Statistical Analysis Plan Cover Page

Official Study Title:	A Randomized, Double-Masked, Parallel-Group, Multicenter Study Assessing the Safety and Efficacy of DE-117 Ophthalmic Solution 0.002% Once Daily and Twice Daily in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - SPECTRUM 6 Study
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STATISTICAL ANALYSIS PLAN

DE-117 SPECTRUM 6 Study

Protocol Title:	A Randomized, Double-Masked, Parallel-Group, Multicenter Study Assessing the Safety and Efficacy of DE-117 Ophthalmic Solution 0.002% Once Daily and Twice Daily in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - SPECTRUM 6 Study
Product:	0.002% DE-117 ophthalmic solution
Protocol Number:	011712IN
Sponsor:	Santen Inc. 6401 Hollis Street, Suite 125 Emeryville, CA 94608 USA
Date:	July 11, 2019
Version:	1.0

CONFIDENTIAL

APPROVAL SIGN-OFF SHEET

A Randomized, Double-Masked, Parallel-Group, Multicenter Study Assessing the Safety and Efficacy of DE-117 Ophthalmic Solution 0.002% Once Daily and Twice Daily in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - SPECTRUM 6 Study

DE-117 SPECTRUM 6 Study

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ABBREVIATIONS

AE	adverse event
ATC	Anatomical-Therapeutic-Chemical
BCVA	Best-corrected visual acuity
CFR	Code of Federal Regulations
CI	confidence interval
CSR	Clinical Study Report
eCRF	electronic Case Report Form
ESI	event of special interest
ET	early termination
FAS	full analysis set
IOP	intraocular pressure
LOCF	last-observation-carried-forward
logMAR	logarithm of the minimum angle of resolution
MedDRA	Medical Dictionary for Regulatory Activities
mmHg	millimeter of mercury
OHT	ocular hypertension
OD	oculus dexter (right eye)
OS	oculus sinister (left eye)
OU	oculus uterque (both eyes)
POAG	primary open-angle glaucoma
PPS	per-protocol set
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan
SAR	suspected adverse reaction
SAS	Statistical Analysis System
SOC	system organ class
WHO-DDE	World Health Organization Drug Dictionary Enhanced

1. INTRODUCTION

This statistical analysis plan (SAP) specifies the statistical methods to be implemented for the analysis of data collected from the SPECTRUM 6 study within the scope of Santen's Protocol 011712IN, "A Randomized, Double-Masked, Parallel-Group, Multicenter Study Assessing the Safety and Efficacy of DE-117 Ophthalmic Solution 0.002% Once Daily and Twice Daily in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - SPECTRUM 6 Study". It applies to the study protocol dated 13 Nov 2018 and provides detailed instructions as to how each analysis will be performed.

Results obtained from the analyses specified in the final approved version of the SAP will become the basis of the clinical study report (CSR) for this study. Any deviations from the final approved version of the SAP must be substantiated by sound statistical reasoning and documented in the CSR.

2. OBJECTIVE(S) AND ENDPOINTS

Table 1: Study Objectives and Endpoints

Study Objectives	**Corresponding Study Endpoints**
Primary Objective: To determine whether DE-117 Ophthalmic Solution 0.002% given BID is superior to QD in reducing the intraocular pressure (IOP) after 6 weeks of treatment in subjects with POAG or OHT.	Primary Efficacy Endpoint: The primary efficacy endpoint is the IOP in the study eye measured at the specified time points: 08:00, 12:00 and 16:00 at Week 2 (Visit 3) and Week 6 (Visit 4).
	Secondary Efficacy Endpoints: • Mean diurnal IOP in the study eye at Week 6 (Visit 4) • Absolute change and percent change from baseline in IOP • Absolute change and percent change from baseline in mean diurnal IOP • Having a mean diurnal IOP reduction ≥ 20%, ≥ 25%, or ≥ 30% from Baseline (Visit 2) at each post-baseline visit • Having a mean diurnal IOP ≤ 18 mmHg at each post-baseline visit

Table 1: Study Objectives and Endpoints (Continued)

Study Objectives	Corresponding Study Endpoints
Safety Objective: To determine the safety of DE-117 ophthalmic solution 0.002% given BID as compared to QD in subjects with POAG or OHT.	**Safety Endpoints:** Safety will be evaluated by the following parameters: • Incidence of ocular and systemic AEs • Best-corrected visual acuity (BCVA) • Slit-lamp biomicroscopy findings: anterior chamber cells, anterior chamber flare, lid hyperemia, lid edema, conjunctival hyperemia, conjunctival chemosis, corneal edema, corneal staining, keratin precipitates, abnormal lens findings, anterior synechiae of iris, posterior synechiae of iris • Ophthalmoscopy

