Statistical Analysis Plan Cover Page

Official Study Title:	A Phase III, Randomized, Double-Masked, Active-Controlled, Parallel-Group, Multi-center Study Assessing the Efficacy and Safety of DE-117 Ophthalmic Solution 0.002% Compared with Timolol Maleate Ophthalmic Solution 0.5% in Subjects with Glaucoma or Ocular Hypertension - Spectrum 3 Study
NCT Number:	NCT03691649
Date of the document:	15 Jan 2020

STATISTICAL ANALYSIS PLAN

DE-117 SPECTRUM 3

Protocol Title:	A Phase III, Randomized, Double-Masked, Active-Controlled, Parallel-Group, Multi-center Study Assessing the Efficacy and Safety of DE-117 Ophthalmic Solution 0.002% Compared with Timolol Maleate Ophthalmic Solution 0.5% in Subjects with Glaucoma or Ocular Hypertension – Spectrum 3 Study
Product:	DE-117
Protocol Number:	011709IN
Sponsor:	Santen Inc. 6401 Hollis Street, Suite 125 Emeryville, CA 94608 USA.
Date:	January 15, 2020
Status:	Version 1.0

CONFIDENTIAL

TABLE OF CONTENTS

TABLE OF CONTENTS 3
LIST OF TABLES 6
LIST OF FIGURES 6
1. INTRODUCTION 9
2. OBJECTIVES AND ENDPOINTS 10
2.1. Objectives 10
2.1.1. Primary Objectives 10
2.1.2. Secondary Objectives 10
2.1.3. Safety Objective 10
2.2. Endpoints 10
2.2.1. Primary Efficacy Endpoint 10
2.2.2. Key Secondary Efficacy Endpoints 10
2.2.3. Other Secondary Efficacy Endpoints 10
2.2.4. Safety Endpoints 11
3. STUDY DESIGN 12
3.1. General Study Design 12
3.2. Randomization and Masking 12
3.3. Sample Size Planning 13
3.4. Visits and Assessments 13
4. DEFINTIONS 18
4.1. Time-Related Terms 18
4.1.1. Baseline Visit 18
4.1.2. Treatment Start Date and Treatment End Date 18
4.1.3. Study Day 19
4.1.4. Out-of-Window Measurements, Analysis Visit, and Analysis Window 19
4.1.5. Extent of Exposure 20
4.2. Endpoint-Related Definitions 20
4.2.1. Study Eye and Fellow Eye 20
4.2.2. Baseline Score 20
4.2.3. Change and Percent Change from Baseline 21
4.3. Efficacy-Related Definitions 21

4.3.1. Response Endpoint and Response Rate 21
4.4. Safety-Related Definitions 21
4.4.1. Adverse Event 21
4.4.1.1. Serious Adverse Event 22
4.4.1.2. Ocular Adverse Event 22
4.4.1.3. Suspected Adverse Reaction 22
4.4.1.4. Events of Special Interest 22
4.4.2. Other Safety Measures 22
4.5. Other Definitions 23
4.5.1. Prior and Concomitant Medications 23
4.5.2. Concurrent Medical Conditions 24
5. STUDY POPULATION 25
5.1. Intent-to-Treat Population 25
5.2. Safety Population 25
5.3. Full Analysis Set 25
5.4. Per-Protocol Set 25
6. GENERAL CONSIDERATIONS 26
6.1. Adjustments for Covariates 26
6.2. Handling of Missing Data 26
6.2.1. Efficacy Measures 26
6.2.2. Safety Measures 26
6.2.3. Dates for Medical Events and Medications 26
6.3. Multi-Center Studies 27
6.4. Multiple Comparisons / Multiplicity 27
6.5. Interim Analysis 28
7. SUMMARY OF STUDY POPULATION DATA 29
7.1. Subject Disposition 29
7.2. Demographics and Baseline Characteristics 29
7.3. Medical and Surgical History 30
7.4. Protocol Deviations 30
7.5. Prior and Concomitant Medications 31
7.6. Treatment Compliance 31
7.7. Exposure to Study Medication 32

8. EFFICACY ANALYSES 33
8.1. Efficacy Analyses for Double-Masked Period 33
8.1.1. Analyses of Primary Endpoint and Key Secondary Endpoints 33
8.1.1.1. Primary Analyses 33
8.1.1.2. Sensitivity Analyses 35
8.1.2. Analyses of Other Secondary Efficacy Endpoints 36
8.1.3. Subgroup Analyses 37
8.2. Efficacy Analyses for Open-Label Period 37
9. SAFETY ANALYSES 38
9.1. Adverse Events 38
9.1.1. Ocular Inflammation 38
9.1.2. Macular Edema 39
9.1.3. Cosmetic Change(s) 39
9.2. Best-Corrected Visual Acuity 40
9.3. Slit-lamp Biomicroscopy 40
9.4. Ophthalmoscopy 40
9.5. Eyelid Sulcus, Eyelid, Eyelash, and Iris Color 41
9.6. Vital Signs 41
10. ADDITIONS AND CHANGES TO THE PROTOCOL-SPECIFIED ANALYSES 42
10.1. Full Analysis Set Definition 42
10.2. Key Secondary Endpoint 42
10.3. Sensitivity Analysis of the Primary and Secondary Efficacy Endpoints 42
11. REFERENCES 43
12. APPENDICES 44

[redacted]

LIST OF TABLES

Table 1: Schedule of Events and Procedures for ADULT Subjects 14
Table 2: Schedule of Events and Procedures Specially for PEDIATRIC Subjects 16
Table 3: Definitions for Treatment Start and End Dates by Treatment Period 18
Table 4: Analysis Visit and Analysis Window 19
Table 5: Response Endpoints 21
Table 6: Safety Assessments 22
Table 7: Handling of Missing Date for Medical Events and Medications 27
Table 8: Overview of Primary and Sensitivity Analysis Methods 36

LIST OF FIGURES

Figure 1: Study Design Diagram 12

Abbreviations

Abbreviation	Explanation
AE	adverse event
ATC	anatomical-therapeutic-chemical
BCVA	best-corrected visual acuity
CI	confidence interval
CRO	contract research organization
CSR	clinical study report
eCRF	electronic case report form
ESI	event of special interest
ET	early termination
FAS	full analysis set
IOP	intraocular pressure
JOAG	juvenile open-angle glaucoma
LOCF	last-observation-carried-forward
LogMAR	logarithm of the minimum angle of resolution
MAR	missing at random
MedDRA	*Medical Dictionary for Regulatory Activities*
MMRM	mixed-effects model for repeated measures
mmHg	millimeter of mercury
MI	multiple imputation
OAG	open-angle glaucoma
OHT	ocular hypertension
OD	oculus dexter (right eye)
OS	oculus sinister (left eye)
OU	oculus uterque (both eyes)
MCMC	markov chain monte carlo
PMM	pattern mixture models
POAG	primary open-angle glaucoma
PPS	per-protocol set
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan

Abbreviations (Continued)

Abbreviation	Explanation
SAR	suspected adverse reaction
SAS	statistical analysis system
SOC	system organ class
TEAE	treatment-emergent adverse event
US	united states
WHO	world health organization

1. INTRODUCTION

This statistical analysis plan (SAP) specifies the statistical methods to be implemented for the analysis of data collected from the SPECTRUM 3 study within the scope of Santen's Protocol 011709IN, "A Phase III, Randomized, Double-Masked, Active-Controlled, Parallel-Group, Multicenter Study Assessing the Efficacy and Safety of DE-117 Ophthalmic Solution 0.002% Compared with Timolol Maleate Ophthalmic Solution 0.5% in Subjects with Glaucoma or Ocular Hypertension – Spectrum 3 Study" It applies to the study protocol Amendment 1 (Version 1.1), dated 13JUL2018, and provides detailed instructions as to how each analysis will be performed.

