticals, Inc. in consultation with SDC and per SDC standard operating procedures (SOPs).

Analyses outlined in this document will be carried out after the following have occurred for the first milestone:

- All data management requirements are met according to SDC SOPs, including data entry, performance of edit and validation checks, documentation and resolution of data queries, generation of coding reports, SAE reconciliation, and database lock with written authorization provided by appropriate SDC and Tarsus Pharmaceuticals personnel.
- Protocol deviations have been identified and status determined as major or minor as well as the potential impact on the primary efficacy endpoint.
- Analysis populations have been determined.
- Randomized treatment codes have been unmasked

At the second milestone the following will occur:

- All data management requirements are met according to SDCSOPs, including data entry, performance of edit and validation checks, documentation and resolution of data queries,

generation of coding reports, SAE reconciliation, and database lock with written authorization provided by appropriate SDC and Tarsus Pharmaceuticals personnel.

- Protocol deviations have been identified and status determined as major or minor.

### 6.2 Output Data

Data from electronic data capture (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.

SDTM will follow the SDTM version 1.7 model and will be implemented using the SDTM Implementation Guide version 3.3 and the latest version of the SDTM Controlled Terminology at the time of study start. ADaM data will follow the ADaM version 2.1 model and will be implemented using the ADaM Implementation Guide version 1.1. Both SDTM and ADaM will be validated using Pinnacle 21 version 3.1.2 or later. Any discrepancies in the validation will be noted in reviewer's guides accompanying the final data transfers.

Define.xml will be created for SDTM and ADaM using the Define-XML version 2.0 model.

## 7. Analysis Populations

### 7.1 Full Analysis Set

The Full Analysis Set (FAS) will include all randomized subjects. This population will be the primary population for efficacy analyses and will be used to summarize all efficacy variables. Subjects in the FAS will be analyzed as randomized.

### 7.2 COVID-19 Analysis Set

The COVID-19 pandemic is continuing during the treatment phase of this study and has impacted the conduct of this clinical study. Challenges from quarantines and potential site closures may lead to difficulties in meeting protocol-specified procedures. Protocol deviations related to COVID-19 will be documented at the subject level. Prior to unmasking, COVID-19-related protocol deviations will be assessed for their significance based on their impact to primary endpoint data.

The COVID-19 analysis set includes all randomized subjects but excludes subjects that discontinued due to COVID-19 complications, subjects that would have discontinued due to COVID-19 had they remained on study, and subjects that had significant COVID-19-related protocol deviations. Subjects that would have discontinued due to COVID-19 had they remained on study will be determined and documented prior to unmasking. Protocol deviations will be assessed prior to unmasking for their significance based on their impact to primary endpoint data. Sensitivity analyses of the primary efficacy and key secondary analyses will be performed on the COVID-19 population and subjects will be analyzed as randomized if at least 5% of subjects met these criteria.

### 7.3 Per-Protocol Set

The per-protocol (PP) set will include subjects in the FAS who do not have significant protocol deviations that potentially could effect the primary endpoint analyses. Protocol deviations will be assessed prior to unmasking. Subjects in the PP set will be analyzed as treated.

This population will be the secondary population for efficacy analyses and will be used to summarize a subset of efficacy variables. If the PP and FAS populations are exactly the same, then additional efficacy analyses on the PP population will not be performed.

### 7.4 Safety Set

The Safety set will include all randomized subjects who have received at least one dose of study medication. This population will be used to summarize safety variables and will summarize subjects as treated.

## 8. General Statistical Considerations

### 8.1 Unit of Analysis

Safety endpoints will be analyzed for both eyes. For subject-level efficacy endpoints, the unit of analysis will be the subject. For efficacy endpoints assessed on each eye individually, the unit of analysis will be the analysis eye. The analysis eye is defined as the eye that met all of the inclusion criteria. If both eyes met all of the inclusion criteria, then the analysis eye will be the eye with the highest *Demodex* density at the Screening visit or, if both eyes have equal *Demodex* density, the right eye.

### 8.2 Missing or Inconclusive Data Handling

Missing data due to intercurrent events will be handled as per Section 2.6. For the primary analysis, missing data due to lack of efficacy or AEs will be imputed as failures, and missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology and is further explained in Section 13.

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.).

### 8.3 Definition of Baseline

Baseline measures are defined as the last measure prior to the initiation of study treatment. Change from Baseline will be calculated as Follow-up Visit measure – Baseline Visit measure.

### 8.4 Data Analysis Conventions

All data analyses will be performed by SDC after the study is completed and the database has been locked and released for unmasking. 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. 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. SDs will be presented to two additional decimal places than reported in the raw values. Summaries for discrete variables will include counts and percentages. All percentages will be rounded to one decimal place (i.e., XX.X%). Differences between the active treatment arm and placebo will be calculated as active minus placebo.