3. STUDY DESIGN

3.1. General Study Design

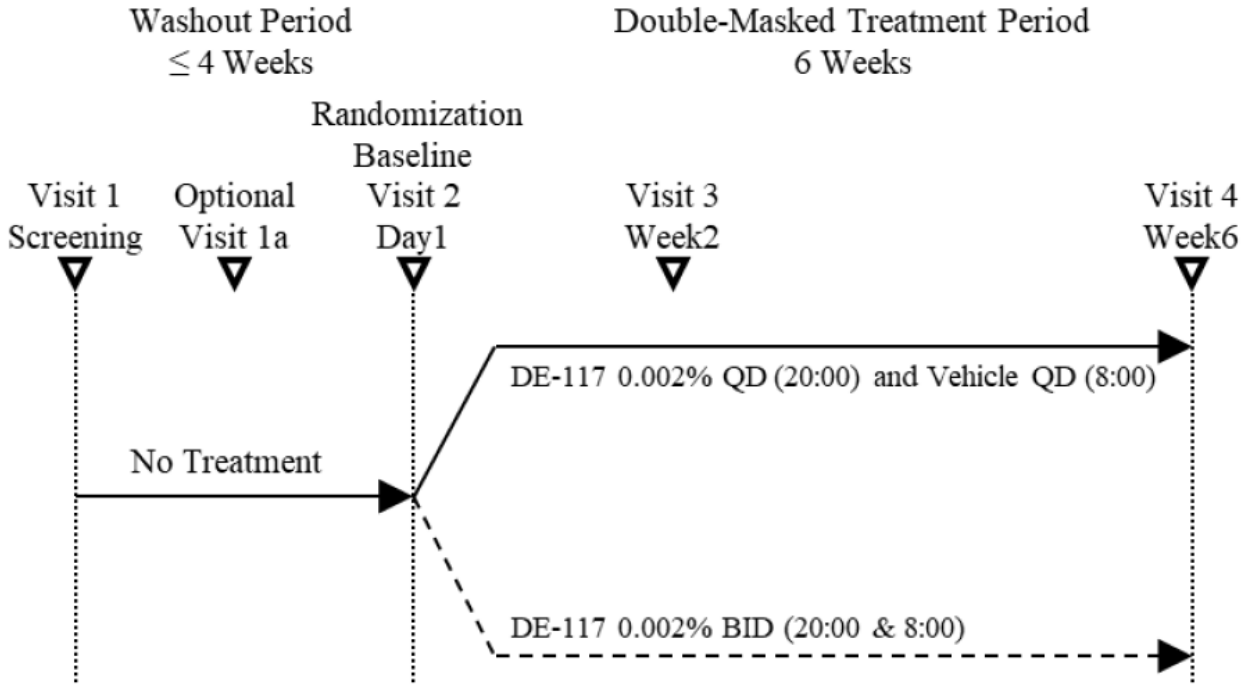
This is a randomized, double-masked, parallel-group, multi-center study. Subjects diagnosed with POAG or OHT who meet eligibility criteria at Visit 1 (Screening) will wash out their current topical IOP lowering medication(s), if any. After completing the required washout period, subjects will return for Visit 2 (Baseline, Day 1). Subjects who meet all eligibility criteria at Visit 2 will be randomized to receive study medication for up to 6 weeks.

Approximately 100 subjects with POAG or OHT will be randomized in a 1:1 ratio to either:

- DE-117 ophthalmic solution 0.002% QD (20:00) and Vehicle QD (08:00), or
- DE-117 ophthalmic solution 0.002% BID (20:00 & 08:00)

As shown in the Study Schema (Figure 1), this study will consist of a screening period of up to 35 days including a washout period of up to 28 days (+ 7 days window), and a 6-week double-masked treatment period.

Figure 1: Study Schema



At Screening (Visit 1), subjects who provide written informed consent and meet all eligibility criteria are required to wash out any IOP-lowering medication(s) they are using, or wait at least one day if they have not used an IOP-lowering medication for the last 28 days, before returning for further eligibility assessments at baseline (Visit 2; Day 1). Subjects who meet all eligibility criteria at baseline will be randomized in a 1:1 ratio to receive either DE-117 0.002% QD in the evening and vehicle QD in the morning or DE-117 0.002% BID for 6 weeks with scheduled Visits 2, 3 and 4 (Baseline, Week 2 and Week 6). Post-baseline safety and efficacy measures will be collected at scheduled visits and Study Exit/Early Termination (ET) according to the Assessment Schedule (Table 2).

3.2. Randomization and Masking

A permuted-block randomization will be employed to randomize eligible subjects in a 1:1 ratio to either DE-117 QD/Vehicle QD arm or DE-117 BID arm. The randomization schedule will be generated and implemented using central randomization via Interactive Response Technology (Medidata Balance/RTSM).

Treatment assignments will be masked to Santen, study subjects, and Clinical Investigator. The appearance of the bottles of DE-117 ophthalmic solution 0.002% and the vehicle will be identical. All subjects will receive bottles labeled “morning” for the morning dose and bottles labeled “evening” for the evening dose. Each eligible subject will be assigned to receive a numbered study medication kit as assigned by central randomization via Interactive Response Technology at Visit 2 (Baseline). In case of a medical emergency, the Principal Investigator may reveal the treatment information by unmasking through Medidata Balance/RTSM to know which treatment the subject has received.

