AR-13324-CS205

A prospective, double-masked, randomized, multicenter, placebo-controlled, parallel-group study assessing the safety and ocular hypotensive efficacy and optimum concentration to be used clinically of netarsudil ophthalmic solution in Japanese/Japanese-American subjects with open-angle glaucoma or ocular hypertension in the United States

NCT03310580

30August2018

APPROVALS

Approved: [redacted] Date: 31 Aug 2018

[redacted] Date: 31-AUG-2018

[redacted] Date: 30/Aug/2018

[redacted] Date: 30-Aug-2018

STATISTICAL ANALYSIS PLAN
Version 1.0
30 August 2018

A prospective, double-masked, randomized, multicenter, placebo-controlled, parallel-group study assessing the safety and ocular hypotensive efficacy and optimum concentration to be used clinically of netarsudil ophthalmic solution in Japanese/Japanese-American subjects with open-angle glaucoma or ocular hypertension in the United States

PROTOCOL NUMBER: AR-13324-CS205

SPONSORED BY

Aerie Pharmaceuticals, Inc.
135 US Highway 206, Suite 15
Bedminster, NJ 07921
Telephone: (908) 470-4320
Fax: (908) 325-0266

PREPARED BY

DOCUMENT VERSION CONTROL

Version Number	Date	Comments/Changes
0.1	16 April 2018	Draft
0.2	23 August 2018	Second Draft/Simplified the analyses for efficacy section due to early study closure; some editorial changes.
1.0	30 August 2018	Final

APPROVALS

Approved:

_____ Date: _____

[redacted]

_____ Date: _____

[redacted]

_____ Date: _____

[redacted]

_____ Date: _____

[redacted]

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BCVA	Best Corrected Visual Acuity
CRF	Case Report Form
CSR	Clinical Study Report
ETDRS	Early Treatment of Diabetic Retinopathy Study
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IOP	Intraocular Pressure
IWR	Interactive Web Response
logMAR	Logarithm of the Minimum Angle of Resolution
LS Mean	Least Squares Mean
ITT	Intent-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
OAG	Open-Angle Glaucoma
OHT	Ocular Hypertension
OU	Both eyes
PP	Per-Protocol
QD	Once-daily
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SD	Standard Deviation
[redacted]	[redacted]
SE	Standard Error
TEAE	Treatment Emergent Adverse Event
VF	Visual Fields

1. PURPOSE OF THE ANALYSES

This statistical analysis plan (SAP) is being developed after review of the Aerie Pharmaceuticals, Inc., protocol number AR-13324-CS205 (dated 17 July 2017) but before any analyses of the data. The SAP contains detailed information to aid in the performance of the statistical analysis and reporting of the study data for use in the final clinical study report (CSR). This SAP is being written with due consideration of the recommendations outlined in the most recent International Conference on Harmonization (ICH) E9 Guideline entitled: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline, entitled: 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. If additional analyses are required to supplement the planned analyses described in this SAP, they may be completed and will be identified in the CSR.

2. PROTOCOL SUMMARY

2.1 Study Objectives

The primary objectives of this study are to evaluate:

- The ocular hypotensive efficacy of netarsudil ophthalmic solution relative to its placebo over a 28-day period
- The ocular and systemic safety of netarsudil ophthalmic solution relative to its placebo over a 28-day period

The secondary objectives of this study are to evaluate:

None.

2.2 Overall Study Design and Plan

This is a 28-day, double-masked, randomized, multicenter, placebo-controlled, parallel-group efficacy and safety study evaluating elevated intraocular pressure (IOP) with netarsudil ophthalmic solution. The purpose of this study is to assess the efficacy and safety of netarsudil ophthalmic solution (0.02% and 0.04%) compared to its placebo in Japanese or Japanese-American subjects that are at least 18 years of age with open-angle glaucoma (OAG) or ocular hypertension (OHT).

All investigational products will be dosed in both eyes (OU) once daily (QD) in the evening (PM). Subjects eligible to be enrolled in this study will be those of Japanese ethnicity within the second generation, with a diagnosis of either OAG or OHT that are on treatment with a topical ocular hypotensive medication. A total of approximately 180 subjects will be enrolled in this study at approximately 35 clinical sites, all in United States, comprising a total of approximately 60 subjects per treatment arm for each of 3 treatment arms. Subjects who are enrolled in this study will be those at least 18 years of age with diagnosed OAG or OHT, each of whom meets all inclusion criteria and none of the exclusion criteria.

There will be a total of 6 visits designated as V1 – V6. For visits 3, 4, 5, and 6, the subjects will be evaluated at multiple time points (08:00, 10:00, and 16:00 hours) within the visit day. These time points will be designated as visit x.0, x.1, and x.2 respectively. The total treatment period is 28 days, starting at Visit 3 (Day 1), and there are follow-up visits at Week 1, Week 2 , and Week 4.

At Screening (Visit 1), an examination will be conducted, including measurements of heart rate and blood pressure (vital signs), urine pregnancy test (for women of child bearing potential), and an ophthalmic examination to include ocular symptoms, best corrected visual acuity (BCVA), central corneal thickness by pachymetry (may be taken within 1 week of Visit 1), intraocular pressure (IOP; before pupil dilation. Medicated IOP must be $\geq$ 15 mmHg and $<$ 30 mmHg in both eyes at the Screening Visit (this also applies to treatment naïve subjects), biomicroscopy, and dilated ophthalmoscopy). Visual fields and gonioscopy may be taken up to three months

prior to randomization (Visit 3). Subject symptoms will be queried and blood samples will be taken for clinical chemistry and hematology. All individuals who are qualified for enrollment will have their current ocular hypotensive therapy reviewed to determine the appropriate washout period, prior to Visit 2, as specified in Section 5.7.1 of the protocol.

