Statistical Analysis Plan Cover Page

Official Study Title:	A Phase IIb Safety and Efficacy Study of DE-126 Ophthalmic Solution in Primary Open-Angle Glaucoma or Ocular Hypertension- Angel Study
NCT Number:	NCT03216902
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STATISTICAL ANALYSIS PLAN

DE-126 ANGEL Study

Protocol Title:	A Phase IIb, Randomized, Observer-Masked, Placebo- and Active-Controlled, Parallel-Group, Multinational and Multicenter Study Assessing the Safety and Efficacy of DE-126 Ophthalmic Solution in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - Angel Study
Product:	0.0005%, 0.001%, 0.002% and 0.003% DE-126 ophthalmic solution
Protocol Number:	012601IN
Sponsor:	Santen Inc. 6401 Hollis Street, Suite 125 Emeryville, CA 94608 USA.
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CONFIDENTIAL

APPROVAL SIGN-OFF SHEET

A Phase IIb, Randomized, Observer-Masked, Placebo- and Active-Controlled, Parallel-Group, Multinational and Multicenter Study Assessing the Safety and Efficacy of DE-126 Ophthalmic Solution in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - Angel Study

DE-126 ANGEL Study

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ABBREVIATIONS

ADaM	Analysis Data Model
AE	adverse event
ANCOVA	analysis of covariance
ATC	Anatomical-Therapeutic-Chemical
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
SDTM	Study Data Tabulation Model
SOC	system organ class
US	United States
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 ANGEL study within the scope of Santen's Protocol 012601IN, "A Phase IIb, Randomized, Observer-Masked, Placebo- and Active-Controlled, Parallel-Group, Multinational and Multicenter Study Assessing the Safety and Efficacy of DE-126 Ophthalmic Solution in Subjects with Primary Open-Angle Glaucoma or Ocular Hypertension - Angel Study". It applies to the study protocol dated 16 June 2017 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

2.1. Objectives

The primary objective of this study is to determine the optimal dose of DE-126 by evaluating the safety and efficacy of four concentrations of DE-126 ophthalmic solution (0.0005%, 0.001%, 0.002%, and 0.003%) when compared to latanoprost (0.005% latanoprost) at Month 3 in subjects with primary open-angle glaucoma (POAG) or ocular hypertension (OHT).

The secondary objectives of this study are:

- to determine if any one of four concentrations of DE-126 (0.0005%, 0.001%, 0.002% and 0.003%) is superior to the placebo (vehicle of DE-126) in lowering intraocular pressure (IOP) at each specified timepoint (09:00, 13:00 and 17:00) at Week 6 compared to baseline;
- to evaluate the dose response of four concentrations of DE-126 ophthalmic solution (0.0005%, 0.001%, 0.002% and 0.003%).

The safety objective of this study is to determine the safety of DE-126 ophthalmic solutions (0.0005%, 0.001%, 0.002% and 0.003%) given once daily as compared to placebo and 0.005% latanoprost given once daily in the study population.

2.2. Endpoints

2.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is IOP in the study eye at each specified timepoint (09:00, 13:00 and 17:00) at Month 3 (Visit 6).

2.2.2. Key Secondary Efficacy Endpoint

The key secondary endpoint is IOP in the study eye at each specified timepoint (09:00, 13:00 and 17:00) at Week 6 (Visit 5).

2.2.3. Other Secondary Efficacy Endpoints

Other secondary endpoints to be assessed include:

- IOP in the study eye at each specified timepoint (09:00, 13:00 and 17:00) at Week 1 and Week 2 (Visit 3 and 4),
- Change and percent change from baseline in IOP in the study eye at the specified timepoints: 09:00, 13:00, and 17:00 at each post-baseline visit (Week 1 [Visit 3], Week 2 [Visit 4], Week 6 [Visit 5], or Month 3 [Visit 6]),
- Mean diurnal IOP in the study eye at each post-baseline visit,
- Change and percent change from baseline in mean diurnal IOP in the study eye at each post-baseline visit,
- Mean diurnal IOP reduction from baseline ≥ 20%, 25% and 30% in the study eye at each post-baseline visit,
- Mean diurnal IOP ≤ 18 millimeter of mercury (mmHg) in the study eye at each post-baseline visit.