Results 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. OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objectives

To determine whether DE-117 Ophthalmic Solution 0.002% given once daily is noninferior to Timolol Maleate Ophthalmic Solution 0.5% given twice daily in reducing the IOP in subjects with glaucoma or OHT after 3 months of treatment.

2.1.2. Secondary Objectives

To determine whether DE-117 Ophthalmic Solution 0.002% is noninferior to Timolol Maleate Ophthalmic Solution 0.5% in reducing mean diurnal IOP in subjects with glaucoma or OHT after 3 months of treatment.

To determine whether DE-117 Ophthalmic Solution 0.002% is superior to that of Timolol Maleate Ophthalmic Solution 0.5% in reducing mean diurnal IOP in subjects with glaucoma or OHT after 1 week of treatment.

2.1.3. Safety Objective

To determine the safety of DE-117 Ophthalmic Solution 0.002% as compared to Timolol Maleate Ophthalmic Solution 0.5% in subjects with glaucoma or OHT.

2.2. Endpoints

2.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the IOP in the study eye measured at the specified time points: 08:00, 10:00 and 16:00 at Week 1, Week 6 and Month 3 (Visits 3, 4 and 5).

2.2.2. Key Secondary Efficacy Endpoints

If noninferiority in the primary endpoint is achieved, then the 3 key secondary endpoints will be tested sequentially (see details in Section 6.4).

1. Mean diurnal IOP in the study eye at Month 3 (Visit 5).
2. IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at Week 1 (Visit 3), Week 6 (Visit 4), and Month 3 (Visit 5) in the study eyes with mean diurnal IOP < 25 mmHg at the Baseline Visit. Note: This key secondary efficacy endpoint was not included in the protocol; this secondary efficacy endpoint addition is addressed in Section 10.2.
3. Mean diurnal IOP in the study eye at Week 1 (Visit 3).

2.2.3. Other Secondary Efficacy Endpoints

The following secondary endpoints will be assessed:

- Change and percent change from baseline in IOP at each scheduled timepoint of each post-baseline visit

- Change and percent change from baseline in mean diurnal IOP at each post-baseline visit
- 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

2.2.4. Safety Endpoints

The safety of DE-117 will be evaluated by:

- Incidence of ocular and non-ocular adverse events (AEs)
- Events of special interest (ESIs, which were defined to be macular edema, medication administration errors, and pregnancy)
- Vital signs: blood pressure and pulse rate
- Best-corrected visual acuity (BCVA)
- Slit-lamp biomicroscopy; severity scores for the following 12 parameters: lid hyperemia, lid edema, conjunctival hyperemia, conjunctival chemosis, corneal staining, corneal edema, keratic precipitate, anterior chamber cells, anterior chamber flare, anterior synechiae of iris, posterior synechiae of iris, and cataract
- Ophthalmoscopy (cup-to-disc ratio, glaucomatous optic nerve severity score, and assessments of retina, macula, choroid, and vitreous)
- Assessments for deepening of the upper eyelid sulcus (DUES) and other changes in eyelid, eyelash, and iris

3. STUDY DESIGN

3.1. General Study Design

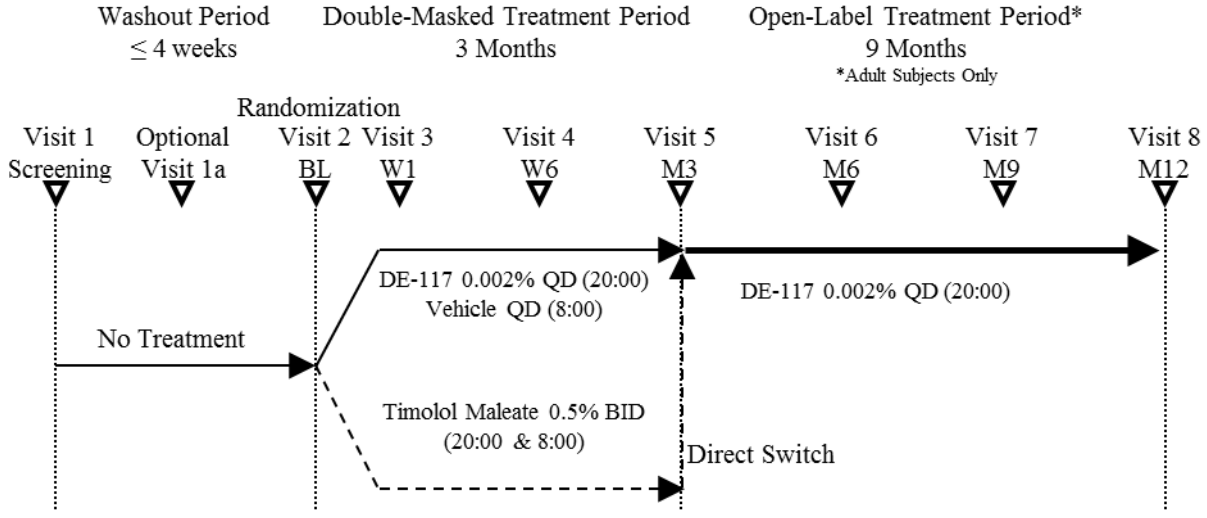
This is a phase 3, randomized, double-masked, active-controlled, parallel-group, multi-center study. Subjects diagnosed with glaucoma or OHT who meet eligibility criteria at Visit 1 (Screening) will discontinue their current topical IOP-lowering medication(s), if any. After completing the required washout period, subjects will return for Visit 2 (Baseline, Day 1). Subjects who meet all eligibility criteria at baseline will be randomized to receive double-masked treatment for 3 months.

Approximately 400 eligible adults and up to 30 eligible pediatric subjects with glaucoma or OHT were planned to be randomized in a 1:1 ratio to receive either:

- **Test regimen – DE-117 0.002% QD**: DE-117 0.002% QD (one drop/eye each day at 20:00) and vehicle QD (one drop/eye each day at 08:00) for the three-month duration of the double-masked treatment period.
- **Control regimen – Timolol 0.5% BID**: Timolol 0.5% BID (one drop/eye each day at 20:00 and one drop/eye each day at 08:00) for the three-month duration of the double-masked treatment period.