At Visit 2 (Qualification #1, 08:00 hours), potential study subjects will be questioned with respect to changes in their health (to be recorded as medical history) and concomitant medication use. Study inclusion/exclusion criteria will be reassessed to confirm eligibility. Symptomatology, vital signs, BCVA, IOP, and biomicroscopy assessments will again be performed. The potential subject must have a post-washout IOP ≥ 15 mmHg (OAG) or ≥ 22 mmHg (OHT) and < 35 mmHg in both eyes to qualify for further participation. [redacted]

Qualified subjects will return for Visit 3 (Qualification #2, Baseline, Day 1) 2 to 7 days after Visit 2 at 3 time points. Inclusion/exclusion criteria will be reviewed again for the qualified individual at all the 3 time points. At Visit 3.0 (08:00 hours), the subject will be questioned regarding any changes in their health and concomitant medication use. The testing will include recording symptomatology, vital signs, BCVA, IOP, and biomicroscopy assessments. Any symptoms reported at this visit should be entered into medical history. The potential subject must have a post-washout IOP ≥ 15 mmHg (OAG) or ≥ 22 mmHg (OHT) and < 35 mmHg in both eyes to qualify for further participation. [redacted]

Qualifying subjects will return for two additional visits on this day (Visit 3.1 at 10:00 hours and Visit 3.2 at 16:00 hours) during which symptomatology, IOP, and biomicroscopy assessments will be performed. The potential subject must have a post-washout IOP ≥ 15 mmHg (OAG) or ≥ 22 mmHg (OHT)and < 35 mmHg in both eyes to qualify for further participation. [redacted]

In summary, the following IOP criteria must be met at the qualifying visits in both eyes for the subject to qualify for the study:

Visit, Day, Time	OAG Eyes IOP Requirement (mmHg)	OHT Eyes IOP Requirement (mmHg)
Visit 2, 8:00 hours	≥ 15 & < 35	≥ 22 & < 35
Visit 3.0, Day 1, 8:00 hours	≥ 15 & < 35	≥22 & < 35
Visit 3.1, Day 1, 10:00 hours	≥ 15 & < 35	≥ 22 & < 35
Visit 3.2, Day 1, 16:00 hours	≥ 15 & < 35	≥ 22 & < 35

For each qualification visit, individuals who do not meet the IOP requirement may return for up to 2 additional unscheduled qualification visits within 1 week of failing the specific qualification visit. Individuals returning at an unscheduled visit within 1 week are required to only re-measure IOP in both eyes. Upon return, such individuals would need to qualify at 08:00, 10:00 and 16:00

hours to continue the study. Individuals who fail due to IOP being ≥ 35 mmHg in either eye (exclusion criterion) MAY NOT return for additional qualification visits.

At this point, eligible subjects will be enrolled and assigned to an investigational product through an IWRS according to a computer-generated randomization list. The study medication kit containing 2 bottles will be dispensed through the IWRS to the eligible subject, along with dosing and storage instructions.

For each subject, both eyes will be treated. Subjects will be:

- Instructed to self-administer their masked medication at home between 20:00-22:00 hours (8 PM - 10 PM) beginning with the evening dose on that day

Subjects will return for post-treatment Visits 4.0, 4.1, and 4.2 (Week 1 [Day 8]; 08:00, 10:00, and 16:00 hours), Visits 5.0, 5.1, and 5.2 (Week 2 [Day 15]; 08:00, 10:00, and 16:00 hours), Visits 6.0, 6.1, and 6.2 (Week 4 [Day 29]; 08:00, 10:00, and 16:00 hours).

Symptomatology/adverse events (AE), review of concomitant medications and non-dilated eye examinations including IOP and biomicroscopy will be conducted at all of these visits. Vital signs and BCVA will also be conducted at Visits 4.0, 5.0 and 6.0. Any symptoms reported, or clinical signs observed which have worsened, either from baseline or from previous visits at these post-dose visits, will be recorded as treatment emergent adverse events (TEAE). At Visit 6 (Week 4 [Day 29]), a urine pregnancy test (for women of child-bearing potential) will be performed and blood will be drawn for clinical labs. A dilated ophthalmoscopy will be performed at Visit 6.2 (Week 4 [Day 29], 16:00 hours).

A study schedule of events table is presented in Appendix 1.

2.3 Study Population

The study population includes subjects aged 18 years of age or older with a diagnosis of OAG or OHT in both eyes (OAG in one eye and OHT in the fellow eye is acceptable). Subjects must be of Japanese ethnicity within the second generation proven by birth certificate or family tree. Japanese generation is defined as follows: 1st generation is defined as born in Japan, immigrated to US; 2nd generation is defined as parents are 1st generation, however, the subject was born in US as an American citizen. Subjects must have medicated intraocular pressure (IOP) ≥ 15 mmHg and < 30 mmHg in both eyes at screening visit (this also applies to treatment naïve subjects). For OAG eyes, unmedicated (post washout) IOP must be ≥ 15 mmHg and < 35 mmHg in both eyes at 2 qualification visits (08:00 hour), 2-7 days apart. At second qualification visit IOP ≥ 15 mmHg and < 35 mmHg at 10:00 and 16:00 hours (in both eyes). For OHT eyes, unmedicated (post washout) IOP must be ≥ 22 mmHg and < 35 mmHg in both eyes at 2 qualification visits (08:00 hour), 2-7 days apart. At second qualification visit IOP ≥ 22 mmHg and < 35 mmHg at 10:00 and 16:00 hours (in both eyes). The specific inclusion and exclusion criteria can be found in Sections 4.2 and 4.3 of the study protocol.