3.3. Sample Size Planning

The sample size calculation does not consider multiplicity from multiple time points. Assuming a two-sided type I error rate of 5% and a standard deviation of 3.5 mmHg, approximately 50 subjects per treatment arm (100 in total) will have about 80% power to detect a 2.0 mmHg between-treatment difference.

3.4. Visits and Assessments

There are 4 scheduled visits for each subject. Assessments at each visit and the time/visit window for each post-baseline assessment are specified in the Assessment Schedule (Table 2). For subjects whose study participation is terminated prior to Week 6 (Visit 4, Day 43 ± 3), to the extent possible, all assessments scheduled for Visit 4 will be performed at the Early Termination (ET) visit.

Table 2: Assessment Schedule

	Washout Period		Double-Masked Treatment Period		
	Visit 1 (Screening)	**Washout Period (up to 4 weeks) Optional Visit 1[k]**	**Visit 2 Eligibility/ Baseline (Day 1)**	**Visit 3 Week 2 (Day 15±2)**	**Visit 4 Week 6 (Day 43±3) Exit or Early Termination[l]**
Informed Consent(s) including the consent for pharmacogenomics/ genomics laboratory research study[a]	X				
Inclusion/Exclusion Criteria	X		X		
Demographics and Medical History	X				
Concomitant Medications / Therapies	X	X	X	X	X
Dosing Compliance				X	X
Adverse Events		X	X	X	X
Pregnancy Test[b]	X		X		X
Refraction[c]	X				
BCVA[c]	X	X	X (prior to 8:00 IOP meas.)	X (prior to 8:00 IOP meas.)	X (prior to 8:00 IOP meas.)
Biomicroscopy[d]	X	X	X (prior to 8:00 IOP meas.)	X (prior to 8:00 IOP meas.)	X (prior to 8:00 IOP meas.)
IOP[e]	X (any time)	X (any time)	08:00 12:00 16:00	08:00 12:00 16:00	08:00 12:00 16:00
Pachymetry[f]	X				
Instill study eye drop after 08:00 IOP measurement				X (after to 8:00 IOP meas.)	X (after to 8:00 IOP meas.)
Gonioscopy[g]	X				
Visual Field[h]	X				
Ophthalmoscopy[i]	X (pupil dilation)		X (after 16:00 IOP meas.)		X (after 16:00 IOP meas., pupil dilation)
Blood Sampling for Pharmacogenomics/genomics[j]				X	
Dispense Study Medication			X		
Collect Study Medication					X

Table 2: Assessment Schedule (Continued)

	Washout Period		Double-Masked Treatment Period		
	Visit 1 (Screening)	**Washout Period (up to 4 weeks) Optional Visit 1[k]**	**Visit 2 Eligibility/ Baseline (Day 1)**	**Visit 3 Week 2 (Day 15±2)**	**Visit 4 Week 6 (Day 43±3) Exit or Early Termination[l]**
Phone call to remind subjects to take morning and evening dose on the day before each visit				X	X

[a] **Informed Consent(s) including the consent for pharmacogenomics/ genomics laboratory research study**
Informed consent form (ICF) must be signed and dated before study procedures are performed. Informed consent for the optional pharmacogenomics/genomics laboratory research study may be obtained at any visit prior to study exit.

[b] **Pregnancy Test**
A urine pregnancy test will be conducted for all female subjects of childbearing potential.

[c] **Refraction and BCVA**
Refraction will be performed at the screening visit. If more than 10 letters in BCVA were lost compared to the screening visit, then refraction should be performed again. BCVA examination will be completed before IOP is measured at 08:00 (±60 min).

[d] **Biomicroscopy**
Biomicroscopy examination must be completed before IOP is measured at 08:00. Aqueous flare and cell evaluation will be performed before fluorescein instillation.

[e] **IOP**
IOP measurements will be performed at 08:00, 12:00 and 16:00 (±60 min) at all visits except for Visit 1 and optional Visit 1a (screening and mid-washout).

[f] **Pachymetry**
Pachymetry will be performed after IOP measurement at Visit 1 (Screening).

[g] **Gonioscopy**
If gonioscopy was performed within 3 months (90 days) prior to screening and was documented in the subject's records, no additional screening gonioscopy examination is necessary.

[h] **Visual Field**
If visual field test was performed within 3 months (90 days) prior to screening and was documented in the subject's records, no additional screening visual field test is necessary.

[i] **Ophthalmoscopy**
Ophthalmoscopy will be performed at Visits 1, 2 and 4 (i.e. Screening, Baseline and Week 6) after the very last IOP measurement. Ophthalmoscopy will be performed with pupil dilation at Visit 1 and Visit 4/ Study Exit or Early Termination. Dilation of the pupil will be performed after the very last IOP measurement at Visit 1 and Visit 4/Study Exit or Early Termination.