At the end of the double-masked period, all adult subjects will enter the open-label period and receive DE-117 Ophthalmic Solution 0.002% for an additional 9 months (Figure 1).

Figure 1: Study Design Diagram



3.2. Randomization and Masking

A blocked randomization stratified by age (< 18 and ≥ 18 years old) will be employed to randomize eligible subjects in a 1:1 ratio to receive either DE-117 or Timolol. The randomization schedule will be generated and implemented by an independent CRO, Medidata Solutions, Inc. (New York City, NY).

During the 3-month double-masked treatment period, the subjects, Investigators, Examiners, and Santen personnel involved in the conduct of the study will be masked to the study treatment. An authorized unmasked study staff member who is not the Investigator or Examiner at the investigative site will dispense and collect study medication(s) and will query about dosing compliance. 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 which treatment the subject has received through the study's interactive web response system (Medidata RTSM).

3.3. Sample Size Planning

The sample size calculation was based on a two-sided Type I error rate of 5% and a non-inferiority margin of 1.5 mmHg. Assuming a treatment difference of 0 mmHg between the DE-117 arm and the Timolol arm, a standard deviation (SD) of 4.0 mmHg, and a correlation coefficient of 0.6 among repeated measures, approximately 400 adult subjects in total (200 subjects per treatment arm) will provide 90% power to demonstrate noninferiority of DE-117 Ophthalmic Solution 0.002% to Timolol Maleate Ophthalmic Solution 0.5% in IOP-lowering effect. Up to 30 pediatric subjects will be enrolled with the agreement from FDA.

The standard deviation of 4.0 mmHg was determined from previous DE-117 studies conducted in the United States: 33-002 and 33-003.

3.4. Visits and Assessments

There are 5 scheduled visits for pediatric subjects and 9 scheduled visits for adult subjects. Assessments at each visit and the time/visit window for each post-baseline assessment are specified in the Assessment Schedule for adult (Table 1) and pediatric subjects (Table 2), respectively.

If the study drug administration is discontinued prior to Visit 5 (end of the double-masked treatment period), the subject should be encouraged to still participate in follow-up study visits until Visit 5 on an observational basis. For subjects whose study participation is terminated prior to Visit 8 (Month 12), to the extent possible, all assessments scheduled for Visit 8 (Month 12) will be performed at the Exit Visit.

Table 1: Schedule of Events and Procedures for ADULT Subjects

	Washout Period		Double-Masked Treatment Period				Open-Label Treatment Period		
	Visit 1 Screening	**Washout Period (up to 4 weeks) Optional Visit 1a**	**Visit 2 Eligibility / Baseline (Day 1)**	**Visit 3 Week 1 (Day 8±2)**	**Visit 4 Week 6 (Day 43 ±3)**	**Visit 5 Month 3 (Day 91±7)**	**Visit 6 Month 6 (Day 181 ±10)**	**Visit 7 Month 9 (Day 271±10)**	**Visit 8 Month 12 (Day 361±10) Exit or Early Termination [n]**
Informed Consent(s) including the optional consent for pharmacogenomics/ genomics laboratory research study [a]	X								
Inclusion/Exclusion Criteria	X		X						
Demographics and Medical History, including prior PGA [b]	X								
Concomitant Medications/ Therapies	X	X	X	X	X	X	X	X	X
Dosing Compliance				X	X	X	X	X	X
AEs		X	X	X	X	X	X	X	X
Pregnancy Test [c]	X		X			X	X	X	X
Vital Signs (blood pressure/pulse rate) [d]	X		X (08:00)			X (08:00)			X (08:00)
Refraction [e]	X								
BCVA[e]	X	X	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)
Biomicroscopy [f]	X	X	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)	X (08:00)
IOP [g]	X	X	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00
Pachymetry [h]	X								
Instill study medication after IOP measurement				X (08:00)	X (08:00)	X (08:00)			
Iris color, eyelash, eyelid [i]			X (photo)			X (photo)	X	X	X (photo)
Gonioscopy [j]	X								
Visual Field [k]	X								
Ophthalmoscopy [l]	X (pupil dilation)		X (16:00)			X (16:00)			X (16:00, pupil dilation)

Table 1: Schedule of Events and Procedures for ADULT Subjects (Continued)

	Washout Period		Double-Masked Treatment Period				Open-Label Treatment Period		
	Visit 1 Screening	**Washout Period (up to 4 weeks) Optional Visit 1a**	**Visit 2 Eligibility / Baseline (Day 1)**	**Visit 3 Week 1 (Day 8±2)**	**Visit 4 Week 6 (Day 43 ±3)**	**Visit 5 Month 3 (Day 91±7)**	**Visit 6 Month 6 (Day 181 ±10)**	**Visit 7 Month 9 (Day 271±10)**	**Visit 8 Month 12 (Day 361±10) Exit or Early Termination [n]**
Blood Sampling for Pharmacogenomics/genomics [m]				X					
Dispense Study Medication			X		X	X	X	X	
Collect Study Medication					X	X	X	X	X
Phone call to remind subject to take evening dose on the day before each visit				X	X	X	X	X	X

a. Informed Consent Form 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's medical records or subject history.
c. A urine pregnancy test will be conducted for all female subjects of childbearing potential.
d. Vital signs (resting blood pressure and pulse rate) will be collected in sitting position anytime at Visit 1 (Screening), and approximately 08:00 for Visit 2 (Baseline), and Visit 5 before the morning dose. Vital signs will also be collected at Visit 8 at approximately 08:00.
e. Refraction will be performed at the screening visit. If more than 10 letters in BCVA are lost compared to the screening visit, then refraction should be performed again. BCVA examination will be completed before IOP measurement at 08:00.
f. Biomicroscopy examination must be completed before IOP is measured at 08:00. Aqueous flare and cell evaluation will be performed before fluorescein instillation.
g. IOP measurements will be performed at 08:00, 10:00 and 16:00 (±60 min) at all visits except for Visit 1 and the optional Visit 1a (screening and mid-washout).
h. Pachymetry will be performed after IOP measurement at Visit 1 (Screening).
i. Eye photograph will be taken at Visits 2 (Baseline), 5 (Month 3) and 8 (Month 12).
j. 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. Gonioscopy will be performed after IOP measurement at Visit 1 (Screening).
k. 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.
l. Ophthalmoscopy will be performed at Visits 1, 2, 5 and 8 (i.e., Screening, Baseline, Month 3 and Month 12) after the 16:00 IOP measurements. Ophthalmoscopy will be performed with pupil dilation at screening and visit 8/exit or early termination. Dilation of the pupil will be performed after the 16:00 IOP measurement.
m. Blood sampling for the pharmacogenomics/genomics laboratory research study may be performed at any visit after pharmacogenomics/genomics informed consent obtained, subject randomized and study drug dosing has begun.
n. Optional at unscheduled visit, required for early terminated subjects.