All statistical tests will be one-sided with a significance level of 0.025 ($\alpha = 0.025$) unless otherwise specified. Confidence intervals (CI) for differences between treatment arms as well as change from baseline will be two-sided at a 95% confidence level. 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 arm and, where appropriate, visit. Listings will be based on all randomized subjects unless otherwise specified, and sorted by subject number, visit/time point, and parameter as applicable.

### 8.5 Adjustments for Multiplicity

A closed hierarchical testing structure will be used where the analysis will be performed for the primary efficacy endpoint and, only if successful, the analysis will be performed for the three secondary efficacy endpoints using the Hochberg testing strategy. Specifically, if the null hypothesis, $H_{01}$, is rejected at a one-sided α of 0.025, the study will be considered a success for clinical cure and the family of three secondary efficacy endpoints will be tested using the Hochberg testing strategy with a familywise α of 0.025. If the secondary endpoint with the largest p-value is significant at the 0.025 level, then all three secondary endpoints will be declared statistically significant. If the secondary endpoint with the largest p-value is not significant at the 0.025 level, then the secondary endpoint with the second largest p-value will be tested at the 0.0125 level. If this secondary endpoint is significant at the 0.0125 level, then it and the endpoint with the smallest p-value will be declared statistically significant. If not, the endpoint with the smallest p-value will be tested at the 0.0083 level. If the null hypothesis, $H_{01}$ is not rejected at a one-sided α of 0.025 testing will stop and the three secondary efficacy hypotheses will not be tested.

## 9. Disposition of Subjects and Protocol Deviations

Subject disposition will be presented in terms of the numbers and percentages of subjects who were randomized to masked study medication with subcategories for those who did or did not dose with the masked study medication; those who were included in the FAS, COVID-19, PP, and Safety sets; and those who completed the study and discontinued from the study. Subjects who do not discontinue from the study will be considered study completers. Subject enrollment and disposition will be summarized by treatment arm and completion/discontinuation will be summarized by treatment arm and site. Percentages will be calculated using randomized subjects as the denominator unless otherwise specified.

The reasons for premature study discontinuation will be summarized by treatment arm for all discontinued subjects. Percentages will be calculated using randomized subjects as the denominator. The reasons for study discontinuation that will be summarized include pregnancy, significant study treatment non-compliance per PI determination, exclusion criterion, reasons relating to COVID-19, AE, and other. Withdrawal due to lack of efficacy will be summarized by treatment arm. A subject listing will be provided that includes the date of and reason for premature study discontinuation.

The number and percentage of subjects with any deviation, major deviation, minor deviation, and the total number of deviations related to COVID-19 will be summarized by treatment arm for all randomized subjects. The protocol deviations that will be summarized include the following categories: informed consent, inclusion/exclusion and randomization, test article/study drug instillation and assignment at site, improper protocol procedures at site, site's failure to report SAE/AE, visit out of window (missed, early, or late), subject non-compliance with test article, subject's use of prohibited concomitant medication, subject's failure to follow instructions, other, COVID-19: visit missed, COVID-19: assessment not performed, and COVID-19: other. A subject listing will be provided that includes the date of the deviation, the deviation code, the deviation description, classification of whether the deviation was judged to be COVID-19 related,

and the classifications of whether the deviations were judged to be major or minor and potential impact on the primary efficacy endpoint based on a masked review.

In addition, subject listings will be provided that include informed consent date, inclusion and exclusion criteria violations, and exclusions from any population, subjects affected by COVID-19, and withdrawal due to lack of efficacy. Details of the study randomization, including randomization date, site, randomized treatment, and actual treatment, will also be included within a subject listing.

Tables and listings for subject disposition and protocol deviations will be generated after the conclusion of Milestone 1 and will be re-run after the conclusion of Milestone 2, as described in Section 2.7.

## 10. Demographic and Pretreatment Variables

### 10.1 Demographic Variables

The demographic variables collected in this study include age, sex, childbearing potential, race, ethnicity, and iris color (right eye [OD] and left eye [OS]). Subjects can select more than one race, and they will be categorized as Multiple Race on summaries. Demographic variables will be summarized for the FAS, COVID-19 Analysis Set, PP set, and Safety set separately. Demographic variables will also be summarized by site for the FAS.

Age (years) will be summarized by treatment arm and overall using continuous descriptive statistics as well as dichotomized into age categories of <65 years and ≥65 years. Age will be reported in years and calculated using the age at last birthday.

The number and percentage of subjects will be presented by treatment arm and overall for sex, childbearing potential, race, ethnicity, and iris color (OD and OS).

A subject listing that includes age, sex, race, ethnicity, and iris color will be provided. Childbearing potential will be listed with urine pregnancy results (see Section 15.2).

Tables and listings for demographics and pre-treatment variables will be generated after the completion of Milestone 1, as described in Section 2.7.

## 11. Medical History and Concomitant Medications

Tables and listings for medical history will be generated after the completion of Milestone 1, as described in Section 2.7.