[j] **Blood Sampling for Pharmacogenomics/genomics**
Blood sampling for the pharmacogenomics/genomics laboratory research study may be performed at any visit after pharmacogenomics/genomics informed consent is obtained, subject is randomized and study drug, DE-117 ophthalmic solution 0.002%, dosing has begun.

[k] **Optional Visit 1a**
An interim safety visit may be performed during the washout period Visit 1a (optional, mid-washout visit), if in the Investigator's opinion a subject's IOP may be of concern. If subjects are treated with a topical CAI during the washout period. Visit 1a (optional, mid-washout visit) is recommended to be performed.

[l] **Exit or Early Termination**
All listed procedures are required for early terminated subjects.

4. DEFINTIONS

4.1. Time-Related Terms

4.1.1. Baseline Visit

The *Baseline visit* is Visit 2 (Day 1) when the subject is randomized.

4.1.2. Treatment Start and End Dates

Treatment start date is the date at which a randomized subject takes the first dose of the study medication.

Treatment end date is the date at which a randomized subject takes the last dose of the study medication. If the date of the last dose is missing, then

- The day before the Visit 4 (Week 6) will be considered the treatment end date for subjects who completed the study, and
- The day before the Exit Visit date will be used for subjects who prematurely discontinued from the study. If the Exit Visit date of a non-completer is not available, then the day before the last available visit date will be considered the treatment end date.

4.1.3. Study Day

The *study day* describes the relative day of an observation starting with the reference date designated as Study day 1. In this study, the Visit 2 (Baseline) date is the reference date. Thus, the study day will be calculated as:

- For days prior to the Visit 2 (Baseline) date, Study Day = Date – Visit 2 (Baseline) Date
- For days on/after the Visit 2 (Baseline) date, Study Day = Date – Visit 2 (Baseline) Date + 1

4.1.4. Out-of-Window Measurements, Analysis Visit, and Analysis Window

Analysis visit is a timing variable to be used for analyses involving visits. For each analysis visit, an *analysis window* is set up to determine the analysis visit to which a measurement should be mapped (Table 3).

The analysis visit of a measurement will be determined based on the study day of the measurement and specified analysis windows and is not necessarily the same as the study visit at which the measurement was collected. For example, an out-of-window measurement collected at the Week 2 study visit will be mapped to the Week 6 analysis visit, if the study day of the measurement falls into the analysis window of Week 6.

Table 3: Analysis Visit and Analysis Window

Analysis Visit (Target Assessment Date)	Protocol Visit Window	Analysis Window
Baseline (Day 1)	[1, 1]	- 1]
Week 2 (Day 15)	[13, 17]	[2, 28]
Week 6 (Day 43)	[40, 46]	[29, 57]

In case that there are two or more visits that fall into the same analysis window, then the visit closest to the target assessment day will be selected for that visit window. In the case that two visits are equidistant to the target assessment day, i.e., one is before and one is after the target assessment day, the later one will be selected for that visit.

For analyses of IOP involving post-baseline visits, if there are two or more visits that fall into the same analysis window of a post-baseline visit, then the visit in which IOP are measured at all the scheduled timepoints (8:00, 12:00, and 16:00) will be selected for that analysis visit first, before applying the above rule.

Furthermore, if applying this analysis window results in missing IOP data, then the nominal visit will be used.

4.1.5. Out-of-Time-Window Measurements, Analysis Timepoint and Analysis Timepoint Window

Analysis timepoint is a timing variable to be used for analyses involving timepoints. In general, the analysis timepoint is the scheduled timepoint per protocol. For this study, the pre-specified IOP measurements time window in the protocol is (8:00±60 mins, 12:00 ±60 mins, 16:00 ±60 mins).

For each analysis timepoint, an *analysis timepoint window* is set up to determine the allowable range (Table 4).

Table 4: Analysis Timepoint and Analysis Timepoint Window

Analysis Timepoint	Timepoint Window	Analysis Timepoint Window
8:00	[7:00, 9:00]	- 9:59]
12:00	[11:00, 13:00]	[10:00, 13:59]
16:00	[15:00, 17:00]	[14:00 -

If there are two or more measurements that fall into the same analysis timepoint window, the measurement closest to the target assessment time will be selected for that timepoint. In the case that two measurements are closest and equidistant to the target assessment time, i.e., one is before and one is after the target assessment time, the later one will be selected for that timepoint.

Furthermore, if applying this analysis timepoint window results in missing IOP data, then the nominal timepoint will be used for that visit.

4.2. Efficacy-Related Definitions

4.2.1. Study Eye and Fellow Eye

The study eye will be the eye that qualifies per eligibility criteria at Visit 2. If both eyes meet the eligibility criteria, the eye with the higher mean diurnal IOP at Visit 2 will be designated as the study eye. If both eyes meet the eligibility criteria and have the same mean diurnal IOP, the right eye will be designated as the study eye. The other eye will be the non-study eye, or fellow eye.

4.2.2. Efficacy Measure

IOP (measured in mmHg) is the efficacy measure for this study. For the IOP at each timepoint of measurement, a mean of two consecutive measurements using the Goldmann applanation tonometer will be used. If the two measurements differ by 2 mmHg or more, then a third measurement will be made, and the median of the three consecutive measurements will be used.