Table 2: Schedule of Events and Procedures Specially for PEDIATRIC Subjects

	Washout Period		Double-Masked Treatment Period			
	Visit 1 Screening	Washout Period (up to 4 weeks) Optional Visit 1a	Visit 2 Eligibility/ Baseline (Day 1)	Visit 3 Week 1 (Day 8±2)	Visit 4 Week 6 (Day 43 ±3)	Visit 5 Month 3 (Day 91±7) Exit or Early Termination [n]
Informed Consent(s) including the optional consent for pharmacogenomics/ genomics laboratory research study [a]	X					
Inclusion/Exclusion Criteria	X		X			
Demographics and Medical History, including prior PGA [b]	X					
Concomitant Medications/ Therapies	X	X	X	X	X	X
Dosing Compliance				X	X	X
AEs		X	X	X	X	X
Pregnancy Test [c]	X		X			X
Vital Signs (blood pressure/pulse rate) [d]	X		X (08:00)			X (08:00)
Refraction [e]	X					
BCVA[e]	X	X	X (08:00)	X (08:00)	X (08:00)	X (08:00)
Biomicroscopy [f]	X	X	X (08:00)	X (08:00)	X (08:00)	X (08:00)
IOP [g]	X	X	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00	08:00 10:00 16:00
Pachymetry [h]	X					
Instill study medication after IOP measurement				X (08:00)	X (08:00)	X (08:00)
Iris color, eyelash, eyelid [i]			X (photo)			X (photo)
Gonioscopy [j]	X					
Visual Field [k]	X					
Ophthalmoscopy [l]	X (pupil dilation)		X (16:00)			X (16:00 pupil dilation)
Blood Sampling for Pharmacogenomics/genomics [m]				X		
Dispense Study Medication			X		X	
Collect Study Medication					X	X

Table 2: Schedule of Events and Procedures Specially for PEDIATRIC Subjects (Continued)

	Washout Period		Double-Masked Treatment Period			
	Visit 1 Screening	Washout Period (up to 4 weeks) Optional Visit 1a	Visit 2 Eligibility/ Baseline (Day 1)	Visit 3 Week 1 (Day 8±2)	Visit 4 Week 6 (Day 43 ±3)	Visit 5 Month 3 (Day 91±7) Exit or Early Termination [n]
Phone call to remind subject to take evening dose on the day before each visit				X	X	X

a. Informed Consent Form and the Assent Form 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's medical records or subject history.
c. A urine pregnancy test will be conducted for all female subjects of childbearing potential.
d. Vital signs (resting blood pressure and pulse rate) will be collected in anytime at Visit 1 (Screening), and approximately 08:00 for Visit 2 (Baseline), and Visit 5 before the morning dose.
e. Refraction will be performed at the screening visit if the pediatric subject is able to cooperate. Autorefraction is also acceptable. If more than 10 letters in BCVA are lost compared to the screening visit level, then refraction should be performed again if the pediatric subject is able to cooperate. BCVA examination will be completed before IOP measurement at 08:00. BCVA will be performed for the pediatric subjects who are able to cooperate using an age-appropriate eye chart and method (e.g., ETDRS chart, LEA symbols chart with logMAR notation, Tumbling E's with logMAR notation, Landolt's broken rings with LogMAR notation, fix and follow) under normal room illumination. Same eye chart should be used for a given subject throughout the study.
f. Biomicroscopy examination must be completed before IOP is measured at 08:00. Aqueous flare and cell evaluation will be performed before instillation of fluorescein.
g. IOP measurements will be performed at 08:00, 10:00 and 16:00 (±60 min) at all visits except for Visit 1 and the optional Visit 1a (screening and mid-washout). IOP will be measured with age-appropriate tonometers (e.g., Goldmann applanation tonometer, a Perkins tonometer, or a tonopen, or iCare tonometer etc.) to the extent the subject is able to cooperate.
h. Pachymetry will be performed after IOP measurement at Visit 1 (Screening) if the subject is able to cooperate. Subject must have a sufficiently clear cornea that allows for a complete ophthalmic exam to be enrolled if pachymetry is not able to be obtained.
i. Eye photograph will be taken at Visits 2 (baseline), and 5 (month 3) if the subject is able to cooperate.
j. Gonioscopy will be performed at Visit 1 (Screening) if the subject is able to cooperate. If unable to cooperate, historical gonioscopy performed within 12 months prior to screening and documented in the subject's records is acceptable. Gonioscopy performed at Visit 1 (Screening) should occur after IOP measurement.
k. Visual field test will be performed if the subject is able to cooperate. 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.
l. Ophthalmoscopy will be performed if the subject is able to cooperate. Ophthalmoscopy will be performed at Visits 1, 2, and 5 (i.e., Screening, Baseline and Month 3) after the 16:00 IOP measurements. Ophthalmoscopy will be performed with pupil dilation at Visit 1 and visit 5/exit or early termination. Dilation of the pupil will be performed after the 16:00 IOP measurement.
m. Blood sampling for the pharmacogenomics/genomics laboratory research study may be performed at any visit after the pharmacogenomics/genomics informed consent is obtained, subject is randomized and study drug dosing has begun.
n. Optional at unscheduled visit, 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 Date and Treatment End Date

Treatment start date and *treatment end date* for each treatment period are defined as follows in Table 3.

Table 3: Definitions for Treatment Start and End Dates by Treatment Period

Treatment Period	Treatment Start Date	Treatment End Date
Double-Masked (DM) Treatment Period	The date at which a randomized subject takes the first dose of the DM study drug	The date at which a randomized subject takes the last dose of the DM study drug. If the date of the last dose is missing and the subject does not receive any OL study drug: • The day before the Visit 5 (Month 3) date will be considered the treatment end date for subjects who completed the Month 3 Visit • The day before the Exit Visit date will be used for subjects who prematurely discontinued from the study before Visit 5 (Month 3). 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
Open-Label (OL) Treatment Period	The date at which a randomized adult subject takes the first dose of the OL study drug	The date at which a randomized adult subject takes the last dose of the OL study drug. If the date of the last dose is missing: • The day before the Visit 8 (Month 12) date will be considered the treatment end date for subjects who completed the Month 12 Visit • The day before the Exit Visit date will be used for subjects who prematurely discontinued from the study during OL period. 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

Table 3: Definitions for Treatment Start and End Dates by Treatment Period (Continued)

Treatment Period	Treatment Start Date	Treatment End Date
Entire Study	The date at which a randomized subject takes the first dose of the DM study drug	The date at which a randomized subject takes the last dose of the study drug. Missing last dose date will be handled the same way as mentioned above.

Notes:
1. Pediatric subjects only participate in the double-masked period.
2. For each treatment period, the treatment end date will be set to missing if the treatment start date is missing.

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 treatment start date is the reference date. Thus, 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

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 4).