As described in Section 2.7, tables, and listings for concomitant medications and procedures with a start date during the on-treatment interval will be generated after Milestone 1. These tables and listings will use all available data and thus, concomitant medications and procedures with start dates during the on-treatment interval and end dates after Day 43/57 will be shown in Milestone 1 tables and listings. After the completion of Milestone 2, the concomitant medication and procedures listings, and the on-treatment interval tables will be re-run to include the Day 90/post-treatment interval data. Additionally, separate tables

for concomitant medications and procedures with start dates during the post-treatment interval will be generated for Milestone 2.

### 11.1 Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0.

Ocular medical history will be summarized using discrete summary statistics and presented by treatment arm at the subject level by System Organ Class (SOC) and Preferred Term (PT) using the Safety set. Non-ocular medical history will be similarly summarized at the subject level. Medical history will be summarized by PT and SOC at the subject level. A subject will only be counted once even if they have multiple histories for a given classification. All SOCs and PTs within an SOC will be sorted in decreasing frequency among all subjects.

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

### 11.2 Concomitant Medications

Concomitant medications will be coded using World Health Organization Drug Dictionary (WHODrug) Global (B3, March 2020) and summarized by therapeutic drug class (Anatomical Therapeutic Chemical [ATC] 4 classification) and preferred name. If the ATC 4 classification is not provided, 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 reported 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.

Concomitant medications will be summarized using the Safety set. Medications will be tabulated for each treatment arm 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 he/she reports one or more medications. Percentages will be based on the number of subjects in each treatment arm. The ATC classes and preferred names within an ATC class will be sorted in decreasing frequency among all subjects. Listings of concomitant medications will be generated separately for ocular and non-ocular data (as specified by the investigator), including indication, route of administration, start date, and end date or ongoing.

### 11.3 Concomitant Procedures

Concomitant procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.0 and summarized by SOC and PT.

Concomitant procedures will be summarized using the Safety set. Procedures will be tabulated for each treatment arm using frequencies and percentages. Subjects may have more than one procedure per PT. At each level of subject summarization, a subject will be counted once if he/she reports one or more procedures. Percentages will be based on the number of subjects in each treatment arm. All SOCs and

PTs within an SOC will be sorted in decreasing frequency among all subjects. Listings of concomitant procedures will be generated separately for ocular and non-ocular data (as specified by the investigator).

## 12. Dosing Compliance

### 12.1 Dosing Compliance

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:

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

The number of actual doses received will be recorded in the CRF (first dose in Study Drug Instillation CRF and remaining doses in Study Drug Diary CRF). Two doses are expected each day except only one dose should be completed on the last day of the study. The number of expected doses that will be used for calculating compliance will be calculated as:

$$2 \times (\text{Date of Last Dose} - \text{Date of First Dose}) + 1$$

for all subjects, regardless of study completion status. Date of last dose will be taken from the last date the study drug was taken from the subject diary or the study drug instillation page of the eCRF, whichever is later. In the case where drug was temporarily withdrawn, the number of days without study drug will be accounted for by subtracting 2 doses per day without study drug.

A categorical dosing compliance variable will also be derived as non-compliant (<80%), compliant (≥80% and ≤125%), and over compliant (>125%).

Dosing compliance (%) will be summarized with continuous descriptive statistics for each treatment arm, as well as by site, sex, age group, and race using the Safety set. The compliance category defined above will be summarized with discrete summary statistics.

A subject listing of dosing compliance will also be produced after Milestone 1.

## 13. Efficacy Analyses

All efficacy analyses, as well as the generation of tables, listings, and figures, will be performed after the completion of Milestone 1, as described in Section 2.7.

Study efficacy assessments performed include the following:

- Assessment of collarette grade
- Eyelash epilation and mite counts
- Assessment of erythema grade

Collarette grade (score) is determined based on number of lashes with collarettes per eyelid (Table 3). The proportion of subjects cured in the upper eyelid of the analysis eye based on collarette grade will be summarized by visit and treatment arm. The percentage of subjects at each grade will be summarized by visit and treatment arm, as well as by site, sex, age group, and race. The mean collarette score and change from Baseline in collarette score will be summarized by visit and treatment arm. The proportion of subjects cured (Collarette Grade 0) in the upper eyelid of the analysis eye, and the change from baseline in the mean collarette score in the upper eyelid of the analysis eye by visit and treatment arm, will be displayed graphically by bar charts.

