[redacted]

STATISTICAL ANALYSIS PLAN

Randomized, Controlled, Multicenter, Double-Masked, Parallel, Phase 2b/3 Trial to Evaluate the Safety and Efficacy of TP-03 for the Treatment of *Demodex* Blepharitis (Saturn-1)

Sponsor: Tarsus Pharmaceuticals, Inc.

Protocol Number: TRS-009

[redacted]

Date: 26MAY2021

[redacted]

NCT04475432

[redacted]

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List of Abbreviations

ADaM	Analysis Data Model
AE	Adverse Event
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
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IOP	Intraocular Pressure
IRB	Institutional Review Board
logMAR	Logarithm of the Minimum Angle of Resolution
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
NCS	Not Clinically Significant
PDF	Portable Document Format
PP	Per Protocol
PT	Preferred Term
RDC	Remote Data Capture
RTF	Rich Text Format
SaaS	Software-as-a-Service
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDC	Statistics & Data Corporation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
SOP	Standard Operating Procedure
TEAE	Treatment-Emergent Adverse Event
WHODrug	World Health Organization Drug Dictionary
WOCBP	Women of Childbearing Potential

1. Introduction

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and reporting for protocol TRS-009, version 3.0 dated 25JAN2021.

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

[redacted]

2.1 Primary Endpoint

The primary efficacy endpoint is the proportion of subjects cured based on their collarette score of 0 at Day 43 of the upper eyelid of the analysis eye. The primary safety endpoint is assessment of treatment-related adverse effects.

2.2 Secondary Endpoints

The secondary efficacy endpoints include the following:

- Proportion of subjects with their *Demodex* mites eradicated, mite density of 0 at Day 43 in the analysis eye
- Proportion of subjects cured based on a composite of collarette score (0) and erythema cure/score (0) of 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:

- Corrected distance visual acuity (CDVA)
- Adverse events (AE)
- Intraocular pressure (IOP) measurement
- Slit lamp biomicroscopy assessment
- Dilated fundus examination
- [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 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 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 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 by treatment with TP-03, 0.25%, is > the proportion with *Demodex* eradicated by treatment with vehicle.

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

H_{03}: The proportion of subjects with a composite of collarette and erythema cure at Day 43 by treatment with TP-03, 0.25%, is ≤ the proportion with a composite of collarette and erythema cure by treatment with vehicle.

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

3. Study Design and Procedures

3.1 General Study Design

This is a randomized, controlled, multicenter, double-masked, parallel, phase 2b/3 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. 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 enrolled in Cohort 2 will also attend Day 57 follow-up. All other subjects are defined as Cohort 1, with final study visit at Day 43.

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

Table 1. Study Visit Windows

Planned Study Day	Day Window
Day -14 to Day 1 (Screening)	N/A
Day 1 (Randomization)	N/A
Day 8	± 3 Days
Day 15	± 3 Days

Planned Study Day	Day Window
Day 22	-3/+4 Days
Day 43	-3/+7 Days
Day 57 (Cohort 2 participants only)	-6/+14 Days

3.2 Schedule of Visits and Assessments

The schedule of visits and assessments is provided in Table 2.

Table 2. Schedule of Visits and Assessments

[redacted]

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
Informed consent	X						
Demographics	X						
Medical/ophthalmic history	X						
Concomitant medication review	X	X	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	X
Corneal staining		X	X	X	X	X	X
Intraocular pressure		X				X	X
Demodex count	X			X	X	X	X
[redacted]							
Dilated fundus examination		X				X	
[redacted]							
Urine pregnancy test[b]	X					X	
Randomization		X					
Dispense study drug; diary		X					
Collection and review of participant diary			X	X	X		
Collection of study drug and return to participant			X	X	X		
Adverse event review and evaluation		X	X	X	X	X	X
Collect study drug; diary						X	
Study exit						X[c]	X[d]

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: For Cohort 1 participants
d: For Cohort 2 participants

4. Study Treatments

4.1 Method of Assigning Subjects to Treatment Groups

A subject who meets all of the inclusion criteria and none of the exclusion criteria and has provided written informed consent is eligible for randomization at Day 1.