For the mean diurnal IOP, the mean of the three scheduled timepoints (8:00, 12:00, and 16:00) will be used.

4.2.3. Baseline Score

The *baseline score* is the observed measurement at Visit 2 (Baseline). If a baseline score is missing, the last observed measurement or derived score prior to the first dose of study medication will be used to impute the baseline score.

4.2.4. Change and Percent Change from Baseline

The change and the percent change from baseline in a measure at a post-baseline visit will be derived as:

- Change = (Score at the Post-Baseline Visit) – (Baseline Score)
- Percent Change from Baseline = 100 × Change / (Baseline Score)

4.2.5. IOP Response Endpoints and Response Rate

At each post-baseline visit (Week 2 or Week 6), the following response endpoints are defined:

- ≥ 20% Reduction Response: the percent reduction from baseline in mean diurnal IOP in the study eye is at least 20%.
- ≥ 25% Reduction Response: the percent reduction from baseline in mean diurnal IOP in the study eye is at least 25%.
- ≥ 30% Reduction Response: the percent reduction from baseline in mean diurnal IOP in the study eye is at least 30%.
- ≤ 18 mmHg Response: the mean diurnal IOP in study eye is equal or below 18 mmHg.

4.3. Safety-Related Definitions

4.3.1. Adverse Event

Under Protocol 011712IN, an AE is defined as any untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness) that occurs in a study subject, regardless of the suspected cause and regardless of timing of study medication administration. An *on-study* AE can occur any time after the date of informed consent through the last study visit. An AE will be considered as *treatment-emergent* if the AE occurred on or after the treatment start date up to the 2 days after treatment end date. Treatment-emergent AEs are a subset of on-study AEs. Both on-study and treatment-emergent AEs will be recorded, but only treatment-emergent AEs will be tabulated.

The severity of each AE will be graded by the Clinical Investigator as Mild, Moderate, or Severe.

Each AE will be classified into a system organ class (SOC) and coded to a preferred term (PT) using Medical Dictionary for Regulatory Activities (MedDRA), version 21.1 published in 2018.

4.3.1.1. Serious Adverse Event

Any AE is considered a SAE if it fulfills one or more of the following criteria:

- Death (i.e., the AE caused or led to death)
- Life threatening (i.e., immediately life-threatening)
- Inpatient hospitalization and/or prolonged hospitalization
- A persistent or significant disability/incapacity (i.e., the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)
- A congenital anomaly/birth defect in the offspring of a study subject who was exposed to study therapy prior to conception or during pregnancy
- Sight threatening event
- Other medically important event (e.g., requires medical or surgical intervention to prevent one or more of the listed above)

4.3.1.2. Ocular Adverse Event

An AE will be counted as an *ocular AE* if the Clinical Investigator selected "OD", "OS", or "OU" on the AE eCRF.

4.3.1.3. Suspected Adverse Reaction

An AE will be counted as a *suspected adverse reaction* (SAR) if the Clinical Investigator selected answered 'Related' to the AE eCRF question 'Relationship to Study Drug'

4.3.1.4. Events of Special Interest

Events of special interest (ESI) in this study is defined as pregnancy, study medication administration error, or macular edema.

4.3.2. Safety Measures

Table 5 lists the safety measures to be evaluated for this study.

Table 5: Safety Assessments

Safety Measures	Note
Slit-lamp biomicroscopy: anterior chamber cells anterior chamber flare lid hyperemia lid edema conjunctival (palpebral and bulbar) hyperemia conjunctival chemosis corneal edema corneal staining (with fluorescein) keratic precipitate anterior synechiae of iris posterior synechiae of iris	Anterior Chamber Cells will be graded as 0 = No cells, 0.5 = 1-5cells, 1 = 6-15 cells, 2 = 16-25 cells, 3 = 26-50 cells, or 4 = > 50 cells. Anterior Chamber Flare will be graded as 0 = None, 1 = Faint, 2 = Moderate, 3 = Marked, or 4 = Intense. The other biomicroscopy parameters will be graded as 0 = None, 1 = Mild, 2 = Moderate, or 3 = Severe.
Ophthalmoscopy glaucomatous optic nerve cup to disc ratio retina macula choroid vitreous	Damage to optic nerve will be graded as 0 = None 1 = Mild, 2 = Moderate, 3 = Severe. Cup/disc ratio will be recorded with two decimal points (e.g., 0.80). Retina, macula, choroid, and vitreous will graded as 0 = Normal, 1 = Abnormal.
Best Corrected visual acuity	Best corrected visual acuity score measures the acuteness or clearness of corrected vision, with a range of [-0.30, 1.10] in logarithm of the minimum angle of resolution (logMAR) scale. A decrease in logMAR score indicates improvements in corrected visual acuity.