**Table 3. Collarette Grading**

| Grade | Clinical Interpretation |
|---|---|
| 0 | 0 to 2 lashes have collarettes per eyelid |
| 1 | 3 to 10 lashes have collarettes per eyelid |
| 2 | More than 10 but less than ⅓ of lashes have collarettes per eyelid |
| 3 | ⅓ or more but less than ⅔ of lashes have collarettes per eyelid |
| 4 | ⅔ or more of lashes have collarettes per eyelid |
| 0.5-unit increments ARE NOT allowed | |

Mite density in the analysis eye and change from baseline will be summarized by visit and treatment arm, as well as by site, sex, age group, and race. Mite density is the total number of mites counted divided by the total number of lashes epilated. The proportion of subjects with *Demodex* eradication will be summarized by visit and treatment arm, as well as by site, sex, age group, and race. The mean mite density and change from Baseline in collarette score will be summarized by visit and treatment arm. The proportion of subjects with *Demodex* eradication (Mite Density 0) in the analysis eye, and the change from baseline in mean mite density in the analysis eye by visit and treatment arm, will be displayed graphically by bar charts.

Erythema of the eyelid margin is graded on a scale from 0 (normal) to 3 (severe erythema) (Table 4). The percentage of subjects at each grade level in the upper eyelid of the analysis eye will be summarized by visit and by treatment arm, as well as by site, sex, age group, and race. The mean erythema score and change from Baseline in collarette score will be summarized by visit and treatment arm. The proportion of subjects with erythema cure (erythema grade 0) in the upper eyelid of the analysis eye, and the change from baseline in mean erythema score in the upper eyelid of the analysis eye by visit and treatment arm, will be displayed graphically with bar charts.

**Table 4. Lid Margin Erythema Grading**

| Grade | Severity | Clinical Interpretation |
|---|---|---|
| 0 | Normal | Normal age-related lid coloration |
| 1 | Mild | Pink capillary involvement along the lid edge, no patches of confluent capillary redness throughout the lid edge |
| 2 | Moderate | Deep pink or red confluent capillary redness present locally along the lid edge |
| 3 | Severe | Deep red, diffuse confluent capillary redness present along the lid edge |
| 0.5-unit increments ARE NOT allowed | | |

The proportion of subjects' composite cure based on collarette of 0 and erythema score of 0 will be summarized by visit and by treatment arm, as well as by site, sex, age group, and race. The proportion of subjects with composite cure in the upper eyelid of the analysis eye by visit and treatment arm will be displayed graphically with bar charts.

### 13.1 Primary Analysis

The primary efficacy endpoint is cure defined as collarette score of 0 for the upper eyelid of the analysis eye at Day 43. Descriptive statistics will be presented by treatment arm. Testing of the percentage of subjects cured at Day 43 based on the collarette grade will be completed using a difference of proportions test.

The primary efficacy endpoint analysis will be conducted on the FAS, with intercurrent events handled as described in Section 2.6. Discontinuation of study medication and non-optimal compliance will be ignored. Missing data due to withdrawal due to lack of efficacy or AEs will be imputed as failure. Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology. The MCMC method simulates missing data under a missing at random assumption while accounting for the uncertainty in the imputed values. Missing values will be imputed with an arbitrary missing pattern. The procedure will use the posterior mode from the EM algorithm as the starting values for the MCMC method. The following SAS® code will be used to generate the multiple imputation datasets:

```
PROC MI DATA=INDATA SEED=19107 NIMPUTE=20 OUT=OUT1 MINIMUM=0 MAXIMUM=4 ROUND=1;
      BY TRT;
      MCMC INITIAL=EM;
      VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
```

where

- INDATA is the name of the input dataset
- OUT1 is the name of the output dataset
- TRT is the name of the treatment group variable
- BASE is the baseline assessment
- DAY08 is the assessment at Day 8
- DAY15 is the assessment at Day 15
- DAY22 is the assessment at Day 22
- DAY43 is the assessment at Day 43

The MI datasets will be used to calculate cure based on a collarette score of 0 at Day 43. The following SAS® code will then be used to calculate the difference of proportions for each imputed dataset and combine the analyses across imputations:

```
PROC SORT DATA=OUT1; BY _IMPUTATION_; RUN;
```

where

```
- OUT1 is the name of the input dataset
- _IMPUTATION_ is the imputation number
```

and

```
PROC FREQ DATA=OUT1;
      BY _IMPUTATION_ ;
      TABLES TRT*CURE / RISKDIFF CL;
      ODS OUTPUT RISKDIFFCOL2=PROP;
RUN;
```

where

```
- OUT1 is the name of the input dataset
- _IMPUTATION_ is the imputation number
- TRT is the name of the treatment group variable
- CURE is the indicator for cure based on a collarette score of 0 at Day 43
- PROP is the name of the output dataset
```

and

```
PROC MIANALYZE DATA=PROP;
      WHERE ROW='Difference';
      MODELEFFECTS RISK;
      STDERR ASE;
      ODS OUTPUT PARAMETERESTIMATES=PEST;
RUN;
```

where

```
- PROP is the name of the input dataset
```

Statistical comparison between treatment arms will be one-sided using an α of 0.025.

In addition to the primary analysis, sensitivity analyses as described in Section 13.1.1 will be conducted to assess the endpoint under different assumptions of missingness.