2.4 Treatment Regimens

There will be 3 treatments in this study:

- Netarsudil 0.02%, QD
- Netarsudil 0.04%, QD
- Placebo, QD

Subjects will be instructed to self-administer their masked medication QD in the evening between 20:00 – 22:00 hours (8pm - 10pm). All treatments will be dosed OU. The treatment period will be 28 days.

2.5 Treatment Group Assignments or Randomization

A randomization code for allocating the treatments was prepared by an independent biostatistician, who was not involved in the day-to-day conduct of the study, and provided in confidence to the unmasked clinical supply manager personnel at Aerie, the unmasked director of quality assurance at Aerie, and the Interactive Web Response (IWR) system personnel. At Visit 3.2 (Day 1), qualified subjects will be randomized in a 1:1:1 ratio to receive netarsudil 0.02%, netarsudil 0.04%, or placebo (stratified by investigative site). Treatment assignments were masked to the Investigator, the Sponsor team members involved in the day-to-day oversight of the clinical study and employees of the CRO administrating the study for the Sponsor, and the study subjects. At the end of the study, the randomized treatment assignments will be presented in a data listing.

2.6 Sample Size Determination

Assuming a 2-sided test with alpha = 0.05 and a common standard deviation (SD) of 3.5 mmHg at each time point yielding a common SD of 3.0 for the diurnal mean assuming a correlation among time points of 0.60, 49 ITT subjects per arm yields at least 90% power to demonstrate superiority of netarsudil (0.02% or 0.04%) to placebo in mean diurnal study eye IOP (average of 08:00, 10:00, and 16:00 hours) at the Week 4 visit assuming a difference of at least 2.0 mmHg in the mean diurnal IOP. The study will be considered a success and superiority of netarsudil to placebo will be concluded if the two-sided p-value ≤ 0.05 at Week 4. With 60 intent-to-treat (ITT) subjects per arm, each test has 95% power to demonstrate superiority of netarsudil (0.02% or 0.04%) to placebo.

3. GENERAL ANALYSIS AND REPORTING CONVENTIONS

This section discusses general policies to be employed in the analysis and reporting of the data from the study. Departures from these general policies may be given in the specific detailed sections of this SAP. When this situation occurs, the rules set forth in the specific section take precedence over the general policies.

All continuous study assessments will be summarized by treatment and time point (as applicable) using descriptive statistics (n, mean, median, standard deviation (SD), minimum, and maximum). All categorical study assessments will be summarized by treatment and time point (as applicable) using frequency counts and percentages.

All study data will be listed by treatment, subject, visit, and time point (as applicable). Hypothesis testing, unless otherwise indicated, will be performed at a 2-sided 0.05 significance level. When applicable, two-sided 95% confidence intervals will be reported. All p-values will be displayed to four decimal places, with p-values less than 0.0001 presented as '<0.0001' and p-values greater than 0.9999 presented as '>0.9999'. Differences between each netarsudil group and placebo will be calculated as netarsudil – placebo.

For diurnally adjusted IOP, baseline will refer to the time-relevant measure at Visits 3.0 through 3.2 (e.g. IOP at 08:00 hours at Visit 3.0 will be the baseline for 08:00 hours at Visits 4.0, 5.0, and 6.0, etc.; IOP at 10:00 hours at Visit 3.1 will be the baseline for 10:00 hours at Visits 4.1, 5.1, and 6.1, etc.). For mean diurnal IOP, baseline will refer to the mean diurnal IOP at Visit 3. For all other variables, baseline is defined as the last measurement prior to the first dose of study medication. Change from baseline will be calculated as follow-up visit – baseline visit.

The unit of analysis for efficacy will be the study eye, defined as the eye with the higher IOP at 08:00 hours on Visit 3. If both eyes have the same IOP at 08:00 hours on Visit 3, then the right eye will be the study eye.

All study data will be listed by subject, treatment, and time point (as applicable). In the listings, individual subjects will be identified by a combination of site number and subject number, e.g., XXX-YYY, where XXX is the site number and YYY is the subject number.
All data analysis will be performed by [redacted] after the study is completed and the database has been locked and released for un-masking. Statistical programming and analyses will be performed using SAS® Version 9.4 or higher. Output will be provided in rtf format for tables and pdf format for tables, listings, and figures.

4. ANALYSIS POPULATIONS

4.1 Randomized Population

The randomized population will include all subjects who were randomized to treatment. Baseline variables and demographic characteristics will be summarized for this population.

4.2 Intent-to-Treat Population (ITT)

The ITT population will include all randomized subjects who have received at least one dose of study medication. 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.

4.3 Per-protocol Population (PP)

The PP population is a subset of the ITT population, which will include those subjects who do not have major protocol violations likely to seriously affect the primary outcome of the study as judged by a masked evaluation prior to the unmasking of the study treatment. 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 ITT 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.

4.4 Safety Population

The safety population 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.

5. STUDY SUBJECTS

5.1 Disposition of Subjects

Subject randomization, analysis populations, study completion, and withdrawal from the study will be summarized and listed for all randomized subjects. The summary table will include the numbers of subjects randomized and included in the analysis populations by randomized treatment group for the ITT population and by actual treatment group for the PP and Safety populations. It will also include the numbers of subjects who completed and discontinued from the study. The reasons for subject discontinuation and test agent discontinuation will be summarized for the applicable subjects. Reasons for subject discontinuation will include adverse event (AE), withdrawal of consent, non-compliance, lost to follow-up, lack of efficacy, disallowed concurrent medication, investigator decision, protocol violation, death, and an "other" category for reasons other than those previously listed.