4.4. Other Definitions

4.4.1. Prior and Concomitant Medications

Non-study medications will be categorized into prior medications and concomitant medications. Specifically, the *prior medication* is defined as any non-study medication taken and ended prior to the treatment start date. The *concomitant medication* is defined as any non-study medication taken concurrently while on the study medication, i.e., the treatment period of a concomitant medication taken by a subject's needs to overlap with his/her treatment period of the study medication.

5. STUDY POPULATION

5.1. Intent-to-Treat Population

The *Intent-to-Treat* (ITT) population will include all randomized subjects.

5.2. Safety Population

The *Safety* population will include all randomized subjects who received at least one dose of the study medication. Safety analyses will be performed using the Safety Population and summarized by actual treatment received.

5.3. Full Analysis Set

The *Full Analysis Set* (FAS) includes all randomized subjects who received at least one dose of the study medication and had at least one post-baseline IOP measurement of the study eye during the study. This will be the population used for efficacy analyses. Efficacy analyses will be performed using the FAS and summarized by treatment as randomized.

5.4. Per-Protocol Set

The Per-Protocol Set (PPS) is a subset of the FAS. It will be the analysis population for sensitivity analyses and will be summarized using treatment as randomized. Any subject affected by a significant protocol deviation that might affect the primary efficacy analysis will be excluded from the PPS. The PPS will be identified before the unmasking of treatment assignments.

Before the unmasking of treatment assignment, Santen's study team will review all protocol deviations, identify subjects with any protocol deviation that could impact the efficacy outcome, and determine whether or not to exclude the subject from the PPS.

6. GENERAL CONSIDERATIONS

All measures will be summarized by treatment (planned or actually received) descriptively. Continuous variables will be summarized using descriptive statistics such as number of observations (n), mean, standard deviation, median, minimum, and maximum. Categorical variables will be tabulated using frequency (n) and percent (%).

Analysis treatment groups are defined as DE-117 QD/Vehicle QD or DE-117 BID.

The statistical testing will be conducted at a significance level of 0.05 (2-sided) unless specified otherwise. No statistical testing will be conducted for safety measures.

All data manipulations, descriptive summaries, and statistical hypothesis testing will be performed using Statistical Analysis System (SAS) Version 9.4 or later.

6.1. Adjustments for Covariates

In general, baseline IOP score will be adjusted in the inferential analysis of each IOP endpoint. Detailed information on covariate adjustment is provided in Section 8.1.

6.2. Handling of Missing Data

6.2.1. Efficacy Measure

For subjects with the use of any non-study IOP lowering therapy to lower IOP, unless otherwise specified, the IOP data collected after the non-study IOP lowering therapy will be censored (treated as missing) in efficacy analyses.

For each IOP endpoint, no imputation is needed for the analysis on observed cases using the mixed-effects model for repeated measures (MMRM).

6.2.2. Safety Measures

Descriptive summaries of safety measures will be based on observed data only. No imputation of missing scores will be implemented.

6.2.3. Dates for Medical Events and Medications

Completely or partially missing onset and resolution dates of medical history (MH), AEs and concomitant medications (CM) will be imputed as follows:

Table 6: Handling of Missing Date for Medical Events and Medications

Date	Type of Missing Date	Handling of Missing Date
Event onset date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied. For AE, the event will be considered treatment-emergent. For CM, the event will be considered concomitant. For MH, the event will be considered to occur prior to Inform Consent date.
	Only YYYY is available	Use the first day of YYYY to impute the missing month and date parts of the onset date
	YYYY and MM are available, but DD is missing	Use the first day of MM to impute the missing date part of the onset date
Event resolution date (e.g., YYYY-MM-DD)	Completely missing	No imputation will be applied. The event will be considered ongoing (i.e., not resolved) at the last visit date.
	Only YYYY is available	Use the last day of YYYY to impute the missing month and date parts of the resolution date
	YYYY and MM are available, but DD is missing	Use the last day of MM to impute the missing date part of the resolution date

Same rules will be followed to impute the completely or partially missing start and end dates of non-study medications.

6.3. Multi-Center Studies

Sites will not be pooled for any inferential analysis or descriptive summary in this study.

6.4. Multiple Comparisons / Multiplicity

No multiplicity adjustment will be applied in this study. Treatment differences in IOP between DE-117 QD arm and DE-117 BID arm for each timepoint will be considered statistically significant if the p-value of the corresponding treatment comparison is smaller than 0.05. Superiority of DE-117 BID to DE-117 QD is achieved if the treatment differences are significantly greater than 0 at all six time points.

6.5. Interim Analysis

No interim analysis is planned for this study.

7. SUMMARY OF STUDY POPULATION DATA

7.1. Subject Disposition

The disposition of all randomized subjects will be summarized by treatment and overall. The summary will include the number of subjects in the FAS population and the numbers and percentages of subjects in the other study populations including the Safety population, and the PPS population. The disposition summary will also include the number and percentage of completers and non-completers at Week 6 (Visit 4), respectively, as well as the number and percentage of non-completers at Week 6 (Visit 4) by the primary discontinuation reason from the study drug and from the study.