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 1 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 4: Analysis Visit and Analysis Window

Analysis Visit Name (Target Day)	Protocol Visit Window	Analysis Window		
		Iris Color, Eyelash, and Eyelid	Ophthalmoscopy and Vital Signs	Other Parameters
Baseline (Day 1)	[1, 1]	- 1]	- 1]	- 1]
Week 1 (Day 8)	[6, 10]	NA*	NA*	[2, 22]
Week 6 (Day 43)	[40, 46]	NA*	NA*	[23, 61]

Table 4: Analysis Visit and Analysis Window (Continued)

Analysis Visit Name (Target Day)	Protocol Visit Window	Analysis Window		
		Iris Color, Eyelash, and Eyelid	Ophthalmoscopy and Vital Signs	Other Parameters
Month 3 (Day 91)	[84, 98]	[2, day of Month 3 Visit]	[2 -, day of Month 3 Visit]	[62, day of Month 3 Visit]
Month 6 (Day 181)	[171, 191]	[First Day of OL period + 1, 225]	NA*	[First Day of OL period + 1, 225]
Month 9 (Day 271)	[261, 281]	[226, 315]	NA*	[226, 315]
Month 12 (Day 361)	[351, 371]	[316 -	[Day of Month 3 Visit + 1, -	[316 -

*Not collected at this visit.

If 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, 10:00, and 16:00) will be selected for that analysis visit first, before applying the above rule.

4.1.5. Extent of Exposure

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

4.2. Endpoint-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. 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.

4.2.3. 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)

4.3. Efficacy-Related Definitions

4.3.1. Response Endpoint and Response Rate

Four IOP response endpoints (Table 5) will be evaluated at each post-Baseline visit during the double masked-period (Week 1, Week 6, or Month 3) and open-label period (Month 6, Month 9, and Month 12) in this study:

Table 5: Response Endpoints

Response Endpoint	Response criteria in mean diurnal IOP
IOP 20% response	Percent reduction from Baseline ≥ 20%
IOP 25% response	Percent reduction from Baseline ≥ 25%
IOP 30% response	Percent reduction from Baseline ≥ 30%
IOP ≤ 18 mmHg response	Mean diurnal IOP ≤ 18 mmHg

For a response endpoint, the response rate at a post-Baseline visit is calculated as the proportion of subjects who met the response criterion at the post-baseline visit.

The open-label analysis of these endpoints will be performed only at the end of this study when all adult subjects complete their Month 12 Visit.

4.4. Safety-Related Definitions

4.4.1. Adverse Event

Under Protocol 011709IN, an AE is defined as any untoward medical occurrence that occurs in a study subject, regardless of the causal relationship with the study treatment. 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 onset date of the AE occurred on or after the treatment start date till up to 2 days (twice the scheduled dosing interval) after treatment end date. Treatment-emergent AEs (TEAEs) are a subset of on-study AEs. Both on-study and TEAEs will be collected, but only TEAEs will be tabulated.

The severity of each AE will be graded by the Clinical Investigator as Mild, Moderate, or Severe. AEs will also be rated by the Investigator as to their causality/relationship to the study drug.

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.

4.4.1.1. Serious Adverse Event

An AE will be counted as a *serious adverse event* (SAE) if the Clinical Investigator selected "Yes" to the question '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)
- It required or prolonged inpatient hospitalization.
- It resulted in 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).
- It resulted in a congenital anomaly/birth defect in the offspring of a study subject who was exposed to study therapy prior to conception or during pregnancy.
- It is a medically significant event(s), which may include "sight-threatening events," that may not meet any of the above serious criteria but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above.

4.4.1.2. Ocular Adverse Event

An AE will be counted as an *ocular AE* if the Clinical Investigator selected "OD", "OS", or "OU" under 'Eye(s) affected' on the AE eCRF.

4.4.1.3. Suspected Adverse Reaction

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

4.4.1.4. Events of Special Interest

Events of special interest (ESI) are pregnancy, clinically significant study medication administration error, and macular edema/cystoid macular edema.

4.4.2. Other Safety Measures

Table 6 lists the safety measures to be evaluated for this study.

Table 6: Safety Assessments

Safety Measures	Note
BCVA	Best-corrected visual acuity will be measured for each eye at each visit under normal room illumination using visual acuity chart (e.g., ETDRS chart) and the logMAR scoring will be recorded in the subject's source document. Increase in logMAR scores means worsening in visual acuity.

Table 6: Safety Assessments (Continued)

Safety Measures	Note
Slit-lamp Biomicroscopy	Slit-lamp biomicroscopy examinations (severity scores for 12 parameters: lid hyperemia, lid edema, conjunctival hyperemia, conjunctival chemosis, corneal staining, corneal edema, keratic precipitate, anterior chamber cells, anterior chamber flare, anterior synechiae of iris, posterior synechiae of iris, and cataract) will be performed and graded immediately before the 8:00 IOP measurement at all visits except Visit 1/1a (Screening or mid-washout visit). Cataract severity will be assessed for phakic eyes.
Ophthalmoscopy	The ophthalmoscopy (fundus) examination will be performed for each eye at Visit 1, Visit 2, Visit 5, and Visit 8/ Study Exit/Early Termination. Variables from ophthalmoscopy are cup-to-disc ratio, glaucomatous optic nerve severity, and assessments of vitreous, retina, macula, and choroid.
Eyelid Sulcus, eyelid, and iris color	The investigator (or his/her designee) will take front- and side-view photographs of each eye at Visit 2 (Baseline). The photographs must include the iris, eye lids and eyelashes of each eye. The photographs taken at Visit 2 (Baseline) will be used to help the Investigator assess any changes ("No changes", "Increased", or "Decreased") from baseline in eyelid, eyelashes, and iris color at each follow-up visit, and assess for DUES ("Yes", "No") at Visit 5, Visit 6, Visit 7, and Visit 8/ Study Exit/Early Termination.
Vital Signs	Resting blood pressure and pulse rate will be collected at Visit 1, Visit 2, Visit 5, and Visit 8/ Study Exit/Early Termination.

4.5. Other Definitions

4.5.1. Prior and Concomitant Medications

Non-study medications will be categorized into prior medications and concomitant medications. Specifically, *prior medication* is defined as any non-study medication taken and ended prior to the study medication start date. *Concomitant medication* is defined as any non-study medication taken concurrently while receiving study medication, i.e., the treatment period (period of time from first dose to last dose) of a concomitant medication taken by a subject must overlap with the treatment period (period of time from first dose to last dose) of the study medication.

All prior and concomitant medications will be coded using the World Health Organization (WHO) Drug Global, Version September 2018, format B3. Each prior or concomitant medication will be classified using the Anatomical-Therapeutic-Chemical (ATC) classification system and mapped to a WHO Drug preferred drug name.

4.5.2. Concurrent Medical Conditions

Concurrent medical conditions are those ongoing conditions or diseases that are present at the signing of informed consent.

5. STUDY POPULATION

During masked data review before database lock, subject 011709IN-8400217-3609 was excluded from all the analysis populations. The background and rationale for excluding this subject can be found in 011709IN-rationale-for-exclusion-of-a-subject.

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 summarizing by actual treatment received.