By-subject listings will include randomization information, actual treatment assigned, first and last dose dates, exposure, study eye, and analysis population inclusion. For subjects who prematurely discontinue following randomization, an additional by-subject listing will be provided that shows treatment assignment, sex, age, date of last visit, date of last dose, treatment duration at time of discontinuation, study day of discontinuation, and reason for discontinuation. Study day of discontinuation will be calculated as (date of discontinuation – date of Visit 3 date + 1). Note that date of first dose is not collected, but will be assumed to be the Visit 3 date. Treatment duration will be calculated as the (Date of Last Dose – Visit 3 Date + 1).

5.2 Protocol Deviations

Protocol deviations will be evaluated for all subjects. Major protocol violations will be judged by a masked evaluation and summarized in writing prior to the unmasking of the study treatment, for the purpose of selecting the PP population. All subjects having a protocol deviation will be identified in a subject data listing. The number of subjects with protocol deviations and level of deviations (major or minor) will be summarized by treatment group and severity of the deviation along with the disposition data.

Failure to meet all Protocol Inclusion Criteria or meeting any Exclusion Criterion will also be considered for categorization of Protocol Deviation. Inclusion and Exclusion Criteria will be presented in a by-subject listing.

6. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

6.1 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized by treatment group and listed for the randomized population. Demographic parameters will include sex, age in years at signing of informed consent form (ICF), Japanese ethnicity (1st or 2nd generation), prior hypotensive therapy, and time on current hypotensive therapy. Baseline characteristics will include study eye diagnosis of OHT or OAG, length of time since study eye diagnosis of OHT or OAG, study eye IOP at screening, and study eye mean diurnal IOP at baseline. Additionally, means for both the study eye and the fellow eye will be summarized separately for the baseline ocular measurements of deviation in visual fields, central corneal thickness, and cup-to-disc ratio.

Age will be reported in years and calculated in SAS using the formula:

Age = Floor ((ICFDT – DOB) / 365.25)

where ICFDT is the date the subject signed the informed consent, DOB is the subject's date of birth, and Floor takes the integer part of the result.

Tests of differences between the treatment groups will be performed for both demographic and baseline characteristics. Categorical responses will be tested using Fisher's exact tests. Continuous measures will be tested using an analysis of variance model with treatment as the only explanatory variable.

6.2 Prior and Concomitant Medications

All medications which the subject has taken within 30 days prior to screening and during the study will be recorded on the CRF. The name of the drug, dose, route of administration, duration of treatment, and indication will be recorded for each medication. For combination products (e.g., Contac®), the brand name is required. For non-combination products, the generic name is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (e.g., fluorescein and local anesthetic) will be allowed, and individual documentation not required. Any change in dosing parameters should also be recorded on the CRF.

All prior and concomitant medications will be coded to preferred drug names and therapeutic drug class using the *WHO Drug Dictionary*, version Enhanced B2, March 2017. Use of concomitant medications will be summarized for each therapeutic drug class and each preferred drug name by treatment group. Subjects will be counted only once under each therapeutic drug class and preferred term for which they have used at least one concomitant medication. All prior and concomitant medication data will also be listed.

The class of prior hypotensive therapy, summarized on the baseline characteristic table and listed on the washout medication listing, will also be derived from the coded medication data. Classes include the following: Prostaglandins, β-adrenoceptor antagonists, adrenergic agonists (including α-agonists such as brimonidine and apraclonidine), Muscarinic agonists (e.g., pilocarpine), Rho Kinase inhibitors, and Carbonic anhydrase inhibitors (topical or oral). For the summary table, categories will be: combination therapy, prostaglandins (monotherapy), other (monotherapy),

and no prior therapy. Additionally, the following two classes will be summarized: prior prostaglandin therapy and no prior prostaglandin therapy.

6.3 Medical and Ocular History

A medical and ocular history will be collected at screening, including diagnosis, start date, and stop date (as applicable) or ongoing. For ocular history, the applicable eye(s) will be noted. Any change in the individual's baseline health status that occur after screening and prior to first dose of study medication should be reported as medical history events.

Ocular surgery and laser procedures will also be collected at screening including the procedure description, procedure date, and affected eye(s).

All medical history and ocular surgery/laser procedures data will be presented in a by-subject listing. Medical and ocular history will be coded to preferred terms and system organ class using the Medical Dictionary for Regulatory Activities (MedDRA), version 20.0. Medical and ocular histories will be summarized for each system organ class and each preferred term by treatment group. Subjects will be counted only once under each system organ class and preferred term for which they have at least one medical/ocular history.

Study eye diagnosis will be derived from the coded ocular history data. The preferred terms of "Ocular hypertension" and "Open angle glaucoma" will be matched to the study eye selection page of the CRF. Analyses based on study diagnosis will use this definition.

7. MEASUREMENTS OF TREATMENT COMPLIANCE

All subjects will be instructed to follow once a day dosing regimen in the evening between 20:00 – 22:00 hours (8pm - 10pm). All treatments will be dosed in both eyes (OU). No formal measure of treatment compliance is planned.

8. EFFICACY EVALUATION

8.1 Overview of Efficacy Analysis Issues

8.1.1 Handling of Dropouts or Missing Data

Any missing, unused, or spurious data will be noted in the final clinical study report. Due to the small sample size caused by early study closure, not all the analyses described in the protocol for the efficacy section will be performed. The selected analyses using the observed data will be performed for informational purposes only. No imputation techniques will be used.

8.1.2 Assessment Time Windows

In general, it is intended that all safety and efficacy data (with some exceptions) will be summarized at each time point collected regardless of assessment time windows. Because subjects may have an early termination visit at any time or may have unscheduled visits, the following conventions will be implemented.

For all safety data, the visit date or start date (e.g., adverse events) will be used to calculate study day, defined as the number of days from the day of first dose. The day of first dose (Visit 3) is considered study day 1, so study day will be computed as (date of data – Visit 3 + 1). Study day will be presented in listings for medical history, concomitant medications, and adverse events.