The safety of DE-126 will be evaluated by

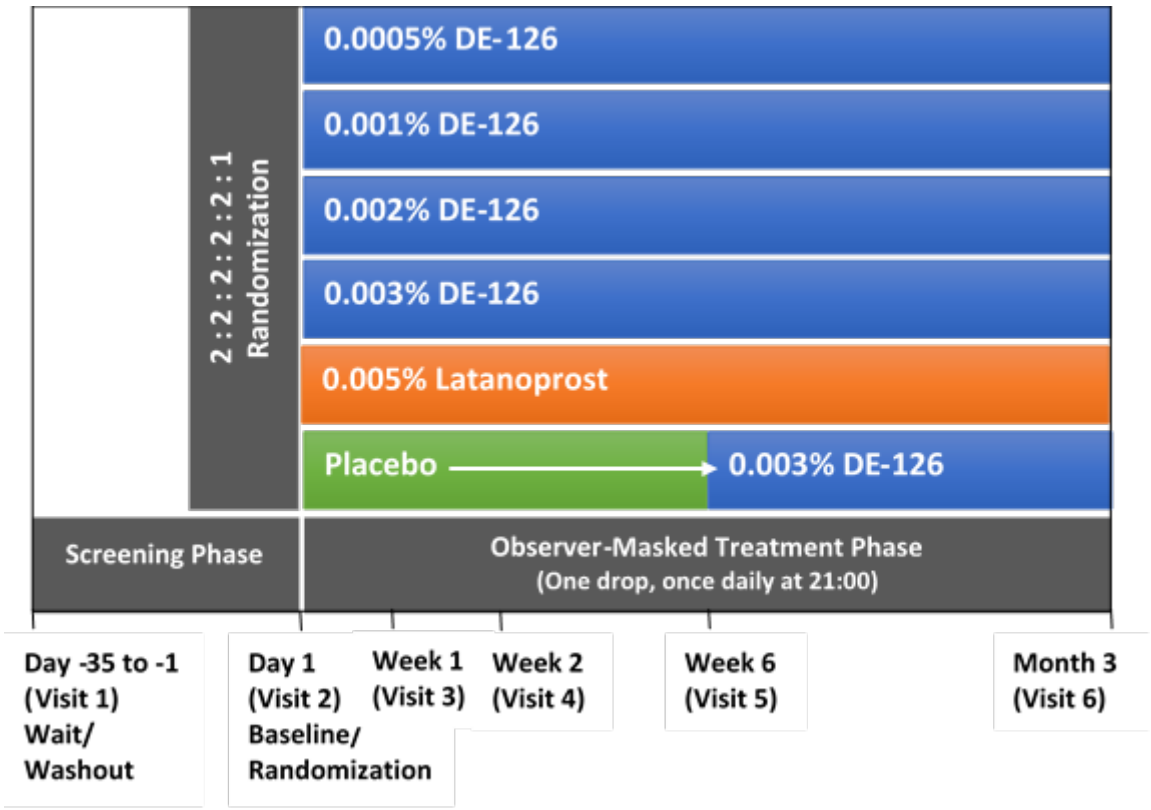
- Incidence of ocular and systemic adverse events (AEs),
- Slit-lamp biomicroscopy,
- Ophthalmoscopy,
- Corrected visual acuity,
- Ocular symptoms,
- Central corneal thickness,
- Iris color/eyelash/eyelid by photographs,
- Vital signs,
- Clinical laboratory tests.

3. STUDY DESIGN

3.1. General Study Design

This is a randomized, observer-masked, placebo- and active-controlled, parallel-group, multinational and multicenter study assessing the safety and efficacy of four concentrations of DE-126 ophthalmic solution (0.0005%, 0.001%, 0.002%, and 0.003%) in subjects with POAG or OHT. Latanoprost ophthalmic solution 0.005% will be used as an active control. As shown in the Study Design Diagram (Figure 1), this study consists of Screening Phase of up to 35 days including a Washout Period of up to 28 days (up to 7 additional days allowed prior to baseline visit), and a 3-month Observer-Masked Treatment Phase. Approximately 220 subjects will be randomized at the United States (US) and Japan sites in a 1:1 ratio.

Figure 1: Study Design Diagram



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 30 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 2:2:2:2:2:1 ratio to receive 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, 0.003% DE-126, latanoprost or placebo at Visit 2. Each subject will use the received study eye drops once daily at 21:00 (± 60 minutes) for 3 months except subjects in the placebo arm. Subjects in the placebo arm will receive placebo eye drops for 6 weeks and then switch to DE-126 0.003% ophthalmic solution for the remaining 6 weeks till study exit. Post-baseline safety and efficacy measures will be collected at Week 1 (Visit 3, Day 8 ± 2), Week 2 (Visit 4, Day 15 ± 2), Week 6 (Visit 5, Day 43 ± 3), Month 3 (Visit 6, Day 91 ± 3), and Study Exit/Early Termination (ET) according to the Assessment Schedule (Table 1).

3.2. Randomization and Masking

A stratified permuted-block randomization will be employed to randomize eligible subjects in a 2:2:2:2:2:1 ratio to receive 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, 0.003% DE-126, latanoprost or placebo. For the randomization procedure, the stratifying factor is country; the block size is 11. The randomization schedule will be generated and implemented by an independent biostatistician at Santen.

Treatment assignments will be masked to Santen (except Drug Supply personnel), study subjects, and Clinical Investigator. In case of a medical emergency, when the unmasking of a subject's

treatment assignment code becomes necessary for the welfare of that subject, the Clinical Investigator may reveal the treatment information by unmasking through Interactive Response Technology (Medidata BALANCE).

3.3. Sample Size Planning

The sample size is planned on the key secondary endpoint instead of the primary efficacy endpoint. The key secondary endpoint is IOP in the study eye at each specified timepoint (09:00, 13:00 and 17:00) at Week 6 (Visit 5). Assuming that the minimal expected treatment difference in IOP across all timepoints is -5.1 mmHg between the optimal DE-126 dose and placebo and the standard deviation of the difference for each timepoint is 3.9 mmHg, with a 2:1 randomization allocation ratio, a sample size of 36 for each DE-126 arm and 18 for placebo will provide 92% power to detect such a difference at all timepoints using a *t*-test (2-sided, α=0.0125 adjusted by Bonferroni correction). To account for the impact of missing data, a sample size of 40 for each DE-126 arm and 20 for the placebo arm is planned.

Note that this sample size will provide less than 20% power to claim the non-inferiority of DE-126 optimal dose to latanoprost with a non-inferiority margin of 1.5 mmHg.