5.3. Full Analysis Set

The Full Analysis Set (FAS) will include all randomized subjects who received at least one dose of study medication and provided Baseline IOP data (at any timepoint) and at least one post-baseline IOP measurement (at any timepoint). 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 that will be conducted for the primary and key secondary efficacy endpoint 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 affect 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 actual 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 (%).

The statistical testing will be conducted at a significance level of 0.05 (two-sided) and the 95% confidence interval will be shown, 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, the inferential analysis of each IOP endpoint will adjust for the baseline IOP. Detailed information on covariate adjustment is provided in Section 8.1.

6.2. Handling of Missing Data

6.2.1. Efficacy Measures

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 in both double-masked and open-label periods.

For efficacy measures during double-masked period,

- For each IOP endpoint, no imputation is needed for the analysis on observed cases using the mixed-effects model for repeated measures (MMRM). Sensitivity analysis will be performed using a Pattern-Mixture Model (PMM) approach.
- In the PMM approach, Multiple Imputation (MI) with mixed missing data mechanisms will be used to investigate the appropriateness of the missing at random (MAR) assumption for missing values resulting from censoring due to the use of IOP-lowering rescue therapy, or resulting from study discontinuation due to lack of efficacy or AE.

For efficacy measures during open-label treatment period, no inferential analysis will be performed. Summary statistics will be provided for IOP and mean diurnal IOP for each scheduled visit, using observed data.

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), AE, and concomitant medications (CM) will be imputed as follows (Table 7):

Table 7: 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 date of YYYY to impute the missing month and day of the onset date.
	YYYY and MM are available, but DD is missing	Use the first date of MM to impute the missing day 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 date of YYYY to impute the missing month and day of the resolution date.
	YYYY and MM are available, but DD is missing	Use the last date of MM to impute the missing day of the resolution date.

6.3. Multi-Center Studies

This is a multi-center study enrolling subjects from 49 US sites. The number of subjects per site might be small. Therefore, there are no analyses adjusting for sites.

6.4. Multiple Comparisons / Multiplicity

To control the overall Type I error rate across the seven hypothesis tests involving the primary endpoint and the key secondary endpoints (listed below) at the 0.05 level (two-sided), a fixed sequence procedure will be implemented. The hypothesis tests will be assessed sequentially as follows:

1. Noninferiority of 0.002% DE-117 to 0.5% timolol maleate for the primary endpoint, IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at Week 1, Week 6, and Month 3.
2. Noninferiority of 0.002% DE-117 to 0.5% timolol maleate for the first key secondary endpoint, mean diurnal IOP at Month 3.
3. Noninferiority of 0.002% DE-117 to 0.5% timolol maleate for the second key secondary endpoint, IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at Week 1, Week 6, and Month 3 in the study eyes with mean diurnal IOP < 25 mmHg at the Baseline Visit.

4. Superiority of 0.002% DE-117 to 0.5% timolol maleate for the third key secondary endpoint, mean diurnal IOP at Week 1.
5. Superiority of 0.002% DE-117 to 0.5% timolol maleate for the first key secondary endpoint, mean diurnal IOP at Month 3.
6. Superiority of 0.002% DE-117 to 0.5% timolol maleate for the second key secondary endpoint, IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at Week 1, Week 6, and Month 3 in the study eyes with mean diurnal IOP < 25 mmHg at the Baseline Visit.
7. Superiority of 0.002% DE-117 to 0.5% timolol maleate for the primary endpoint, IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at Week 1, Week 6, and Month 3.

The second hypothesis testing 2) will be tested only if the first hypothesis 1) is rejected. The third hypothesis testing 3) will be tested only if the second hypothesis 2) is rejected, and so on.

6.5. Interim Analysis

No interim analysis is planned for this study.

Analysis of the double-masked period data will be conducted when all the randomized subjects complete their Month 3 Visit, data cleaned and frozen. All efficacy and safety data from open-label period at the time of this data cut will also be analyzed and reported. The 011709IN Data Cut Plan details how data collected during open-label period will be cut for this “snapshot”.

7. SUMMARY OF STUDY POPULATION DATA

7.1. Subject Disposition

The disposition of ITT population (i.e., all randomized subjects) will be summarized by treatment period (double-masked and open-label) and by planned treatment (for double-masked period) and overall.

For the double-masked period, the summary will include the number and percentage of ITT subjects in the Safety population, FAS, and PPS. The disposition summary will also include the number and percentage of subjects who completed the study drug, subjects who discontinued the study drug prematurely but continued with study participation until the Month 3 Visit and among which who completed or not completed the Month 3 Visit, as well as the number and percentage of subjects who discontinued from the study or discontinued from the study drug prior to Month 3 Visit by the primary discontinuation reason.

For the open-label period, the summary will include the number and percentage of subjects who received any study treatment during open-label period, and subjects who are still on the study at the time of data cut (for reports other than the final clinical study report). The disposition summary will also include the number and percentage of subjects who completed the study drug, and subjects who discontinued from the study prior to Month 12 Visit by the primary discontinuation reason.

7.2. Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be descriptively summarized for the FAS, adult subjects, pediatric subjects, Safety population, and subjects who received open-label treatment period therapy with DE-117, separately, by planned treatment (for double-masked period). Specifically, for subject demographics, the following variables will be summarized:

- Age at randomization (continuous and categorical: (< 18 years, ≥ 18 and < 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 and fellow eye, separately by treatment period (double-masked and open-label):

- Primary ocular diagnosis [categorical: POAG, Pseudoexfoliative Glaucoma, Pigmentary Glaucoma, or OHT for adult subjects, and juvenile open angle glaucoma (JOAG) for pediatric subjects]
- Prior use of IOP-lowering medications (categorical: oral/topical carbonic anhydrase inhibitors, alpha agonists, beta-blockers, prostaglandin/prostaglandin analogues, or none)
- Prostaglandin naive (Yes or No)

- Baseline lens status (categorical: phakic or pseudophakic/aphakic)
- Baseline mean diurnal IOP score and baseline IOP score at each scheduled timepoint (08:00, 10:00, and 16:00)
- BCVA (logMAR)
- Central corneal thickness (μm)
- Glaucomatous optic nerve findings (categorical: none, mild, moderate, or severe)
- Anterior chamber angle classification (Shaffer scale; categorical: approximately 5 degrees or less, approximately 10 degrees, approximately 20 degrees, approximately 30 degrees, or approximately 40 degrees or more)
- Iris Color (categorical: Blue/Gray, Blue/Gray with Slightly Brown, Blue/Gray – Brown, Green, Green with Slightly Brown, Green – Brown, Yellow – Brown, or Brown)
- Visual field (parameters: glaucoma Hemifield test, visual field mean deviation by device, and visual field pattern standard deviation by device)

7.3. Medical and Surgical History

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

The medical and surgical history will be summarized for the FAS. 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
- Randomization
- Safety reporting
- Time Window
- Other

A protocol deviation is considered significant if it may affect the subject's rights, safety, or well-being, and/or the completeness, accuracy, or reliability of the study data. Santen's study team will review all protocol deviations and determine the list of significant protocol deviations prior to database lock. All randomized subjects with any significant protocol deviation(s) will be tabulated by treatment period (double-masked and open-label) and by deviation category for each planned treatment (for double-masked period) and overall. In addition, two listings will be provided for double-masked period: (1) all significant protocol deviations and (2) subjects excluded from the per protocol population. All significant protocol deviations will also be listed for open-label period.