In all by-visit safety assessments, end of study visits and early termination visits will be combined in order to present all data available for each subject; early termination visits will not be windowed into the nearest fitting study visit. Each subject will have one end of study visit.

For efficacy outcomes, early termination data will not be combined with end of study visit information as the timing of the outcome measure is integral to the analysis. Instead, the efficacy outcome will be windowed into the nearest study visit, where Week 1 visit has a ± 2 day window and Week 2 and Week 4 visits have a ± 3 day window.

8.2 Efficacy Variables and Primary Hypotheses

1. The primary efficacy outcome will be the comparison of each netarsudil group to placebo for:
   - Mean diurnal IOP within a treatment group at the Week 4 study visit (Day 29) by Goldmann Applanation Tonometry.

2. Secondary efficacy endpoints will include the comparison of each netarsudil group to placebo for:
   - Mean diurnal IOP at Weeks 1 and 2 (Days 8 and 15, respectively)
   - Mean change from baseline in mean diurnal IOP at each post-treatment visit
   - Mean percent change from baseline in mean diurnal IOP at each post-treatment visit
   - Mean IOP at each post-treatment time point
   - Mean change from diurnally adjusted baseline IOP at each post-treatment time point

- Mean percent change from diurnally adjusted baseline IOP at each post-treatment time point
- Percentages of subjects achieving pre-specified mean, mean change, and percent mean change diurnal IOP levels at each post-treatment time point:
    - Diurnal mean IOP of ≤22, ≤21, ≤20, ≤19, ≤18, ≤17, ≤16, ≤15, ≤14
    - IOP reduction from baseline of ≥4, ≥6, ≥8, ≥10, ≥12 (IOP reduction at a visit from baseline was calculated as IOP [baseline] - IOP [visit], using mean [integral or non-integral] IOP values)
    - IOP percent reduction from baseline of ≥5, ≥10, ≥15, ≥20, ≥25, ≥30, ≥35, ≥40 (IOP percent reduction at a visit from baseline was calculated as [IOP reduction from baseline / IOP (baseline)] * 100%)

3. The primary hypotheses are:

- H_{01}: The difference between study eyes treated with netarsudil 0.02% and study eyes treated with placebo (netarsudil 0.02% - placebo), in mean diurnal IOP at Week 4 is equal to 0.

- H_{11}: The difference between study eyes treated with netarsudil 0.02% and study eyes treated with placebo (netarsudil 0.02% - placebo), in mean diurnal IOP at Week 4 is not equal to 0.

- H_{02}: The difference between study eyes treated with netarsudil 0.04% and study eyes treated with placebo (netarsudil 0.04% - placebo), in mean diurnal IOP at Week 4 is equal to 0.

- H_{12}: The difference between study eyes treated with netarsudil 0.04% and study eyes treated with placebo (netarsudil 0.04% - placebo), in mean diurnal IOP at Week 4 is not equal to 0.

Due to the early study closure, the sample size is expected to be approximately 10 ITT subjects per arm. The study will not be able to achieve the adequate power for primary hypotheses as planned. Therefore, the analyses will be performed based upon the observed data for informational purposes only. No statistical inference will be made. No subgroup analyses will be performed.

There will be no adjustment for the multiplicity of the 2 active concentrations (0.02% and 0.04%) tested against placebo in this Phase 2 study.

8.3 Analysis Methods

All continuous study assessments will be summarized by treatment and time point (as applicable) using descriptive statistics (n, mean, median, SD, minimum, and maximum). All categorical study assessments will be summarized by treatment and time point (as applicable) using frequency counts and percentages. All statistical tests will be performed at a 2-sided 5%

significance level. For the IOP measurements, 2-sided tests and 2-sided 95% confidence intervals will be reported.

Mean diurnal IOP values will be constructed by averaging the three IOP measurements on each of Week 1, Week 2, and Week 4. Mean diurnal baseline IOP will be constructed as the average of the three Day 1 IOP measurements. Mean change from mean baseline diurnal IOP will be created by taking the average of the three-time points on each of Week 1, Week 2, and Week 4 and subtracting the mean baseline diurnal IOP measurement.

For diurnally adjusted IOP measures, baseline will refer to the time-relevant measure at Visits 3.0 through 3.2 (e.g., IOP at 08:00 hours at Visit 3.0 will be the baseline for 08:00 hours at Visits 4.0, 5.0, and 6.0; IOP at 10:00 hours at Visit 3.1 will be the baseline for 10:00 hours at Visits 4.1, 5.1, and 6.1). For mean diurnal IOP, baseline will refer to the mean diurnal IOP at Visit 3. For all other variables, baseline is defined as the last measurement prior to the first dose of study medication. Change from baseline will be calculated as follow-up visit – baseline visit.

Percent change from diurnally adjusted baseline IOP will be determined by dividing the change from diurnally adjusted baseline by the corresponding baseline IOP value, such that a negative change from baseline will produce a negative percent change from baseline.

An abbreviated list of primary and secondary efficacy variables, along with the planned analysis methods for those variables, are given in Table 8-1. Note that each subject will have one eye designated as the study eye. Only study eyes will be evaluated for all the efficacy measures; however, both eyes will be treated. Fellow eyes will be evaluated separately for the primary analysis of the primary efficacy measure.