3.4. Visits and Assessments

There are 6 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 1). For subjects whose study participation is terminated prior to Month 3 (Visit 6, Day 91 ± 3), to the extent possible, all assessments scheduled for Visit 6 will be performed at the ET visit. Subjects who have an on-going study medication-related serious adverse event (SAE) at study completion or at ET from the study will be followed by the Clinical Investigator until the event is resolved or determined to be irreversible, chronic, or stable.

Table 1: Assessment Schedule

	Screening Phase		Treatment Phase				
	Visit 1 Screening	Washout Period (optional Visit 1a)	Visit 2 Baseline (Day 1)	Visit 3 Week 1 (Day 8±2)	Visit 4 Week 2 (Day 15±2)	Visit 5 Week 6 (Day 43±3)	Visit 6 Month 3 (Day 91±3, Exit) or Early Term
Informed Consent(s)[a]	X						
Inclusion/Exclusion Criteria	X		X				
Demographics and Medical History (incl. Prostaglandin Analogs naïve[b])	X						
Concomitant Medications/Therapies	X	X	X	X	X	X	X
Dosing Compliance				X	X	X	X
Adverse Events	X	X	X	X	X	X	X
Pregnancy Test[c]	X		X				X
Urinalysis	X						X
Hematology and Serum Biochemistry	X						X
Vital signs (Blood pressure/heart rate)[d]	X		X				X
Refraction[e]	X		X	X	X	X	X
Corrected VA[f]	X	X	X (09:00)	X (09:00)	X (09:00)	X (09:00)	X (09:00)
Ocular Symptoms questionnaire[g]	X		X	X	X	X	X
Biomicroscopy (including conjunctival hyperemia, aqueous flare, cells)	X	X	X (09:00)	X (09:00)	X (09:00)	X (09:00)	X (09:00)
Intraocular Pressure (IOP[f])[h]	X	X	09:00 13:00 17:00	09:00 13:00 17:00	09:00 13:00 17:00	09:00 13:00 17:00	09:00 13:00 17:00
Pachymetry (Central Corneal Thickness)[i]	X		X (17:00)			X (17:00)	X (17:00)
Iris, Eyelash, Eyelid assessment and photograph[j]			X			X	X
Gonioscopy[k]	X						
Visual Field[k]	X						
Ophthalmoscopy[l]	X		X (17:00)			X (17:00)	X (17:00)
Pharmacogenomics/genomics[m]			X				
Dispense Study Medication			X			X	
Collect Study Medication						X	X

[a] Informed Consent and authorization as appropriate for local privacy regulations 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] Prostaglandin naive subjects are defined as subjects who are not known to have used prostaglandin as their glaucoma treatment. The previous use of prostaglandin should be confirmed by either subject medical records or subject history.

[c] A urine pregnancy test will be conducted for all female subjects of childbearing potential.

[d] Vital signs should be recorded at same timepoint at each visit, where possible.

[e] Refraction will be required if the prescription is not up to date within 12 months at the screening visit. If more than 10 letters in corrected visual acuity were lost compared to the screening visit, then refraction must be performed.

[f] Corrected VA and biomicroscopy examination will be completed before IOP is measured at 09:00. Aqueous flare and cell evaluation will be performed before fluorescein instillation. If the Clinical Investigator evaluates for possible torn posterior lens capsule by biomicroscopy under dilation in subjects with pseudophakic eye(s) based on his/her decision at visit 1 (screening), please dilate pupil and evaluate after all other ocular procedures have been completed.

[g] Ocular symptoms questionnaire should be performed at the screening visit, and at the beginning of each post-screening visit (Visits 2 to 6).

[h] IOP Measurements will be taken at 09:00 ±60min, 13:00 ±60min and 17:00 ±60min at all visits except for Visit 1 (Screening).

[i] Pachymetry will be performed after IOP measurement at Visit 1 (if pachymetry is contact type), and after the 17:00 IOP measurement at Visits 2, 5, and 6.

[j] Iris, eyelash and eyelid photograph will be maintained as source documentation.

[k] Gonioscopy and visual field measurement will be performed if not done within 3 months prior to Visit 1. Gonioscopy will be performed after IOP measurement.

[l] Ophthalmoscopy will be performed after IOP measurement at Visit 1 and after the 17:00 IOP measurement at Visits 2, 5, and 6. If the Clinical Investigator performs ophthalmoscopy under dilation based on his/her decision, please dilate pupil and perform after all other ocular procedures have been completed.

[m] Saliva collection for the pharmacogenomics/genomics laboratory research study may be collected at any visit after informed consent obtained and subject randomized.

4. DEFINTIONS

4.1. Time-Related Terms

4.1.1. Screening Visit

The *Screening visit* is Visit 1 when the subject started washout period.

4.1.2. Baseline Visit

The *Baseline visit* is Visit 2 (Day 1) when the subject is randomized. The Screening visit will be used as baseline for visual field, gonioscopy and laboratory tests.

4.1.3. Treatment Start and End Dates, Extent of Exposure (Days)

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 same study medication. For all treatment arms except the placebo arm, if the date of the last dose is missing, then

- The date prior to Month 3 (Visit 6) will be considered the treatment end date for subjects who completed the study, and
- the date prior to ET visit will be used for subjects who prematurely discontinued the study. If the ET visit date of a non-completer is not available, then the date prior to last available visit date will be considered the treatment end date.