7.5. Prior and Concomitant Medications

For this study, non-study medications, including prior and concomitant medications for double-masked period and concomitant medications for open-label period, will be coded using World Health Organization (WHO) Drug Global, Version September 2018, format B3. Each non-study medication will be classified using the Anatomical-Therapeutic-Chemical (ATC) classification system and mapped to a WHODrug 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.

For the same concomitant medications that are used in both double-masked and open-label treatment periods, they will be summarized in both double-masked and open-label periods.

7.6. Treatment Compliance

For the purpose of compliance calculation, there will be six study intervals:

- Baseline Visit to Week 1
- Week 1 to Week 6
- Week 6 to Month 3
- Month 3 to Month 6
- Month 6 to Month 9
- Month 9 to Month 12

The compliance rate for a subject will be calculated as follows for each study interval during double-masked period:

$$\text{Compliance Rate (\%)} = (2*\text{Duration} - \sum\text{Miss}) / (2*\text{Duration}) \times 100$$

And, for open-label period:

$$\text{Compliance Rate (\%)} = (\text{Duration} - \sum\text{Miss}) / (\text{Duration}) \times 100$$

Where

Duration: The number of days subject should have administered study medication, calculated as:

Study Interval	Equation for Duration Calculation
Baseline Visit to Week 1	Week 1 Visit date – Date of first drug dispensation
Week 1 to Week 6	Week 6 Visit date – Week 1 Visit date
Week 6 to Month 3	Month 3 Visit date – Week 6 Visit date
Month 3 to Month 6	Month 6 Visit date – Month 3 Visit date
Month 6 to Month 9	Month 9 Visit date – Month 6 Visit date
Month 9 to Month 12	Month 12 Visit date – Month 9 Visit date

Miss: The number of missed doses since the last visit.

For subjects in the FAS, the compliance rate will be summarized by treatment period (double-masked and open-label), post-baseline study interval, and by planned treatment (for double-masked treatment period).

7.7. Exposure to Study Medication

The duration of exposure to a study medication is measured by days on treatment as derived in Section 4.1.5. 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 actual treatment received for double masked period and for the entire study separately. For the entire study period, the duration of exposure to overall DE-117 treatment will also be provided, which will combine exposure to DE-117 from both double-masked arms. For subjects who were in the Timolol arm during double-masked treatment period, their duration of exposure to DE-117 will be the total duration on the study minus the number of days they were exposed to Timolol treatment.

The duration of exposure categories are:

- Double-masked period: 1-30, 31-60, or ≥ 61 days
- Entire study period: 1-30, 31-60, 61-90, 91-120, 121-150, 151-180, 181-210, 211-240, 241-270, 271-300, 301-330, or ≥ 331 days

8. EFFICACY ANALYSES

Unless specified otherwise, efficacy analyses will be performed on the study eye, based on the FAS, and summarized by planned treatment, irrespective of the actual treatment received.

Unless otherwise specified, for subjects who receive IOP-lowering rescue therapy, any IOP values collected after the receipt of rescue therapy will be censored (i.e., treated as missing) in the analyses.

8.1. Efficacy Analyses for Double-Masked Period

8.1.1. Analyses of Primary Endpoint and Key Secondary Endpoints

8.1.1.1. Primary Analyses

8.1.1.1.1. Primary Endpoint

The primary efficacy endpoint is IOP in the study eye at each scheduled timepoint (08:00, 10:00, and 16:00) at each of three follow-up visits, Week 1, Week 6, and Month 3. The nine combinations of three scheduled timepoints at each of three follow-up visits are referred to subsequently as nine timepoints. For the primary endpoint, the following null (versus alternative) hypothesis will be tested:

$$H_0: \mu_T - \mu_C > \Delta, \textit{ for at least one timepoint}$$

versus

$$H_A: \mu_T - \mu_C \leq \Delta \textit{ at all nine timepoints}$$

where μ_T and μ_C denote the mean values of the primary endpoint in the DE-117 arm and the Timolol arm, respectively, and Δ denotes the noninferiority margin of 1.5 mmHg or 1.0 mmHg as specified below.

The primary analysis of the primary efficacy endpoint will be performed using a MMRM of IOP based on the FAS. A separate MMRM will be used for IOP at each scheduled timepoint (8:00, 10:00, or 16:00). For each scheduled timepoint, the model will include treatment (T_i), analysis visit (V_j), and treatment-by-visit interaction (TV_{ij}) as fixed effects, and baseline IOP at the scheduled timepoint as a covariate. Correlations of IOP measurements within-subject will be modeled using an unstructured covariance matrix. The mathematical model is given by:

$$IOP_{ijn} = \mu + T_i + V_j + TV_{ij} + \varepsilon_{ijn}$$

where μ denotes the overall mean, and $\boldsymbol{\varepsilon}$ denotes the random error associated with the individual subject. The above model allows treatment effect and its variability to vary over study visits.

If there are convergence issues, covariance matrix structures other than unstructured will be tried in the following order: (1) heterogeneous Toeplitz (TOEPH), (2) heterogeneous autoregressive of order 1 (ARH(1)), (3) heterogeneous compound symmetry (CSH), and (4) compound symmetry (CS). The first covariance structure that converges will be used as the primary analysis. Least squares mean IOP values for each treatment arm and differences between least squares treatment arm means and associated 95% confidence intervals will be reported for each of the nine timepoints.

The primary analysis will be repeated on pediatric subjects.

Noninferiority criteria for primary endpoint: With respect to the primary study objective, noninferiority of DE-117 to Timolol is achieved if the upper limit of the two-sided 95% confidence interval for the difference in the mean IOP (DE-117 minus Timolol) is ≤ 1.5 mmHg at all nine timepoints and ≤ 1.0 mmHg at a majority (five or more) of the nine timepoints.

Superiority of DE-117 to Timolol for the primary efficacy endpoint is achieved if the upper limit of the two-sided 95% confidence interval for the difference in the mean IOP (DE-117 minus Timolol) is < 0 mmHg at all nine timepoints.

8.1.1.1.2. Key Secondary Endpoints

8.1.1.1.2.1. Mean Diurnal IOP at Month 3

The first key secondary endpoint is the mean diurnal IOP in the study eye at Month 3. The following null (versus alternative) hypothesis will be tested:

$$H_{0S1}: \mu_{T1} - \mu_{C1} > \Delta \text{ versus } H_{AS1}: \mu_{T1} - \mu_{C1} \leq \Delta$$

where μ_{T1} and μ_{C1} denote the mean diurnal IOP at Month 3 in the DE-117 and Timolol arm, respectively, and Δ denotes the noninferiority margin of 1.5 mmHg. The primary analysis of this key secondary efficacy endpoint will be performed using a MMRM based on the FAS. The model will include treatment, analysis visit (Week 1, Week 6, or Month 3), and treatment-by-visit interaction as fixed effects, and baseline mean diurnal IOP at as a covariate. Correlations of mean diurnal IOPs within-subject will be modeled using an unstructured covariance matrix. The mathematical model is analogous to the model in the primary efficacy endpoint.