Table 8-1 Summary of Efficacy Variables and Analysis Methods

	Two Sample T-test[a]	ANCOVA[b]	MMRM[c]	Fisher's Exact Test[d]	Analysis Population	Data Used for Analysis
Primary Analysis						
Mean diurnal IOP at Week 4		X			ITT	Observed data
Secondary Analyses						
Mean diurnal IOP at Week 1 and Week 2	X	X			ITT	Observed data
Mean change from baseline in mean diurnal IOP at each post-treatment visit	X				ITT, PP	Observed data
Mean percent change from baseline in mean diurnal IOP at each post-treatment visit	X				ITT	Observed data
Mean IOP at each post-treatment time point	X	X			ITT, PP	Observed data
Mean IOP at each post-treatment time visit			X		ITT	Observed data
Mean change from diurnally adjusted baseline IOP at each post-treatment time point	X				ITT, PP	Observed data
Mean percent change from diurnally adjusted baseline IOP at each post-treatment time point	X				ITT	Observed data
Percentages of subjects achieving pre-specified mean, mean change, and percent mean change diurnal IOP levels				X	ITT	Observed data

[a] Two Sample T-test comparing actual mean IOP value at each time point between each netarsudil group and placebo.

[b] ANCOVA model including treatment as the main effect and baseline as the covariate. Individual models will be fit for each visit and time point.

[c] Mixed Model Repeated Measures analysis including treatment as the main effect, and baseline IOP, visit, time point, treatment*visit, treatment*time point, visit*time point, and treatment*visit*time point as model terms. Repeated measures will be used to account for the correlation among measures within a subject. The model will include all post-dose visits and time points.

[d] Fisher's exact test comparing the incidence in each category at each time point between each netarsudil group and placebo.

8.3.1 Primary Efficacy Analyses

Only observed data will be used for efficacy analyses. The primary analysis of the primary outcome will employ a linear model with mean diurnal IOP at Week 4 as the response, baseline mean diurnal IOP as a covariate, and treatment as a main effect, using the ITT population. The treatment differences (netarsudil – placebo) between each netarsudil group and placebo will be

tested. The least squares mean differences (netarsudil – placebo) will be presented separately for netarsudil 0.02% and 0.04% as well as 2-sided p-values and 95% confidence intervals (CIs). However, no statistical inference will be made.

8.3.2 Secondary Efficacy Analyses

The secondary efficacy analyses will include additional analyses of the primary efficacy endpoint as well as other analyses of the secondary endpoints, as outlined below.

The primary efficacy analysis will be repeated on the PP population.

Secondary analyses of the primary endpoint will be completed using individual two-sample t-tests and 95% t-distribution confidence intervals for each comparison (each netarsudil group vs placebo) using the ITT and PP populations.

Similar analyses will be completed on the secondary endpoints based on the ITT and PP populations: mean change from baseline in mean diurnal IOP at each post-treatment visit, mean IOP at each post-baseline time point, and mean change from diurnally adjusted baseline IOP at each post-treatment time point. Models adjusting for baseline will only be performed on the mean IOP response variable as inference is identical between this response and the change from baseline IOP response variable in such a model based on the PP and ITT populations.

Additionally, for the mean IOP values at each post-treatment time visit, mixed model repeated measures will be run with mean IOP baseline as the covariate; treatment, visit, time point, treatment by visit, treatment by time point, and treatment by visit by time point as the fixed effects; and subject as the repeated measure. An unstructured covariance structure will be used model covariances. This model will be run including the Week 1, Week 2, and Week 4 visits based on the ITT population only.

Graph will also be provided for mean IOP values by treatment group, visit, and time point.

Percent change from baseline in mean diurnal IOP at each post-treatment visit and percentage change from diurnally adjusted baseline IOP at each post-treatment time point will be analyzed using two-sample t-tests, between each netarsudil group and placebo, at each time point and visit, including two-sample t-tests and 95% t-distribution confidence intervals on the difference (netarsudil – placebo). The analysis will be based on ITT population only.

Analyses of IOP will also include summarizing the number and percentage of study eyes achieving mean diurnal IOP reduction from baseline of ≥ 4 to ≥ 12 mmHg in 2 mmHg increments and percent reduction from baseline of $\geq 5\%$ to $\geq 40\%$ in 5% increments at Week 1, Week 2, and Week 4. Additionally, the number and percentage of study eyes attaining a mean diurnal IOP of ≤ 22 to ≤ 14 mmHg in 1 mmHg increments will be summarized at Week 1, Week 2, and Week 4. Fisher's exact test (2-sided p-values) will be used to test the pair wise differences between treatment groups for each category at each visit. These analyses will be presented for the ITT population only.

9. SAFETY EVALUATION

9.1 Overview of Safety Analysis Methods

The assessment of safety and tolerability is the secondary objective of this study. All safety analyses will be carried out using the Safety Population and will include the study eye and fellow eye separately, where applicable.

The assessment of safety will be evaluated by:

- Adverse events
- Heart rate and blood pressure
- Biomicroscopy of anterior segment including evaluation of cornea, conjunctiva and anterior chamber. (Fluorescein staining to be used.)
- Dilated ophthalmoscopy
- Best Corrected ETDRS Visual Acuity
- Visual fields
- IOP
- Clinical chemistry, hematology and urinalysis laboratory findings

All safety variables will be descriptively summarized by treatment group at each assessment time and for relevant changes from baseline.

For complete inclusion of subjects who withdraw from the study early, the End of Study visit for safety outcomes will be defined as either Visit 6 or Early Discontinuation.

9.2 Extent of Exposure

Summary statistics will be presented for treatment exposure. Note that date of first dose is not collected, but will be assumed to be the Visit 3 date. Treatment exposure will be defined as the number of days that the subject was exposed to study treatment as calculated using the formula:

Treatment exposure = Date of Last Dose – Visit 3 Date + 1.

9.3 Adverse Events

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. All AEs occurring during the study (i.e. once the subject has received one dose of study drug) will be defined as treatment emergent adverse events (TEAEs) and must be documented, regardless of the assumption of causal relationship, on the respective AE CRF. All adverse events should be documented from the time the subject receives the first dose of study drug until the subject's participation in the study has been completed. If a subject has ongoing AEs at the time of study completion, the ongoing AEs must be followed-up and provided appropriate medical care until the event has resolved or stabilized. Documentation of AEs includes onset date, severity, action(s) taken, study medication relationship, outcome, resolution date, and seriousness.