For the placebo arm, two sets of treatment start date and end date will be defined for the medications placebo and 0.003% DE-126 respectively.

The *extent of exposure* to study medication will be assessed by duration of treatment exposure, derived as:

- Duration of treatment exposure = (Treatment end date – Treatment start date) + 1.

4.1.4. Study Day

The *study day* describes the relative day of an observation starting with the reference date The study day describes the relative day of an observation starting with the reference date designated as Study Day 1.

In this study, for efficacy analyses, the randomization date is the reference day and the study day will be calculated as:

- For days prior to the randomization date, Study Day = Date – Randomization Date.
- For days on/after the randomization date, Study Day = Date – Randomization Date + 1.

For safety analyses, the treatment start date is the reference day and the study day will be calculated as:

- For days prior to the treatment start date, Study Day = Date – Treatment Start Date.
- For days on/after the treatment start date, Study Day = Date – Treatment Start Date + 1.

Note that there is no Study Day 0.

4.1.5. Days on Study

The *days on study* measures the duration of stay since randomization in the study. For each randomized subject, the days on study will be derived as:

- Days on Study = (Study Exit date – Baseline Visit Date) + 1.

4.1.6. Analysis Periods

Analysis periods for safety events such as adverse events and concomitant medications are defined in Table 2. Subperiods will be used for the placebo arm for the analyses since the placebo arm will switch to 0.003% DE-126 at Week 6 (Visit 5).

Table 2: Analysis Periods for Safety Events

Treatment Arm	***Analysis Period***	***Analysis Period Start Date***	***Analysis Period End Date***[a]
Placebo	Subperiod 1	The date at which a randomized subject took the first dose of the study medication.	The last dose of placebo or ET visit[b] for subjects who prematurely discontinued the study.
	Subperiod 2	The date of first dose of 0.003% DE-126.	Month 3 (Visit 6) for subjects who completed the study, and ET visit[b] for subjects who prematurely discontinued the study.
Latanoprost 0.0005% DE-126 0.001% DE-126 0.002% DE-126 0.003% DE-126	Entire Study	The date at which a randomized subject took the first dose of the study medication.	Month 3 (Visit 6) for subjects who completed the study, and ET visit[b] for subjects who prematurely discontinued the study.

[a] For analysis with AEs, the analysis period end date is 2 days (twice the scheduled dosing interval) after the last dose.
[b] If the ET visit date of a non-completer is not available, then the date prior to last available visit date will be considered the treatment end date.

For each analysis period, the treatment end date will be set to missing if the treatment start date is missing.

4.1.7. Out-of-Window Measurements, Analysis Timepoints, Analysis Visit, and Analysis Window

For this study, a measurement collected at a visit is an *out-of-window* measurement if the study day of the visit falls outside of a visit window specified in Assessment Schedule (Table 1), or a *within-window* measurement otherwise.

Analysis timepoint is a timing variable to be used for analyses involving timepoints. In general, the analysis timepoint is the scheduled timepoint per protocol. Subjects whose IOP measurements are collected outside of the pre-specified time window in the protocol (9:00±60 mins, 13:00 ±60 mins, 17:00 ±60 mins) may be excluded from the Per-Protocol (PP) population.

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 a measurement should be mapped to. 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 with the study visit where the measurement was collected. For example, an out-of-window measurement collected at the Week 1 study visit will be mapped to the Week 2 analysis visit, if the study day of the measurement falls into the analysis window of Week 2.

The following *analysis windows* for post-baseline visits will be applied to minimize the amount of missing data for analysis purposes:

Table 3: Post-Baseline Analysis Visit and Analysis Window

Post-Baseline Analysis Visit (Target Assessment Date)	***Visit Window***	***Analysis Window***
Week 1 (Day 8)	[6, 10]	[5, 11]
Week 2 (Day 15)	[13, 17]	[12, 22]
Week 6 (Day 43)	[40, 46]	[36, 50]
Month 3 (Day 91)	[88, 94]	[71, 111]

For analyses involving post-baseline visits, if there are two or more measurements that fall into the same analysis window of a post-baseline visit, then a visit in which IOP are measured at all the scheduled timepoints (9:00, 13:00 and 17:00) will be selected for that analysis visit. In case that there are two or more such visits within the same analysis window, the measurement closest to the target assessment day will be selected for that analysis visit. In the case that two measurements are closest and 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.

4.2. Efficacy-Related Definitions

4.2.1. Study Eye and Fellow Eye

The *study eye* will be the eye that qualifies per inclusion/exclusion criteria at randomization. For example, IOP must be ≥ 22 mmHg at all IOP measurement time-points (09:00, 13:00 and 17:00). See Section 8.1 and 8.2 of protocol for all inclusion/exclusion criteria. If both eyes meet eligibility criteria, the eye with the higher diurnal IOP at Visit 2 will be designated as the study

eye. If both eyes meet eligibility criteria and have the same mean diurnal IOP, the right eye will be designated as the study eye. The *fellow eye* is the non-study 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 3 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 (9:00, 13:00, and 17:00) will be used. If there are one or more missing IOP measurement at timepoints, the mean diurnal IOP will not be calculated.

To assess the efficacy of DE-126, the mean/median IOP at each scheduled timepoint (9:00, 13:00, 17:00) and the mean diurnal IOP in the study eye will be analyzed. In addition, changes and percent changes from baseline in IOP at each timepoint and the mean diurnal IOP in the study eye will also be analyzed.