7.2. Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be descriptively summarized for the FAS population by planned treatment, Safety population by actual treatment, and overall. Specifically, for subject demographics, the following variables will be summarized:

- Age at randomization (continuous and categorical: < 65 years or ≥ 65 years)
- Sex (categorical: Male or Female)
- Ethnicity (categorical: Hispanic/Latino or Not)
- Race (categorical: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or Other)

For baseline characteristics, the following variables will be summarized for study eye:

- Primary ocular diagnosis (categorical: POAG, or OHT)
- Prior use of Prostaglandin analogs (Yes or No)
- Baseline mean diurnal IOP score and baseline IOP score at each scheduled timepoint (08:00, 12:00, and 16:00)

- Visual acuity
- Glaucomatous optic nerve findings (categorical: none, mild, moderate, or severe)

7.3. Medical and Surgical History

For this study, medical and surgical history will be coded using MedDRA 21.1, 2018. Each medical event will be classified into a SOC and mapped to a PT.

The medical and surgical history will be summarized for the FAS population. Subjects reporting any medical and surgical history at baseline will be tabulated by SOC and PT for each planned treatment and overall.

7.4. Protocol Deviations

In this study, protocol deviations are categorized as follows:

- Informed Consent
- Inclusion/Exclusion Criteria
- Concomitant Treatment
- Investigational Product
- Procedures/Tests/Assessments/ Laboratory
- Safety Reporting
- Time Window
- Withdrawal Criteria
- Other deviations

A protocol deviation is considered major if it may affect the subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study data. Santen's study team will review all protocol deviations and determine the list of major protocol deviations prior to database lock. All randomized subjects with any major protocol deviation(s) will be tabulated by deviation category for each planned treatment and overall. In addition, protocol deviations will also be listed.

7.5. Prior and Concomitant Medications

For this study, non-study medications, including prior and concomitant medications, will be coded using World Health Organization Drug Dictionary Enhanced (WHO-DDE) (March 2017). Each non-study medication will be classified using the Anatomical-Therapeutic-Chemical (ATC) classification system and mapped to a WHO-DDE preferred drug name.

Non-study medications will be summarized for the Safety population. Subjects taking any prior medications will be tabulated by ATC level 3, level 4 and preferred drug name for each actual treatment received and overall. A subject will be counted at most once for each prior medication, even if the subject took the same prior medication on multiple occasions. Subjects

taking any concomitant medications will be tabulated similarly. In addition, prior medications and concomitant medications will also be listed, separately.

7.6. Treatment Compliance

A subject will be considered fully compliant to treatment if the he/she responded "0" at all post-baseline visits to the eCRF question "How many doses were missed since the previous visit?"

The treatment compliance rate for a subject at a visit will be as following:

- Compliance Rate (%) = (2*Duration – ∑Miss) / (2*Duration) × 100

Where:

Duration: The number of days subject should have administered study medication, calculated as treatment end date – Baseline date.

Miss: The number of missed doses since the last visit day(s)

For subjects in the FAS, the compliance rate will be summarized by post-baseline analysis visit for each planned treatment.

7.7. Exposure to Study Medication

The duration of exposure to a study medication is measured by days on treatment as derived in Section 4.1.3. For subjects in the Safety Population, the duration of exposure will be summarized using descriptive statistics, and frequency and percentage of subjects will be tabulated by duration category (1-14 days, 15-28 days, or > 28 days) for each actual treatment received.

8. EFFICACY ANALYSES

Unless specified otherwise, the efficacy analyses will be performed on the FAS, where subjects are classified by planned treatment, irrespective of the actual treatment received.

Unless specified otherwise, all efficacy analyses will be performed on the study eye, and the data on fellow eye will not be used.

8.1. Analyses of Primary Endpoint

8.1.1. Primary Analyses

The primary efficacy endpoint is IOP in the study eye at each scheduled timepoint (08:00, 12:00 and 16:00) at Weeks 2 and 6 (Visits 3 and 4). The comparison between DE-117 BID arm and QD arm will be performed with the following hypotheses:

$$H_{0i}: \mu_{Bi} = \mu_{Qi}$$

versus

$$H_{1i}: \mu_{Bi} \neq \mu_{Qi}$$

where μ_B and μ_Q denote the mean values of the primary endpoint in DE-117 BID arm and QD arm at i =1,2, …6 timepoints, with i = 1, 2, 3 represent 08:00, 12:00 and 16:00 at Week 2 and i = 4, 5, 6 represent 08:00, 12:00 and 16:00 at Week 6, respectively.

The primary analyses will be performed on the FAS. A mixed-effect model for repeated measures (MMRM) will be carried out for each timepoint. Each model will include treatment, visit, and treatment-by-visit interaction as fixed effects, baseline IOP as a covariate. Within-subject errors will be modeled using an unstructured covariance matrix. Least squares mean of the endpoint within each treatment arm will be reported. The 95% confidence intervals (CIs) for the difference in means between DE-117 BID arm and QD arm at each timepoint and the corresponding p-values will be provided.