Verbatim descriptions of AEs will be mapped to MedDRA, Version 20.0, thesaurus terms and be presented in a data listing. Ocular and non-ocular treatment emergent AEs, those that occur after the first dose of study medication, will be summarized by treatment group using frequencies and proportions for each system organ class (SOC) and preferred term (PT) within each SOC. Additionally, separate summaries will be presented for serious TEAEs, TEAEs that are related to the study drug (marked as "possibly related" or "related" in the CRF) and TEAEs resulting in study drug discontinuation. For these summaries, Fisher's exact tests will be used to test the difference in proportions of subjects with each TEAE between treatment groups, SOC, and PT. Another table will be presented summarizing TEAEs by maximum severity.

An overall summary table will be developed to report the number of events and the incidence of subjects having at least one event in the following categories:

- TEAEs
- Ocular TEAEs
- Non-Ocular TEAEs
- Serious TEAEs (SAEs)
- Treatment-Related TEAEs (reported as possibly related or related to the study drug)
- Treatment-Related SAEs
- TEAEs by severity
- TEAEs leading to study drug discontinuation
- TEAEs resulting in death

While the overall summary of TEAEs will present both the number of TEAEs and the incidence of TEAEs, the other summaries will only report the incidence of TEAEs. When reporting the number of TEAEs, if the same TEAE occurs for a subject on multiple occasions the event will be counted once for each occurrence. When reporting the incidence, a subject will only be counted once if they ever experience an event within the SOC and ever experience the individual PT. Summaries will be performed using the actual treatment received.

9.4 Deaths, Serious Adverse Events, and Other Significant Adverse Events

An adverse event 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 or sight-threatening adverse event, 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. Serious adverse events and deaths will be listed and summarized separately for the Safety population.

9.5 Clinical Laboratory Evaluation

Clinical laboratory results, including clinical chemistry, hematology and urinalysis, will be presented in data listings. Abnormal results, those above or below the normal range, will be flagged with "H" (high), "HP" (high panic), "L" (low), "LP" (low panic) or "AB" (abnormal) based on the laboratory ranges provided by the lab and included in the listings. Descriptive statistics will be used to summarize continuous measures and will be presented in tabular form

including change from baseline to the Week 4 visit. Shifts from baseline to the Week 4 visit will also be presented in tabular form for clinical chemistries, hematology and urinalysis parameters by treatment group and laboratory domain. Summary tables also will be presented for the number of subjects with abnormal values within each laboratory parameter at the end of the study. Clinical laboratory results, including clinical chemistry, hematology, urinalysis and urine pregnancy test will also be listed by subject.

9.6 Vital Signs, Ophthalmic Exam Findings, and Other Safety Outcomes

9.6.1 Vital Signs

Heart rate and blood pressure will be listed by treatment group, subject, and visit including observed values and changes from baseline. Measurements and change from baseline measurements will be summarized by treatment group and visit. Paired t-tests will be used to test within treatment group changes from baseline in vital sign parameters. Two-sided 95% CIs will be provided.

9.6.2 Intraocular Pressure

Intraocular pressure will be presented in data listings. Intraocular pressure data will be summarized at each visit and time point using continuous summaries, including change from baseline, for both the study eye and the fellow eye.

9.6.3 Visual Acuity

Visual acuity scores will be presented in data listings. Subjects who lost three or more lines will be presented in an additional listing. Visual acuity data will be summarized at each visit using continuous summaries, including change from baseline, for both the study eye and the fellow eye. Additionally, discrete summaries of the worst change from baseline will be presented for both the study eye and the fellow eye with the following groupings based on the logMAR scores: 0 or less, >0 to +0.09, +0.10 to +0.19, +0.20 to 0.29, +0.30 or more.

9.6.4 Dilated Ophthalmoscopy and Cup-to-Disc Ratio

Dilated ophthalmoscopy and cup-to-disc ratio results from Screening and Week 4 visits will be presented in data listings. A separate listing will be created for those subjects with a criterion change, defined as a change from “Normal” to “Abnormal” or a change from “Abnormal – Not Clinically Significant” to “Abnormal – Clinically Significant”. Frequencies and percentages of normal, abnormal – not clinically significant, and abnormal – clinically significant ophthalmoscopy results will be created for the following fields: Retina, Macula, Choroid, Optic Nerve, and Vitreous Humor. Abnormal results will further be broken down by clinical significance. A shift table of study eye and fellow eye ophthalmoscopy results will also be presented by treatment group.

Vertical cup-to-disc ratio at Screening will be summarized for the study eye and for the fellow eye by treatment group in the baseline characteristics table. Additionally, vertical cup-to-disc ratio will be summarized at Week 4 visit and change from baseline to Week 4 visit using

continuous summary statistics. A listing of subjects with increases of ≥ 0.2 in either eye at end of study will be presented.

9.6.5 Biomicroscopy

Biomicroscopy results will be listed for both eyes at each visit and time point. A separate listing will be presented for subjects with a criterion change, defined as a +1 unit increase from baseline. Summaries of biomicroscopy results will also be presented for study eyes and fellow eyes by treatment group, visit, and time point in tabular form.

A summary table of the number and percentage of subjects with at least a +1 unit increase in score from baseline will be presented by region, finding, time point and eye (study eye and fellow eye). In addition to each time point, there will be summaries for "At the Final Visit" and "At Any Visit".