4.2.3. Baseline Score

For any measure, the *baseline score* is the last observed measurement or derived score prior to the first dose of study medication.

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 1 [Visit 3], Week 2 [Visit 4], Week 6 [Visit 5], or Month 3 [Visit 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.

The response rate for each response endpoint is calculated as the percentage of responders.

4.3. Safety-Related Definitions

4.3.1. Adverse Event

Events reported on the AE electronic Case Report Form (eCRF) will be assessed according to the recently amended Food and Drug Administration regulations 21 CFR Parts 312 and 320.

Under Protocol 012601IN, 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 (twice the scheduled dosing interval) 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 20.0 published in 2017.

4.3.1.1. Serious Adverse Event

An AE will be counted as a *serious adverse event* (SAE) if the Clinical Investigator selected "Yes" under Item 5 (Is the adverse event serious?) on the AE eCRF. 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" under 'Item 2 (Eye(s) affected)' 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 Item 10 (Relationship to Study Drug).

4.3.1.4. Events of Special Interest

Events of special interest (ESI), such as pregnancy, study medication administration error, and AEs leading to study discontinuation or unmasking, will be identified throughout the study.

4.3.2. Safety Measures

Table 4 lists the safety measures to be evaluated for this study.

Table 4: 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.
Corrected visual acuity	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.
Ocular symptoms: burning/stinging foreign body Sensation tearing	The severity of an ocular symptom will be graded as 0=None, 1=Mild, 2=Moderate, or 3=Severe.

itching photophobia pain	
Central corneal thickness	The central corneal thickness of an eye will be recorded in micrometers (μm).
Iris Eyelash Eyelid	The iris color of each eye will be assessed as 1 = Blue/gray, 2 = Blue/gray with slightly brown, 3 = Blue/gray - brown, 4 = Green, 5 = Green with slightly brown, 6 = Green - brown, 7 = Yellow - brown, 8 = Brown. Any changes from baseline in iris color, eyelash (e.g., length, thickness, pigmentation and number) and eyelid (e.g., pigmentation and hair growth) will be assessed as 1 = No change, 2 = Increased, 3 = Decreased.
Lens Phakic lens severity score	The lens of an eye will be classified as phakic, aphakic, or pseudophakic. The status of a phakic lens will be graded as 0=None, 1=Mild, 2=Moderate, or 3=Severe.
Vital signs: systolic blood pressure diastolic blood pressure pulse rate	Systolic blood pressure and diastolic blood pressure will be measured in mmHg. Pulse rate will be measured by beats/minute.
Laboratory test: urinalysis hematology serum biochemistry	Urinalysis: urine glucose, urine protein, and urobilinogen; Hematology: erythrocytes, leukocytes, hemoglobin, hematocrit, platelet count, leukocyte differentiation Including neutrophil, eosinophil, basophil, monocyte, and lymphocyte; Serum biochemistry: aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, total bilirubin, total protein, albumin, cholesterol (total, high-density lipoprotein, low-density lipoprotein and triglycerides), urea nitrogen, creatinine, uric acid, sodium, potassium, and chloride.

	All parameters will be assessed in the scale of 1 = Normal, 2 = Not Clinically Significant Abnormality, 3 = Clinically Significant Abnormality, 4 = No Results/Invalid Results, 5 = Sample Not Collected

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. Safety Population

The *Safety* population will include all randomized subjects who received at least one dose of the study medication. It will be the analysis population for safety analyses to be performed with subjects as treated.

5.2. Full Analysis Set

The *Full Analysis Set* (FAS) includes all randomized subjects who received at least one dose of the study medication (test or control medication) and had at least one post-baseline efficacy assessment of the study eye during the study. This will be the population used for efficacy analyses. Unless specified otherwise, subjects in efficacy analyses are classified by planned treatment, irrespective of the actual treatment received.

5.3. Per-Protocol Set

The *Per-Protocol Set* (PPS) will be a subset of the FAS population, restricted to the subjects who fulfill the protocol in the terms of the eligibility, interventions, and outcome assessment. It will be the analysis population for some sensitivity analyses.

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 or certain data point 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 follows.

Table 5: Analysis Treatment Groups

Analysis	Analysis Treatment Groups
Study Population Data (Subject Disposition etc.)	Placebo, latanoprost, 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, 0.003% DE-126, and Overall
Efficacy	Week 6: placebo, latanoprost, 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, and 0.003% DE-126 Month 3: latanoprost, 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, and 0.003% DE-126
Safety	Placebo, latanoprost, 0.0005% DE-126, 0.001% DE-126, 0.002% DE-126, 0.003% DE-126, 0.003% DE-126 Total[a], and DE-126 Total[b]

[a] 0.003% DE-126 Total includes subjects who ever received 0.003% DE-126 during the entire study.
[b] DE-126 Total includes subjects who ever received any dose of DE-126 during the entire study.

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 each IOP endpoint, no imputation is needed for the analysis on observed cases using the mixed-effects model for repeated measures (MMRM). Sensitivity analyses using last observation carried forward (LOCF) may be performed. For IOP at each timepoint, the last observation at the same timepoint will be carried forward. For the mean diurnal IOP, the last available mean diurnal IOP will be carried forward.