Furthermore, the primary endpoint of the proportion of subjects cured based on a collarette score of 0 will be analyzed at all visits in the upper eyelid of the analysis eye, the upper eyelid of the fellow eye, the lower eyelid of the analysis eye, and the lower eyelid of the fellow eye using observed data only. Separate stratified analyses will be conducted for the upper eyelid of the analysis eye by site, sex, age group, and race on the FAS using observed data only, unless p-values are not calculable due to sparsity of sample size limitations. In addition, if the COVID-19 Analysis Set consists of ≤95% of study subjects, the analysis will be conducted for the upper eyelid of the analysis eye at Day 43 on the COVID-19 Analysis Set with observed data only. These analyses will be conducted using similar SAS® code as described in Section 13.1.1.3.

### 13.1.1 SENSITIVITY ANALYSES

The following sensitivity analyses will be conducted on the primary efficacy endpoint.

#### 13.1.1.1 SENSITIVITY ANALYSIS I

The primary efficacy analysis will be performed on the FAS set using the difference of proportions test. The hypothetical strategy introduced in Section 2.6 will be used for the intercurrent event, where missing data will be imputed assuming missing not at random (imputing from the vehicle treatment arm). Discontinuation of study medication and non-optimal compliance will be ignored. The following SAS® code will be used to generate the MI datasets:

```
PROC MI DATA=INDATA SEED=43623 NIMPUTE=20 OUT=MDATA MINIMUM=0 MAXIMUM=4 ROUND=1;
       BY TRT;
       MCMC IMPUTE=MONOTONE;
       VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
PROC MI DATA=MDATA SEED=346238 NIMPUTE=1 OUT=OUT1 MINIMUM = . 0 0 0 0 0 MAXIMUM
= . 4 4 4 4 4 ROUND = . 1 1 1 1 1;
       BY _IMPUTATION_;
       CLASS TRT;
       MONOTONE REG(DAY08 = BASE/DETAILS);
       MONOTONE REG(DAY15 = BASE DAY08/DETAILS);
       MONOTONE REG(DAY22 = BASE DAY08 DAY15/DETAILS);
       MONOTONE REG(DAY43 = BASE DAY08 DAY15 DAY22/DETAILS);
       MNAR MODEL(BASE DAY08 DAY15 DAY22 DAY43 / MODELOBS=(TRT='Vehicle'));
       VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
```

#### 13.1.1.2 SENSITIVITY ANALYSIS II

The primary efficacy analysis will be performed on the FAS set using the difference of proportions test. The hypothetical strategy introduced in Section 2.6 will be used for the intercurrent event, where missing data will be imputed assuming missing at random (imputing from the same treatment arm as the subject with missing data) using similar code as provided in Section 13.1 for the primary efficacy analysis. Discontinuation of study medication and non-optimal compliance will be ignored.

#### 13.1.1.3 SENSITIVITY ANALYSIS III

The primary efficacy analysis will be performed on the PP set with observed data only using the difference of proportions test. In addition, a Fisher's exact test will be performed. The following SAS® code will be used to generate the analyses:

```
ODS OUTPUT CHISQ = OUT_CHISQ FISHERSEXACT = OUT_F;
PROC FREQ data = INDATA;
      TABLES TRT * OTCM / FISHER EXPECTED OUTEXPECT OUT = OUT_EXP;
RUN;
```

Where

```
- INDATA is the name of the input dataset
- TRT is the name of the treatment group variable
- OTCM is the indicator for cure based on a collarette score of 0 at Day 43
- OUT_CHISQ is the name of the output dataset that will contain the p-value for
the difference of proportions test when all expected cell counts are at least 5
- OUT_F is the name of the output dataset that will contain the p-value for the
Fisher's exact test and the difference of proportions test when any expected cell
count is < 5
- OUT_EXP is the name of the output dataset that will contain the expected cell
counts
```

#### 13.1.1.4 SENSITIVITY ANALYSIS IV

The primary efficacy analysis will be performed on the FAS set with observed data only using the difference of proportions test. In addition, a Fisher's exact test will be performed. Analyses will be conducted using similar SAS® code as described in Section 13.1.1.3.

#### 13.1.1.5 SENSITIVITY ANALYSIS V

The primary efficacy analysis will be performed on the FAS set using the difference of proportions test. The hypothetical strategy introduced in Section 2.6 will be used for the intercurrent event where missing data will be imputed as failure. Discontinuation of study medication and non-optimal compliance will be ignored. In addition, a Fisher's exact test will be performed.

### 13.2 Secondary Analyses

The secondary efficacy endpoints are the proportion of subjects with *Demodex* eradication (mite density of 0 mites/lash) at Day 43; the proportion of subjects cured based on a composite collarette and erythema score of 0 at Day 43; and the proportion of subjects cured based on an erythema score of 0 at Day 43. All secondary endpoint analyses will be conducted on the FAS.