Another summary table will be presented with the number and percentage of subjects who had a finding judged to be clinically significant by region, finding, time point and eye (study eye and fellow eye). Fisher's exact tests will be used to compare incidence between treatment groups in both tables. Additionally, Conjunctival Hyperemia will be presented using continuous summary statistics for each visit, for the change from baseline to each post-baseline visit. Differences between treatment groups will be tested using two-sample t-tests as well as Wilcoxon rank sum tests.

9.6.6 Visual Field Examination, Pachymetry, and Gonioscopy

Visual field examination results will be collected at Screening and listed for each subject. Visual field mean deviation (dB) at Screening will be summarized using continuous summary statistics for the study eye and fellow eye separately in the baseline characteristics summary.

Central corneal thickness, collected at Screening will be listed for each subject. Central corneal thickness will be summarized using continuous summary statistics by treatment group for the study eye and fellow eye separately in the baseline characteristics summary.

Gonioscopy will be collected at screening for each eye and listed for each subject. Possible values will be Open Angle, Narrow Angle, and Closed Angle.

10. PHARMACOKINETIC EVALUATION

Not applicable.

11. OTHER ANALYSES

Any additional analyses conducted will be considered exploratory and enumerated in the CSR.

12. INTERIM ANALYSES AND DATA MONITORING

No planned interim analysis.

13. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

Due to the small sample size caused by early study closure, not all the analyses listed in the protocol for the efficacy section will be performed. Instead, only the selected analyses using the observed data will be performed for information purposes only. No statistical inference will be made. No subgroup analyses will be performed.

14. REFERENCES

US Federal Register. (1998) International Conference on Harmonization; Guidance for Industry: Statistical Principles for Clinical Trials. Department of Health and Human Services: Food and Drug Administration. Federal Register, Vol. 63, No. 179, September 16, 1998, page 49583. (E9)

US Federal Register. (1996) International Conference on Harmonization; Guidance for Industry: Structure and Content of Clinical Study Reports. Department of Health and Human Services: Food and Drug Administration. Federal Register Vol. 61, July 17, 1996, page 37320. (E3)

15. APPENDICES

Appendix 1: Schedule of Visits and Examinations

						Post Day 1 Treatment								
Day (D)/Week (W)	Screening	Qual. #1	Qual. #2 (Day 1)			W1 (Day 8±2)			W2 (Day 15±3)			W4 (Day 29±3)		
Visit	1	2	3.0	3.1	3.2	4.0	4.1	4.2	5.0	5.1	5.2	6.0	6.1	6.2
Hour (XY = XY:00)		08	08	10	16	08	10	16	08	10	16	08	10	16
Informed Consent	X													
Inclusion/Exclusion	X	X	X	X	X									
Washout[1]	X													
Demography	X													
Medical/Ophthalmic History	X	X	X											
Concomitant Medications	X	X	X			X			X			X		
HR/BP	X	X	X			X			X			X		
Urine Pregnancy Test[2]	X												X	
Clinical Labs (Chem/Hem/UA)	X[3]												X	
Symptoms/AEs[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Visual Acuity	X	X	X			X			X			X		
IOP	X	X[5]	X[5]	X[5]	X[5]	X	X	X	X	X	X	X	X	X
Biomicroscopy	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gonioscopy[6]/Ultrasound Pachymetry[7]	G/P													
Visual Field[8]	X													
Ophthalmoscopy (dilated)	X													X
Eye-Drop Instillation Evaluation	X													
Study Medications Dispensed					X									
Study Medications Collected												X[9]		

						Post Day 1 Treatment								
Day (D)/Week (W)	Screening	Qual. #1	Qual. #2 (Day 1)			W1 (Day 8±2)			W2 (Day 15±3)			W4 (Day 29±3)		
Visit	1	2	3.0	3.1	3.2	4.0	4.1	4.2	5.0	5.1	5.2	6.0	6.1	6.2
Hour (XY = XY:00)		08	08	10	16	08	10	16	08	10	16	08	10	16
Study Completed														X

Abbreviations: HR/BP = heart rate/blood pressure; Chem/Hem/UA = Chemistry/Hematology/Urinalysis; AE = adverse event; IOP = Intraocular pressure.

Early Discontinuation: Visit 6.0 procedures are to be completed plus a dilated ophthalmoscopy examination.

Visit Requirements: IOP measurements at all visits are to be made within ±½ hour of the protocol-specified times of 08:00, 10:00, and 16:00 hours with the exception of the Visit 1 (Screening).

[1] Subjects currently using ocular hypotensive medications must undergo a minimum washout period (see Protocol Section 5.7.1).

[2] Urine pregnancy test for women of childbearing potential is required.

[3] For subjects who are unable or unwilling to have blood drawn and urine collected for clinical labs at Visit 1 (Screening), the blood and urine sample may be drawn at Visit 2 (Qualification Visit #1) so long as the results of the clinical labs are available for that subject prior to Visit 3 (Qualification Visit #2).

[4] Ocular symptoms: Subjects will be queried at each visit "How are you feeling?" and treatment emergent AEs beginning at Visit 4 will be documented on the AE form. Additional symptoms reported after screening and before randomization will be documented on the medical history form. Adverse events will be recorded for every study visit (i.e., at 08:00, 10:00, and 16:00 hours) as needed.

[5] Individuals returning at an Unscheduled Visit within 1 week to re-attempt IOP qualification are required to only re-measure IOP in both eyes.

[6] Gonioscopy evaluation up to 3 months prior to randomization is acceptable.

[7] Ultrasound pachymetry within 1 week prior to screening is acceptable.

[8] Entry visual field evaluation up to 3 months prior to randomization is acceptable. Visual field collection must meet the requirement for automated threshold visual field assessment (e.g., 30-2 or 24-2 Humphrey) and reliability.

16. ATTACHMENTS

- AR-13324-CS205 Listing Shells
- AR-13324-CS205 Table Shells