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 events, i.e., medical history events and AEs will be imputed in a conservative fashion 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.
	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

This is a multi-center study enrolling subjects from approximately 40 sites total in the US and Japan. The number of subjects per site is small. Therefore, sites will not be pooled for any inferential analysis or descriptive summary.

6.4. Multiple Comparisons / Multiplicity

Both analyses with and without multiplicity adjustment will be provided for the key secondary endpoint. For the analysis with multiplicity adjustment, to control the overall Type I error rate associated with the 4 comparisons (DE-126 each dose vs. placebo) on the key secondary endpoint at the 0.05 level (2-sided), the step-up Hochberg procedure (1988) will be followed.

Hochberg procedure begins with the least significant p-value and examines the other p-values in a sequential manner until it reaches the most significant one. Let H_{0i} denote the hypothesis test for the comparison between DE-126 of dose arm i and placebo arm at a timepoint. Consider testing the family of equally weighted hypotheses $H_{0i}, i = 1, \ldots, 4$. Let $p_i, i = 1, \ldots, 4$, denote the p-values of the four comparisons between each DE-126 dose arm i and placebo arm. Let $[1], \ldots, [4]$ denote the random indices such that $p_{[1]} \leq \cdots \leq p_{[4]}$. The decision rule for the Hochberg procedure is defined as follows:

Step 1. If $p_{[4]} < 0.05$, then reject $H_{0[1]}, \ldots, H_{0[4]}$, and stop; otherwise go to Step 2.

Step 2. If $p_{[3]} < 0.05/2$, then reject $H_{0[1]}, H_{0[2]}, H_{0[3]}$, and stop; otherwise go to Step 3.

Step 3. If $p_{[2]} < 0.05/3$, then reject $H_{0[1]}, H_{0[2]}$, and stop; otherwise go to Step 4.

Step 4. If $p_{[1]} < 0.05/4$, then reject $H_{0[1]}$, and stop; otherwise retain $H_{0[1]}$.

[redacted]

Treatment differences in IOP between a DE-126 dose arm and the placebo arm for each timepoint will be considered statistically significant if the adjusted p-value of the corresponding treatment comparison is smaller than 0.05.

6.5. Interim Analysis

No formal 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 Month 3 (Visit 6), respectively, as well as the number and percentage of non-completers at Month 3 (Visit 6) by the primary discontinuation reason.

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)
- Race (categorical: Asian, Black or Africa American, or White)
- Country (categorical: Japan or US)

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

- Primary ocular diagnosis (categorical: POAG or OHT)
- Prior use of IOP-lowering medication(s) (categorical: ß-adrenergic antagonist, prostamide or prostaglandin analogue, α-adrenergic agonist, carbonic anhydrase inhibitors, miotic agent, ROCK inhibitor, other, or none)

- Baseline mean diurnal IOP score and baseline IOP score at each scheduled timepoint (09:00, 13:00, and 17:00)
- Baseline visual acuity
- Baseline central corneal thickness
- Glaucomatous optic nerve findings (categorical: none, mild, moderate, or severe)
- Baseline degree of angle closure in Shaffer scale (ordinal: 0, 1, 2, 3, or 4)
- Baseline glaucoma hemifield test result by device (categorical: within normal limits, borderline, or outside normal limits)
- Baseline visual field global indices by device
    - mean deviation
    - pattern standard deviation

7.3. Medical and Surgical History

For this study, medical and surgical history (i.e., medical events) will be coded using MedDRA 20.0, 2017. 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:

- Safety issues
- Consent issues
- Enrollment issues
- Protocol implementation issues
- Study medication-related errors
- 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.

- Treatment Compliance Rate (%)

  = (Duration – Total Number of Missed Dose) / Duration × 100 if the subject did not withdraw the study medication before this visit,

Or = (Duration – Total Number of Missed Dose - (Current Visit Date – Treatment End Date)) / Duration × 100 if the subject withdrew the study medication before this visit.

Where

- Duration is the number of days subjects should have administered study medication, calculated as current visit date – baseline date.
- Total number of missed dose is the total number of days that subject did not follow the proper dosing procedures or dosing schedule since baseline.

This calculation of treatment compliance is a conservative way because it does not take into consideration if a subject stops the study medication because of AE.

For FAS subjects, treatment compliance will be summarized by post-baseline analysis visit for each planned treatment and overall.

7.7. Exposure to Study Medication

The duration of exposure to a study medication is measured by days on treatment as derived in Section 4.1.8. For Safety subjects, the duration of exposure will be summarized using descriptive statistics for actual treatment received and overall. Frequency and percentage of subjects will also be tabulated by analysis visit.

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 and Key Secondary Endpoint

8.1.1. Primary Analyses

8.1.1.1. Primary Endpoint

The primary efficacy endpoint is IOP in the study eye at each specified timepoint (09:00, 13:00 and 17:00) at Visit 6 (Month 3). No formal statistical hypothesis testing will be implemented for the primary endpoint. Descriptive summaries will be provided by each planned treatment arm.

The primary analyses will be performed on the FAS. Besides the summary tables, plots of IOP measures versus analysis time/analysis visit will be provided.

An optimal dose of DE-126 will be specified based on the descriptive summary. If there is any evidence showing non-inferiority of DE-126 optimal dose to Latanoprost, statistical hypotheses testing may be performed.