In this study, subjects will be instructed to administer a single drop of the study product twice a day (e.g., 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. Subjects will be asked to wash their hands prior to administration. Following drop instillation, the subject will be advised to close their eyes for approximately 15 to 30 seconds and apply gentle pressure to the upper lid to express the medication across the upper and lower lid margins. The subject should be instructed to let the medication air dry on the lid without dabbing with a tissue.

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.

4.2 Masking and Unmasking

All subjects, investigators, and study personnel involved with the conduct of the study 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 informed consent form (ICF) will be assigned a 3-digit screening number. Screening numbers will be assigned in a sequential order beginning with 101. 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 assigned to receive the next available study drug kit. The subject will 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.

Throughout the study, both the subject and site personnel performing study assessments 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.

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 a Pearson chi-squared test with a one-sided 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.

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

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. If in addition to success for clinical cure, H_{02} is rejected in favor of H_{12}, the study will also claim superiority of TP-03, 0.25%, to vehicle in *Demodex* eradication. Similarly, if in addition to success for clinical cure, H_{03} is rejected in favor of H_{13}, the study will also claim superiority of TP-03, 0.25%, to vehicle in composite collarette and erythema cure. With this sample size, the study will have > 95% power to claim success for clinical cure and superiority for both *Demodex* eradication and collarette and erythema cure.

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™ v1.191.0 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. Therefore, 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, 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.

All analyses outlined in this document will be carried out after the following have occurred:

- All data management requirements are met according to SDC standard operating procedures (SOP), including data entry, performance of edit and validation checks, documentation and resolution of data queries, 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.

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 SDTM Controlled Terminology version 2020-11-06. 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 3.1.0. 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 and will summarize subjects as randomized.

7.2 COVID-19 Analysis Set

The Coronavirus Disease 2019 (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 this criteria.

7.3 Per-Protocol Set

The per-protocol (PP) set is a subset of the FAS, which will include those subjects (and their visits) who do not have a protocol deviation likely to seriously affect the primary outcome of the study as judged by a masked evaluation prior to the unmasking of the study treatment, including non-compliance of study drug. Additionally, masked evaluators will determine if the data associated with the deviation will be excluded from the PP population. 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. The PP population will summarize subjects as treated.

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

The primary efficacy analysis will be conducted with missing data and intercurrent events handled in the following manner:

- 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].

In the event that the percentage of missing data 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 repeated in a separate analysis.

Sensitivity analyses on the primary efficacy endpoint are described in the Efficacy Analysis section.

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 – Baseline Visit.

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. Standard deviations 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 group 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 groups as well as change from baseline will be two-sided at 95% confidence. All p-values will be rounded to 4 decimal places; p-values less than 0.0001 will be presented as "<0.0001"; p-values greater than 0.9999 will be presented as ">0.9999."

Unless otherwise specified, summaries will be presented by treatment group and, where appropriate, visit. Listings will be based on all randomized subjects unless otherwise specified, and sorted by subject number, 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 two 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 two secondary efficacy endpoints will be tested using the Hochberg testing strategy with an α of 0.025. If the null hypothesis, H_{01} is not rejected at a one-sided α of 0.025, testing will stop and the two secondary efficacy hypotheses will be deemed not statistically significant.

9. Disposition of Subjects

Subject disposition will be presented in terms of the numbers and percentages of subjects who were randomized to masked study medication with subcategories of dosed with the masked study medication, did not dose with masked study medication; who were included in the following analysis populations: FAS, PP set, safety set; and who completed the study and discontinued from the study. Subjects who are not discontinued from the study will be considered study completers. Disposition will be summarized by treatment group and for all subjects. Subject enrollment and disposition will be summarized by treatment group 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 group for all discontinued subjects. Percentages will be calculated using randomized subjects as the denominator. The reasons for study discontinuation that will be summarized include: AE, significant study treatment non-compliance per PI determination, pregnancy, exclusion criteria, reasons relating to COVID-19, and other. 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, and minor deviation will be summarized by treatment group 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, late), subject's non-compliance with test article, subject's use of prohibited concomitant medication, subject's failure to follow instructions, COVID-19: visit missed, COVID-19: assessment not performed, COVID-19: other, or other. A subject listing will be provided that includes the date of the deviation, the deviation category, 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 the PP set. Details of the study randomization, including randomization date, randomized treatment and actual treatment, will also be included within a subject listing.