As specified in Section 8.2, missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology. The following SAS® code will be used to generate the MI datasets:

```
PROC MI DATA=INDATA SEED=10719 NIMPUTE=20 OUT=OUT1 MINIMUM=0 MAXIMUM=4 ROUND=1;
       BY TRT;
       MCMC INITIAL=EM;
       VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
```

The MI datasets will be used to calculate cure based on each respective secondary endpoint. The following SAS® code will then be used to calculate the difference of proportions for each imputed dataset and combine the analyses across imputations:

```
PROC SORT DATA=OUT1; BY _IMPUTATION_; RUN;
PROC FREQ DATA=OUT1;
       BY _IMPUTATION_ ;
       TABLES TRT*CURE / RISKDIFF CL;
       ODS OUTPUT RISKDIFFCOL2=PROP;
RUN;
PROC MIANALYZE DATA=PROP;
       WHERE ROW='Difference';
       MODELEFFECTS RISK;
       STDERR ASE;
       ODS OUTPUT PARAMETERESTIMATES=PEST;

RUN;
```

Comparisons will be one-sided using an α of 0.025.

### 13.2.1 SECONDARY EFFICACY ENDPOINTS

The first secondary efficacy endpoint is the proportion of subjects cured at Day 43, as defined by *Demodex* eradication (mite density of 0 mites/lash) in the analysis eye. Descriptive statistics will be presented by treatment arm.

Analysis will be conducted on the FAS, with intercurrent events handled as described in Section 2.6. Discontinuation of study medication and non-optimal compliance will be ignored. Missing data due to withdrawal due to lack of efficacy or AEs will be imputed as failure. Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology as described in Section 13.1. Testing of the percentage of subjects cured at Day 43 based on the *Demodex* eradication will be completed using a difference of proportions test.

In addition, a sensitivity analysis will be conducted in which all missing data will be imputed as failure. The difference of proportions test and Fisher's exact test will be used in this sensitivity analysis. Furthermore, the proportion of subjects cured based on *Demodex* eradication will be analyzed at all visits in both the analysis and fellow eye using observed data only. Stratified analyses will be conducted for the upper eyelid of the analysis eye by site, sex, age group, and race on the FAS using observed data only, unless p-values are not calculable due to sparsity or sample size limitations. In addition, if the COVID-19 Analysis Set consists of ≤95% of study subjects, the analysis will be conducted for the upper eyelid of the analysis eye at Day 43 on the COVID-19 Analysis Set with observed data only. These analyses will be conducted using similar SAS® code as described in Section 13.1.1.3.

The second secondary efficacy endpoint is the proportion of subjects cured at Day 43, as defined by a composite score of 0 based on collarette and erythema scores in the analysis eye. Descriptive statistics will be presented by treatment arm.

Analysis will be conducted on the FAS, with intercurrent events handled as described in Section 2.6. Discontinuation of study medication and non-optimal compliance will be ignored. Missing data due to withdrawal due to lack of efficacy or AEs will be imputed as failure. Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology as described in Section 13.1. Testing of the percentage of subjects cured at Day 43 based on a composite score of 0 will be completed using a difference of proportions test.

In addition, a sensitivity analysis will be conducted in which all missing data will be imputed as failure. The difference of proportions test and Fisher's exact test will be used in this sensitivity analysis. Furthermore, the proportion of subjects cured based on a composite score of 0 for collarette and erythema scores will be analyzed at all visits in both the analysis and fellow eye, and both the upper and lower eyelid, using observed data only. Stratified analyses will be conducted for the upper eyelid of the analysis eye by site, sex, age group, and race on the FAS using observed data only, unless p-values are not calculable due to sparsity or sample size limitations. In addition, if the COVID-19 Analysis Set consists of ≤95% of study subjects, the analysis will be conducted for the upper eyelid of the analysis eye at Day 43 on the COVID-

19 Analysis Set with observed data only. These analyses will be conducted using similar SAS® code as described in Section 13.1.1.3.

The third secondary efficacy endpoint is the proportion of subjects cured at Day 43, as defined by an erythema score of 0 in the analysis eye. Descriptive statistics will be presented by treatment arm.

Analysis will be conducted on the FAS, with intercurrent events handled as described in Section 2.6. Discontinuation of study medication and non-optimal compliance will be ignored. Missing data due to withdrawal due to lack of efficacy or AEs will be imputed as failure. Missing data without withdrawal or withdrawal due to reasons other than lack of efficacy or AEs will be imputed employing MI using randomized treatment-based MCMC methodology as described in Section 13.1. Testing of the percentage of subjects cured at Day 43 based on an erythema cure of 0 will be completed using a difference of proportions test.