8.1.1.2. Key Secondary Endpoint

The key secondary endpoint is IOP in the study eye at each scheduled timepoint (09:00, 13:00 and 17:00) at Week 6 (Visit 5). For the key secondary endpoint, the comparison between DE-126 of each dose arm (i) and placebo arm (p) will be performed with the following pair of testing hypotheses:

$$H_{0i}: \mu_i = \mu_p$$

versus

$$H_{1i}: \mu_i \neq \mu_p$$

where μ_i and μ_p denote the mean values of the primary endpoint in DE-126 treatment arms i (i=1,2,3,4) and placebo p, 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, country, visit, and treatment-by-visit point interaction as fixed effects, baseline IOP as a covariate, and subject as a random effect. 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-126 of each dose arm (0.0005%, 0.001%, 0.002% and 0.003%) and the placebo arm at each timepoint will be estimated. Superiority of a DE-126 dose to placebo is established if the upper limit of the 95% CI for the difference is less than 0 mmHg for IOP at all timepoints at Week 6 (Visit 5).

If the model with unstructured covariance matrix fails to converge, models with different covariance structures will be fitted until the convergence criteria are met.

Besides the p-values from MMRM models, adjusted p-values from Hochberg procedure described in Section 6.4 to control the overall Type I error rate associated with the four set of treatment comparisons between DE=126 and placebo at the 0.05 level (2-sided) will also be provided.

8.1.2. Sensitivity Analyses

8.1.2.1. Primary Endpoint

To assess the robustness of the results from the primary analysis for primary endpoint, the efficacy analyses will be repeated on the PPS population with observed cases, and FAS population with missing data imputed by the LOCF approach.

8.1.2.2. Key Secondary Endpoint

To assess the robustness of the results from the primary analysis for key secondary endpoint, the following sensitivity analyses will be performed:

Table 7: Sensitivity Analyses for Key Secondary Endpoint

Statistical Method	Analysis Population	Handling of Missing Data
MMRM	PPS	Observed cases
ANCOVA (factors: treatment; covariate: time-matched baseline IOP)	FAS	LOCF
	PPS	LOCF

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 and ANCOVA is provided in Appendix 12.1 and Appendix 12.2, respectively.

8.2. Analyses of Other Secondary Endpoints

For continuous secondary endpoints (IOP, mean diurnal IOP, change and percent change from baseline in IOP/mean diurnal IOP) at Week 1(Visit 3), Week 2 (Visit 4) and Week 6 (Visit 5), both MMRM and descriptive summaries will be performed. The MMRM will be the same as the one for key secondary endpoint but without multiplicity adjustment. For continuous secondary endpoints at Month 3 (Visit 6), only descriptive summaries will be provided.

For binary secondary endpoints, the responder rates will be summarized at each post-baseline visit. Each DE-126 arm will be compared the placebo and latanoprost using Fisher's exact test, and the differences between DE-126 arms and the placebo/latanoprost arm will be reported along with 95% CIs.

8.3. Subgroup Analyses

To assess the homogeneity of treatment effects among subgroups, descriptive summaries by age group (< 65, or ≥ 65 years), sex (males or females), country (Japan or US), primary ocular diagnosis (POAG or OHT), prior use of IOP lowering medication (ß-adrenergic antagonist, prostamide or prostaglandin analogue, α-adrenergic agonist, carbonic anhydrase inhibitors, miotic agent, ROCK inhibitor, other, or none), and mean diurnal IOP at baseline (< 25 or ≥ 25 mmHg) may be conducted for mean diurnal IOP.

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

9. SAFETY ANALYSES

The safety-related measures collected in this study include AEs, slit-lamp biomicroscopy, ophthalmoscopy, corrected visual acuity, ocular symptoms, central corneal thickness, iris color, eyelash, and eyelid by photographs, vital signs, and clinical laboratory tests. The Safety population will be used for all safety summaries, where subjects will be classified by actual treatment received. If a subject was administered a different treatment than that which was assigned in the study, this subject will be included in the higher dose of DE-126 arm or latanoprost arm if the subject didn't receive any DE-126 in the study.

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.

9.1. Adverse Event

Subjects with any AEs will be tabulated by type of AEs for each actual treatment received, 0.003% DE-126 Total and DE-126 Total. Besides, subjects with any AEs will be tabulated by SOC and PT. A subject who experienced multiple AEs within a SOC or PT will be counted only once for that SOC or PT. SAEs, SARs, and Serious SARs will be tabulated similarly.

Ocular AEs will be summarized. Any ocular AE that occurred simultaneously to both eyes will be counted once. Non-ocular AEs will be tabulated by SOC and PT.

AE, SARs, ocular and non-ocular AEs will also be summarized by country.

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

9.2. Slit-lamp Biomicroscopy

For each biomicroscopy parameter, frequency and percentage of severity rating score will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. In addition, subjects with improvement (decrease) or worsening (increase) or maintaining from baseline in severity rating score will be tabulated by analysis visit and biomicroscopy parameter. Subjects with worsening of 2 units or more from baseline will be listed.

9.3. Ophthalmoscopy

For cup to disc ratio, scores and changes from baseline will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately.

For glaucomatous optic nerve, frequency and percentage of severity rating score will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. Subjects with improvement or worsening or maintaining from baseline in severity rating score will be tabulated by analysis visit. Subjects with worsening of 2 units or more from baseline will be listed.

For the other ophthalmoscopy parameters including retina, macula, choroid and vitreous, abnormality (Normal or Abnormal) will be summarized by actual treatment received and analysis

visit for study eyes and fellow eyes, separately. In addition, subjects with any change from Normal at baseline to Abnormal in status will be tabulated and listed by ophthalmoscopy parameter.