10. Demographic and Pretreatment Variables

10.1 Demographic Variables

The demographic variables collected in this study include age, sex, child-bearing potential, race, ethnicity, and iris color (OD and OS). Demographic variables will be summarized for the FAS, COVID-19 Analysis Set, PP set, and safety set, separately. Demographic variables will be summarized by site for the FAS.

Age (years) will be summarized, overall and by treatment, using continuous descriptive statistics as well as dichotomized into age <65 years and age ≥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, overall and by treatment, for sex, childbearing potential, race, ethnicity, and iris color (OD and OS).

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

11. Medical History and Concomitant Medications

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 group 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. Summaries will be sorted in decreasing frequency.

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 to the 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 listed as having been taken (1) prior to initiation of study drug administration and continuing for any period of time following the first administration of study drug or (2) at any time following the first administration of study drug.

Concomitant medications will be summarized using the safety set . Medications will be tabulated for each treatment group using frequencies and percentages. Subjects may have more than one medication per ATC text. At each level of subject summarization, a subject will be counted once if he/she reports one or more medications. Percentages will be based on the number of subjects in each treatment group.

Summaries will be sorted in decreasing frequency. Listings of concomitant medications will be generated separately for ocular and non-ocular data, 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 group 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 group. Summaries will be sorted in decreasing frequency. Listings of concomitant procedures will be generated separately for ocular and non-ocular data.

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.

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 group, as well as by site, sex, age, 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.

13. Efficacy Analyses

The efficacy assessments performed include the following:

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

Collarette grade 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 group. The percentage of subjects at each grade will be summarized by visit and treatment group, as well as by site, sex, age, and race. The proportion of subjects cured in the upper eyelid of the analysis eye by visit and treatment group 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	

The observed value and change from Baseline in collarette score will be summarized by visit and treatment group. 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 group, as well as by site, sex, age, and race. The change from baseline in mean collarette score and in mean erythema score in the upper eyelid of the analysis eye by visit and treatment group will be displayed graphically by bar charts.

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

Mite density in the analysis eye and change from Baseline will be summarized by visit and treatment group, as well as by site, sex, age, and race. The proportion of subjects with *Demodex* eradication will be

summarized by visit and treatment group, as well as by site, sex, age, and race. The proportion of subjects with *Demodex* eradication and change from baseline in mean mite density in the upper eyelid of the analysis eye by visit and treatment group will be displayed graphically by bar charts.

13.1 Primary Analysis

The primary efficacy endpoint is cure based on collarette score of 0 for the upper eyelid of the analysis eye at Day 43. The primary efficacy endpoint will be conducted on the FAS, with intercurrent events handled as described in Section 8.2. 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.

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 multiple imputation datasets:

```
PROC MI DATA=INDATA SEED=56636 NIMPUTE=20 OUT=OUT1 MINIMUM=0 MAXIMUM=4 ROUND=1;
      BY TRT;
      MCMC INITIAL=EM;
      VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
```