If the model with unstructured covariance matrix fails to converge, the following structures other than unstructured are to be used in order of 1) heterogeneous toeplitz (TOEPH), 2) heterogeneous autoregressive of order 1 (ARH(1)), 3) heterogeneous compound symmetry (CSH), 4) compound symmetry (CS) and the first (co)variance structure that converges would be used as the primary analysis.

8.1.2. Sensitivity Analyses

To assess the robustness of the results from the primary analysis for primary endpoint, the sensitivity analysis will be performed using the same MMRM model on PPS. All the primary and sensitivity analyses will be implemented using SAS PROC MIXED. The main part of the SAS code for the specified MMRM analysis is provided in Appendix 12.1.

8.2. Analyses of Secondary Endpoints

For continuous secondary endpoints (mean diurnal IOP, change and percent change from baseline) at Week 2 (Visit 3) and Week 6 (Visit 4), MMRM and descriptive summaries will be performed. The MMRM will be the same as the one for primary endpoint.

For binary secondary endpoints, the responder rates will be summarized at each post-baseline visit. Pearson's Chi-square test will be used for comparison between DE-117 BID arm and QD arm.

8.3. Subgroup Analyses

To assess the homogeneity of treatment effects among subgroups, descriptive summaries by age groups (< 65 vs ≥ 65 years), sex (males or females), race (White vs Non-white), primary ocular diagnosis (POAG or OHT), and mean diurnal IOP at baseline (< 25 or ≥ 25 mmHg) will be reported for primary efficacy endpoint.

Other subgroup analyses may be performed as suggested by the data.

9. SAFETY ANALYSES

Safety of DE-117 will be primarily assessed by AEs, BCVA and evaluation with slit-lamp biomicroscopy and ophthalmoscopy. The Safety population will be used for all safety summaries, where subjects will be classified by actual treatment received.

All the safety-related measures will be summarized descriptively by actual treatment received. Except AEs, the descriptive summary of each safety-related measure and the change from baseline in that measure will be performed if applicable.

9.1. Adverse Event

Subjects with any AE(s), SAE(s), SAR(s) serious SAR(s) and significant AE (AE leading to study drug discontinuation) will be tabulated by type of AE(s) for each actual treatment received.

Besides the overall AE summary, subjects with any AE(s) and any SAR(s) will be tabulated by SOC and preferred term. A subject who experienced multiple AEs within a SOC or preferred term will be counted only once for that SOC or preferred term.

The same analysis above will be performed for ocular AEs and non-ocular AEs.

AEs, AEs leading to death, AEs leading to study drug discontinuation, SAEs, SARs and ESIs, if any, will be listed separately.

9.2. Slit-lamp Biomicroscopy

For each biomicroscopy parameter, frequency and percentage of rating scores will be summarized by analysis visit for study eyes and non-study eyes, separately. In addition, any clinically significant worsening (increase) from baseline will be summarized and listed.

9.3. Ophthalmoscopy

Frequency and percentage of rating scores will be summarized by treatment and analysis visit for study eyes and non-study eyes, separately. In addition, any worsening (increase) of ≥ 2 units from baseline will be summarized and listed.

9.4. Best Corrected Visual Acuity

Corrected visual acuity scores measured in logMAR scale and changes from baseline will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. In addition, any change such as worsening or improvement of at least 0.2 logMAR (2 lines) or maintained within 0.2 from baseline will be summarized. Subjects with worsening of at least 0.2 from baseline will be listed.

10. ANALYSES OF OTHER MEASURES

None.

11. REFERENCES

None.

12. APPENDICES

12.1. PROC MIXED for MMRM

```
PROC MIXED data = dataset_name METHOD=REML;
        WHERE fasfl='Y' AND paramcd=" IOP " AND avisitn IN (2, 6) AND steyefl='Y' AND  atpt IN
('8:00', '12:00', '16:00');
        By atpt;
        CLASS usubjid trtpn avisitn;
        MODEL aval = base trtpn avisitn trtpn*avisitn/DDFM =KR;
        REPEATED avisitn/SUBJECT = usubjid TYPE= UN;
        LSMEANS trtpn*avisitn/DIFF CL;
        ESTIMATE "0.002% DE-117 QD – 0.002% DE-117 BID at Week 2" trtpn 1 -1
                trtpn*avisitn 1 0 -1 0 /CL;
        ESTIMATE " 0.002% DE-117 QD – 0.002% DE-117 BID at Week 6" trtpn 1 -1
                trtpn*avisitn 0 1 0 -1 /CL;
RUN;
```

where

- ✓ fasfl: Full Analysis Set Population Flag
- ✓ paramcd: Parameter Code
- ✓ avisitn: Analysis Visit (N)
- ✓ steyefl: Study Eye Flag
- ✓ usubjid: Unique Subject ID
- ✓ trtpn: Planned Treatment (N) for 0.002% DE-117 QD and 0.002% DE-117 BID
- ✓ aval: Post baseline IOP score
- ✓ base: Baseline Value

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