9.4. Lens

For phakic lens only, frequency and percentage of cataract severity score will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. Subjects with improvement or worsening or maintaining from baseline in severity rating score will be tabulated by analysis visit. Subjects with worsening of 2 units or more from baseline will be listed.

9.5. 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.

9.6. Ocular Symptoms

For each parameter, frequency and percentage of severity rating score will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. Subjects with improvement or worsening or maintaining from baseline in severity rating score will be tabulated by analysis visit and ocular symptom. Subjects with worsening of 2 units or more from baseline will be listed.

9.7. Central Corneal Thickness

Central corneal thickness scores and changes from baseline will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately. In addition, subjects with any worsening of 50 μm or more from baseline will be tabulated and listed.

9.8. Iris, Eyelash, Eyelid

For each parameter, frequency and percentage of incidence (Increased/No Change/Decreased) in iris color, eyelash length, etc. will be summarized by actual treatment received and analysis visit for study eyes and fellow eyes, separately.

9.9. Vital Signs

Blood pressures and pulse rate, and changes from baseline may be summarized by actual treatment received and analysis visit.

9.10. Laboratory Test

For each parameter, descriptive summaries may be performed by actual treatment received and analysis visit. In addition, any abnormalities outside the reference range may be summarized and listed.

10. ANALYSES OF OTHER MEASURES

None.

11. REFERENCES

Hochberg, Y. (1988). A Sharper Bonferroni Procedure for Multiple Tests of Significance. Biometrika 75:800-2.

12. APPENDICES

12.1. PROC MIXED for MMRM

```
PROC MIXED data = dataset_name METHOD=REML;
        WHERE fasfl='Y' AND fasrfl = 'Y' AND anl03fl = 'Y' AND paramcd='IOP' AND avisit
IN ('Week 1', 'Week 2', 'Week 6') AND index(dtype, 'LOCF')=0 AND atpt IN ('9:00', '13:00',
'17:00');
        By atpt;
        CLASS usubjid trt01pn avisitn country;
        MODEL aval = base trt01pn country avisitn trt01pn*avisitn/DDFM =KR;
        REPEATED avisitn/SUBJECT = usubjid TYPE= UN;
        LSMEANS trt01pn*avisitn/ CL;
        ESTIMATE "0.0005% DE-126 – Placebo at Week 6" trt01pn -1 1 0 0 0 0
                trt01pn*avisitn 0 0 -1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0/CL;
        ESTIMATE "0.001% DE-126 – Placebo at Week 6"  trt01pn -1 0 1 0 0 0
                trt01pn*avisitn 0 0 -1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 /CL;
        ESTIMATE "0.002% DE-126 – Placebo at Week 6" trt01pn -1 0 0 1 0 0
                trt01pn*avisitn 0 0 -1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 /CL;
        ESTIMATE "0.003% DE-126 – Placebo at Week 6" trt01pn -1 0 0 0 1 0
                trt01pn*avisitn 0 0 -1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 /CL;
RUN;
```

where

- fasfl: Full Analysis Set Population Flag
- fasrfl: Full Analysis Set Record Level Flag
- anl03fl: Study Eye Flag
- country: country
- paramcd: Parameter Code
- avisitn: Analysis Visit (N)
- usubjid: Unique Subject ID
- trt01pn: Planned Treatment for Period 1 (N) = 0, 1, 2, 3, 4, or 5 for Placebo, 0.0005%DE-126, 0.001%DE-126, 0.002%DE-126, 0.003% DE-126, or Latanoprost
- aval: Analysis Value
- base: Baseline Value

12.2. PROC MIXED for LOCF-ANCOVA

```
PROC MIXED data = dataset_name METHOD=REML;
        WHERE fasfl='Y' AND fasrfl = 'Y' AND anl03fl = 'Y' AND paramcd='IOP' AND avisit
= 'Week 6' AND index(dtype, 'LOCF')>=0 AND atpt IN ('9:00', '13:00', '17:00');
        By atpt;
        CLASS trt01pn country avisitn;
        MODEL aval = base country trt01pn;
        LSMEANS trt01pn /DIFF CL;
        ESTIMATE "0.0005% DE-126 – Placebo at Week 6" trt01pn -1 1 0 0 0 0/CL;
        ESTIMATE "0.001% DE-126 – Placebo at Week 6" trt01pn -1 0 1 0 0 0/CL;
        ESTIMATE "0.002% DE-126 – Placebo at Week 6" trt01pn -1 0 0 1 0 0/CL;
        ESTIMATE "0.003% DE-126 – Placebo at Week 6" trt01pn -1 0 0 0 1 0/CL;
RUN;
```

where

- fasfl: Full Analysis Set Population Flag
- fasrfl: Full Analysis Set Record Level Flag
- anl03fl: Study Eye Flag
- country: country
- paramcd: Parameter Code
- avisitn: Analysis Visit (N)
- dtype: Derivation Type
- trt01pn: Planned Treatment for Period 1 (N) = 0, 1, 2, 3, 4, or 5 for Placebo, 0.0005%DE-126, 0.001%DE-126, 0.002%DE-126, 0.003% DE-126, or Latanoprost
- aval: Analysis Value
- base: Baseline Value

DE-126 STATISTICAL ANALYSIS PLAN (Approved, v1.0)

[redacted]