The multiple imputation 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;
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;
```

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

In addition to the primary analysis, supplementary analyses are proposed under different assumptions for the estimand to assess efficacy in patients who have missing data.

13.1.1 Sensitivity Analyses

The following sensitivity analyses will be conducted.

13.1.1.1 SENSITIVITY ANALYSIS I

The hypothetical strategy will be used for the intercurrent event. All missing data will be imputed assuming missing not at random (imputing from the vehicle treatment arm). The following SAS code will be used to generate the multiple imputation 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 hypothetical strategy will be used for the intercurrent event. All 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.

13.1.1.3 SENSITIVITY ANALYSIS III

The primary efficacy analysis will be performed using the PP set with observed data only. Treatment comparisons will also be made using the difference of proportion test and Pearson's chi-squared test or Fisher's exact test if any of the expected cell counts are less than five as an additional sensitivity analysis.

13.1.1.4 SENSITIVITY ANALYSIS IV

The primary efficacy analysis will be performed using the FAS set with observed data only. Treatment comparisons will also be made using the difference of proportion test and Pearson's chi-squared test or Fisher's exact test if any of the expected cell counts are less than five as an additional sensitivity analysis.

13.1.1.5 SENSITIVITY ANALYSIS V

The hypothetical strategy will be used for the intercurrent event. All missing data will be imputed as failure. Treatment comparisons will also be made using the difference of proportion test and Pearson's chi-squared test or Fisher's exact test if any of the expected cell counts are less than five as an additional sensitivity analysis.

13.2 Secondary Analyses

The secondary efficacy endpoints proportion of subjects with *Demodex* eradication at Day 43 and proportion of subjects composite cure based on collarette and erythema scores at Day 43 and 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 multiple imputation datasets:

```
PROC MI DATA=INDATA SEED=56636 NIMPUTE=20 OUT=OUT1 MINIMUM=0 MAXIMUM=4 ROUND=1;
      BY TRT;
      MCMC INITIAL=EM;
      VAR BASE DAY08 DAY15 DAY22 DAY43;
RUN;
```

The multiple imputation 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 First Secondary Efficacy Endpoint

The first secondary efficacy endpoint is cure based on proportion of subjects with *Demodex* eradication of the analysis eye at Day 43, with intercurrent events handled as described in Section 8.2. Descriptive statistics will be presented by treatment arm. 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 proportion test and Pearson's chi-squared (or Fisher's exact test if any of the expected cell counts are less than five) will be used in this sensitivity analysis.

13.2.2 Second Secondary Efficacy Endpoint

The second secondary efficacy endpoint is cure based on proportion of subjects composite cure based on collarette and erythema scores of the analysis eye at Day 43, with intercurrent events handled as described in Section 8.2. Descriptive statistics will be presented by treatment arm. 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 proportion test and Pearson's chi-squared (or Fisher's exact test if any of the expected cell counts are less than five) will be used in this sensitivity analysis.

[redacted]

14. Safety Analyses

All safety analyses will be conducted using the safety set.

14.1 Treatment Exposure

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

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

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

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

Extent of treatment exposure for each subject exposed to study drug will be summarized with continuous descriptive statistics for each treatment group using the safety set. A subject listing of treatment exposure 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. All AEs will be coded using the MedDRA version 23.0.

Treatment-emergent adverse events (TEAE) are defined as any event that occurs or worsens on or after the day that randomized study treatment is initiated. It is not anticipated that any AEs will occur before treatment. However if in the unlikely event an AE recorded in the eCRF which began prior to treatment will not be included in the summary tables but will be included in the AE data listings.

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

- **Mild** - Events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate** - Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** - Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

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

- **Definitely Related** – Evidence indicates a reasonable temporal sequence of the event with the study drug administration exists or that the association of the event with study drug administration is unknown and the event is not reasonably supported by other conditions such that:
    - There is a clinically plausible time sequence between onset of the AE and study treatment administration; and/or
    - There is a biologically plausible mechanism for study treatment causing or contributing to the AE; and
    - The AE cannot be reasonably attributed to concurrent/underlying illness, other drugs or procedures.

- **Potentially Related** – Evidence indicates a possible relationship to the study drug such that:
  - The event occurs within a reasonable period of time relative to the treatment that makes a causal relationship possible, but plausible explanations may also be provided by other causes such as other drugs, products, chemicals, underlying disease, environment, etc.
  - The event is possibly related to the study treatment.
- **Not Related** – Evidence indicates no plausible direct relationship to the study drug such that:
  - A clinically plausible temporal sequence is inconsistent with the onset of the AE and drug administration; and/or
  - A causal relationship is considered biologically implausible.
  - The AE can be attributed to concurrent/underlying illness, other drugs, or procedures.

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

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious adverse events include drug deficiencies that might have led to a SAE if a) suitable action had not been taken or b) intervention had not been made or c) if circumstances had been less fortunate.

A planned hospitalization for a pre-existing condition without a serious deterioration in health is not considered to be a SAE. Hospitalization for less than 24 hours is not considered a SAE unless the precipitating event was unanticipated and also related to the investigational drug.

Events that are considered sight-threatening in the opinion of the investigator will be considered SAEs.

An overall summary will be presented that includes the number of events and the number and percentage of subjects who experienced at least one TEAE, by treatment group and over all subjects. This summary will also include breakdowns of TEAEs further categorized as ocular or non-ocular, treatment-related TEAEs, serious adverse events (SAE), TEAEs leading to death, and TEAEs by maximum severity.

Ocular and non-ocular TEAEs will be summarized using discrete summary statistics and presented by treatment group 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 AEs:

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

Summaries of TEAEs by maximum 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 group. 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 maximum severity.

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

14.3 Corrected Distance Visual Acuity

For CDVA testing, subjects will wear their own spectacles or may use a pinhole occluder for correction using 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.

Visual acuity should 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, this prescription can be placed in a trial frame or a pinhole can be used.

The observed and change from Baseline CDVA will be summarized for each eye using continuous descriptive statistics by visit for each treatment group. 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 change from Baseline of ≥0.22 on the logMAR scale.

14.4 Slit-Lamp Biomicroscopy Examination

A slit-lamp biomicroscopy examination will be performed and the following structures will be assessed for pathology defined as normal, abnormal NCS, or abnormal CS:

- Eyelids and lashes (i.e., meibomian glands, lid margin, puncta, lashes)

- Cornea
- Conjunctiva
- Anterior chamber
- Iris
- Crystalline lens (Lens Opacities Classification System III for grading)
- Eyelids and Eyelashes

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

14.5 Dilated Fundus Examination

An examination of the posterior segment of the eye with dilation will be performed using indirect ophthalmoscopy. Pupils should be dilated adequately to perform a thorough fundus evaluation. The following structures will be assessed for pathology defined as normal, abnormal NCS, or abnormal CS:

- Vitreous
- Optic nerve
- Macula
- Retina
- Choroid

The results will be summarized using counts and percentages for each treatment group at each visit for each eye. Percentages will be based on the number of subjects in each treatment group with responses. Shift tables for the dilated fundus exam parameters will also be provided comparing each follow-up visit to Baseline. A subject listing of the dilated fundus exam parameters will also be produced.

[redacted]

14.7 Intraocular Pressure

Subjects' IOP will be assessed by applanation tonometry in each eye at each visit. 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 group. 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.

[redacted]

14.9 Urine Pregnancy Test

At Screening and at the final treatment visit, a urine pregnancy test should be administered to women of childbearing potential (WOCBP). WOCBP includes any females who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or are not postmenopausal at least 12 months since last menses. 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 group. Percentages will be based on the number of subjects in each treatment group with responses. A subject listing of the urine pregnancy test result will also be produced.

[redacted]

14.12 Corneal Staining

Corneal Staining is assessed at Days 1, 8, 15, 22, 43 and 57 (Cohort 2 only). The following will be assessed:

- Inferior
- Superior
- Central
- Temporal

- Nasal
- Corneal Sum

The observed values and change from baseline for corneal sum will be summarized for each eye using continuous descriptive statistics for each treatment group. All other measures will be summarized using counts and percentages for each treatment group at each visit. Percentages will be based on the number of subjects in each treatment group with responses. A subject listing of the corneal staining will also be produced.

15. Interim Analyses

No interim analysis is planned.

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
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