In addition, a sensitivity analysis will be conducted in which all missing data will be imputed as failure. The difference of proportions test and Fisher's exact test will be used in this sensitivity analysis. Furthermore, the proportion of subjects based on an erythema cure of 0 will be analyzed at all visits in both the analysis and fellow eye, and both the upper and lower eyelid, using observed data only. Stratified analyses will be conducted for the upper eyelid of the analysis eye by site, sex, age group, and race on the FAS using observed data only, unless p-values are not calculable due to sparsity or sample size limitations. In addition, if the COVID-19 Analysis Set consists of ≤95% of study subjects, the analysis will be conducted for the upper eyelid of the analysis eye at Day 43 on the COVID-19 Analysis Set with observed data only. These analyses will be conducted using similar SAS® code as described in Section 13.1.1.3.

[redacted]

[redacted]

## 14. Safety Analyses

All safety analyses will be conducted using the Safety set. Safety analyses will be conducted for each milestone as described in Section 2.7 and in the sections below.

### 14.1 Treatment Exposure

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

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

Duration of treatment exposure for subjects who were lost to follow-up will be calculated in days using the following:

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

After the completion of Milestone 1, duration of treatment exposure for each subject exposed to study drug will be summarized with continuous descriptive statistics for each treatment arm using the Safety set. A subject listing of treatment exposure, study drug installation, study drug accountability, and study drug kit assignment and replacement will also be produced.

### 14.2 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not the event is considered drug-related (21 CFR 312.32 (a)). All AEs will be coded using the MedDRA Version 23.0.

Treatment-emergent adverse events (TEAEs) are defined as any event that occurs or worsens on or after the day that study treatment is initiated. It is not anticipated that any AEs will occur before treatment. However, if in the unlikely event an AE which begins prior to treatment is recorded in the eCRF, the AE 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

[redacted]

of relationship to study drug or seriousness of the event and should be categorized with possible values: “Mild”, “Moderate”, or “Severe”.

The relationship of each AE to the study drug should be determined by the Investigator with possible values: “Definitely Related”, “Potentially Related”, and “Not Related”.

Definitely and potentially related TEAEs are considered treatment-related TEAEs.

Drop instillation-related AEs are AEs that are determined by the investigator to be related to the instillation of the study drug.

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 arm and over all subjects. This summary will also include breakdowns of TEAEs further categorized as ocular or non-ocular; treatment-emergent serious adverse events (TE-SAEs) for ocular, non-ocular, and combined; TEAEs by maximal severity; treatment-related TEAEs by relatedness; drop instillation-related TEAEs; TEAEs leading to premature treatment discontinuation; TEAEs leading to temporary treatment discontinuation; and TEAEs leading to death.

Ocular and non-ocular TEAEs will be summarized using discrete summary statistics and presented by treatment arm at the subject and event level by SOC and PT. If a subject reports the same PT multiple times, that PT will only be counted once within that SOC. As with the PT, if a subject reports multiple conditions within the same SOC, that SOC will only be counted once. In the summary, SOCs will be summarized 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 TEAEs:

- Ocular TEAEs by SOC and PT
- Non-ocular TEAEs by SOC and PT
- Ocular TEAEs by SOC, PT, and Maximal Severity
- Non-ocular TEAEs by SOC, PT, and Maximal Severity
- Ocular Treatment Related TEAEs by SOC and PT
- Non-ocular Treatment Related TEAEs by SOC and PT
- Ocular TEAEs by SOC, PT, Maximal Severity, and Strongest Relationship to Study Drug
- Non-ocular TEAEs by SOC, PT, Maximal Severity, and Strongest Relationship to Study Drug
- TEAEs That Led to Premature Treatment Discontinuation
- TEAEs that Led to Temporary Treatment Discontinuation
- Treatment-emergent SAEs

Summaries of TEAEs by maximal severity will be presented for ocular AEs and non-ocular AEs separately. The number of subjects with any TEAEs (along with percentages) will be tabulated by SOC and PT within

each SOC by treatment arm. To count the number of subjects with any TEAEs, if a subject has multiple TEAEs coded to the same PT, the subject will be counted once under the maximal severity.

All AEs and TEAEs will be presented in a subject listing. TEAEs that led to premature treatment discontinuation, TEAEs that led to temporary treatment discontinuation and AEs that led to study discontinuation will be listed separately. SAEs will be presented in a separate listing. In addition, a separate listing will be generated for drop instillation-related AEs.

As described in Section 2.7, tables, and listings for adverse events with a start date during the on-treatment interval will be generated after Milestone 1. These tables and listings will use all available data and thus, adverse events with start dates during the on-treatment interval and end dates after Day 43/57 will be shown in Milestone 1 tables and listings. After the completion of Milestone 2, the adverse events listings, and the on-treatment interval tables will be re-run to include the Day 90/post-treatment interval data. Additionally, separate tables for adverse events with start dates during the post-treatment interval will be generated for Milestone 2.

### 14.3 Corrected Distance Visual Acuity (CDVA)

Corrected distance visual acuity will be evaluated near the beginning of each study visit (i.e., prior to slit lamp examination). Subjects should use their most recent correction to attain their CDVA; if they forget their spectacles, their prescription can be placed in a trial frame or a pinhole can be used with the Early Treatment Diabetic Retinography Study (ETDRS) Fast procedure under standard illumination. The subject will be asked to focus on an ETDRS visual acuity chart 4 meters away, checking one eye at a time. The number of letters read correctly will be used to compute the subject's logarithm of the minimum angle of resolution (logMAR) score for CDVA. The procedure used will be consistent with the recommendations provided for using the ETDRS-Fast method.

The observed and change from baseline CDVA will be summarized for each eye using continuous descriptive statistics by visit and treatment arm. The proportion of subjects at each visit that had ≥ 0.22 logMAR change from baseline will be summarized separately for improvement (change from baseline of ≤ -0.22 logMAR) or worsening (change from baseline of ≥ 0.22 logMAR) by treatment and eye. A subject listing of visual acuity will also be produced. This listing will include a variable that indicates if a subject had a visual acuity logMAR change from baseline (worsening or improvement).

As described in Section 2.7, tables and listings for CDVA will be generated after the completion of Milestone 1 for the on-treatment interval (excluding Day 90) and will be re-run and updated to include Day 90 data after the completion of Milestone 2.

### 14.4 Slit-Lamp Biomicroscopy Examination

At each study visit, a slit-lamp biomicroscopy examination will be performed. The following structures will be assessed for pathology defined as normal, abnormal (not clinically significant [NCS]), or abnormal (clinically significant [CS]):

- Cornea
- Conjunctiva
- Anterior chamber
- Iris
- Eyelids and lashes

The results will be summarized using counts and percentages for each treatment arm and for all actively treated subjects at each visit for each eye. Percentages will be based on the number of eyes in each treatment arm with responses. Shift tables for the slit-lamp biomicroscopy parameters will be provided comparing each follow-up visit to baseline. A subject listing of the slit-lamp biomicroscopy parameters will also be produced.

As described in Section 2.7, tables and listings for slit-lamp biomicroscopy examinations will be generated after the completion of Milestone 1 for the on-treatment interval (excluding Day 90) and will be re-run and updated to include Day 90 data after the completion of Milestone 2.

### 14.5 Corneal Staining

Corneal fluorescein staining will be assessed at the Day 1 (baseline), 8, 15, 22, 43, and 57 study visits using the National Eye Institute (NEI)/Industry Scale. The NEI/Industry Scale uses a standardized grading system of 0 to 3 for each of the five areas on each cornea. Grade 0 will be specified when no staining is present. The maximum score is 15. The following will be assessed:

- Inferior
- Superior
- Central
- Temporal
- Nasal

The above measures will be summarized using counts and percentages for each treatment arm at each visit. Percentages will be based on the number of eyes in each treatment arm with responses.

In addition, the observed values and change from baseline for total corneal staining, sum of the above regions, will be summarized for each eye using continuous descriptive statistics for each treatment arm. A subject listing of the corneal staining will also be produced.

As described in Section 2.7, tables and listings for corneal staining will be generated after Milestone 1 and corneal staining listings will only be re-run after Milestone 2 if performed during an unscheduled visit during the post-treatment interval.

### 14.6 Intraocular Pressure (IOP)

At the Day 1 (baseline), 43, and 57 study visits, IOP will be assessed by applanation tonometry in each eye. Results will be taken from a single measurement and will be 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 arm. A subject listing of IOP will also be produced. This listing will include a variable that indicates if a subject had an IOP increase from baseline of ≥6 or ≥10 mmHg.

As described in Section 2.7, tables and listings for IOP will be generated after Milestone 1 and IOP listings will only be re-run after Milestone 2 if performed during an unscheduled visit during the post-treatment interval.

[redacted]

## 15. Other Analyses

[redacted]

[redacted]

[redacted]

### 15.2 Urine Pregnancy Test

At Screening and Day 43 study visit, a urine pregnancy test should be administered to women of childbearing potential. The pregnancy test result should be recorded as positive or negative on the eCRF. The results will be summarized using counts and percentages for each treatment arm. Percentages will be based on the number of subjects in each treatment arm with responses. A subject listing of the urine pregnancy test result will also be produced with childbearing potential and reasons for no childbearing potential on the same listing.

As described in Section 2.7, urine pregnancy results tables and listings will be generated after Milestone 1. Urine pregnancy result listings will only be re-run after Milestone 2 if performed during an unscheduled visit during the post-treatment interval.

[redacted]

[redacted]

[redacted]

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

[redacted]

[redacted]

[redacted]

## 16. Interim Analyses

No interim analysis is planned.

## 17. Changes from Protocol-Stated Analyses

There were no changes from protocol-stated analyses in the development of this Statistical Analysis Plan.

[redacted]