If there are convergence issues, covariance matrix structures other than unstructured will be tried in the following order: (1) heterogeneous Toeplitz (TOEPH), (2) heterogeneous autoregressive of order 1 (ARH(1)), (3) heterogeneous compound symmetry (CSH), and (4) compound symmetry (CS). The first covariance structure that converges will be used as the primary analysis. Least squares mean diurnal IOP values at Month 3 for each treatment arm and the difference between least squares treatment group means and its 95% confidence interval will be reported.

Noninferiority of DE-117 to Timolol is achieved for this endpoint if the upper limit of the 95% confidence interval for the difference between DE-117 and Timolol (DE-117 minus Timolol) in the mean diurnal IOP at Month 3 is ≤ 1.5 mmHg. Superiority of DE-117 to Timolol is achieved for this endpoint if the upper limit of the 95% confidence interval is < 0 mmHg.

This key secondary endpoint analysis will be repeated on pediatric subjects.

8.1.1.1.2.2. IOP at Nine Timepoints in Study Eyes with Baseline Mean Diurnal IOP < 25 mmHg

The second key secondary endpoint is IOP at each scheduled timepoint (08:00, 10:00, and 16:00) at each of three follow-up visits, Week 1, Week 6, and Month 3, in the study eyes with mean diurnal IOP < 25 mmHg at the Baseline Visit. If noninferiority of DE-117 to Timolol for the primary endpoint and the first key secondary endpoint is achieved, the following null (versus alternative) hypothesis will be tested for the second key secondary endpoint:

$$H_{0\,S2}: \mu_{T2} - \mu_{C2} > \Delta, \text{ for at least one timepoint}$$

versus

$$H_{A\,S2}: \mu_{T2} - \mu_{C2} \leq \Delta \text{ at all nine timepoints}$$

where μ_{T2} and μ_{C2} denote the mean IOP values in the DE-117 arm and the Timolol arm, respectively, and Δ denotes the noninferiority margin of 1.5 mmHg or 1.0 mmHg as specified below.

The same MMRM used in the analysis of the primary efficacy endpoint will be used for the analysis of the second key secondary endpoint. Noninferiority of DE-117 to Timolol for this endpoint (i.e., for subjects with baseline mean diurnal IOP < 25 mmHg) is achieved if the upper limit of the two-sided 95% confidence interval for the difference in the mean IOP (DE-117 minus Timolol) is ≤ 1.5 mmHg at all nine timepoints and ≤ 1.0 mmHg at a majority (five or more) of the nine timepoints. Superiority of DE-117 to Timolol for this endpoint (i.e., for subjects with baseline mean diurnal IOP < 25 mmHg) is achieved if the upper limit of the two-sided 95% confidence interval for the difference in the mean IOP (DE-117 minus Timolol) is < 0 mmHg at all nine timepoints.

8.1.1.1.2.3. Mean Diurnal IOP at Week 1

The third key secondary endpoint is mean diurnal IOP in the study eye at Week 1. If noninferiority of DE-117 to Timolol for the primary and the first two key secondary endpoints is achieved, then the following null (versus alternative) hypothesis will be tested for the third key secondary endpoint:

$$H_{0S3}: \mu_{T3} = \mu_{C3} \text{ versus } H_{AS3}: \mu_{T3} \neq \mu_{C3}$$

where μ_{T3} and μ_{C3} denote the mean diurnal IOP at Week 1 in the DE-117 and Timolol arm, respectively. The primary analysis of this key secondary endpoint will be performed using a MMRM analogous to the model used for the first key secondary efficacy endpoint (mean diurnal IOP at Month 3).

For the primary and key secondary efficacy endpoints, the testing sequence and multiplicity adjustment details are provided in Section 6.4.

This key secondary endpoint analysis will be repeated on pediatric subjects.

8.1.1.2. Sensitivity Analyses

For the primary endpoint and the key secondary endpoints, sensitivity analyses will be performed to assess the robustness of the results from the primary analysis. The sensitivity analyses consist of two types: (1) assessment based on the PPS and (2) a pattern-mixture model (PMM) approach (Table 8). Unless otherwise specified, for subjects who receive IOP-lowering rescue therapy, any IOP values collected after the receipt of rescue therapy will be censored (i.e., treated as missing) in the analyses.

Table 8: Overview of Primary and Sensitivity Analysis Methods

Primary or Sensitivity Analysis	Statistical Method	Analysis Population	Handling of Missing Data
Primary Analysis	MMRM	FAS	Observed cases
Sensitivity Analyses	MMRM	PPS	Observed cases
	PMM with delta-adjustment	FAS	MI

Abbreviations: FAS = full analysis set; MI = multiple imputation; MMRM = mixed-effects model for repeated measures; PMM = pattern-mixture model; PPS = per protocol set.

To assess the sensitivity to departure from MAR assumption, a PMM with delta-adjustment will be applied, as follows:

a. Use Markov chain Monte Carlo (MCMC) to create monotone missingness first. Then obtain standard multiple imputations under MAR assumptions for missing IOP values using monotone regression. The missing data are filled in and 50 complete datasets are created. The seed that will be used in the SAS program is 13546.
b. For each IOP value that had been missing because of (1) censoring after having received IOP-lowering rescue therapy; or because of (2) early study discontinuation due to lack of efficacy or AE, regardless of treatment arm, adjust these values by adding delta to the imputed values, where delta = 1.0 mmHg. The seed that will be used in the SAS program is 13546.
c. Each of the 50 complete datasets will then be analyzed separately using the same MMRM as used for the primary analysis.
d. The estimates obtained from the MMRM analysis of each complete dataset are combined for inference purposes.
e. Repeat step b, c, d, with delta = 2.0, 3.0, 4.0, and 5.0 mmHg. The larger the value of delta that is required to reverse the conclusion of the primary analysis, the more robust the conclusion is considered to be.

The main part of the SAS code for the specified MMRM analysis and PMM are provided in Appendix A and Appendix B, respectively.

For the primary endpoint, in addition to the two types of sensitivity analyses described above, if the overall rate of study drug discontinuation due to lack of efficacy or AE is greater than 10% and the difference between the rate in the DE-117 and Timolol arms is greater than 5 percentage points, a trimmed mean analysis (Permutt et al., 2017) will be conducted as a sensitivity analysis.

8.1.2. Analyses of Other Secondary Efficacy Endpoints

Other secondary endpoints to be assessed include:

- Change and percent change from baseline in IOP at each timepoint/post-baseline visit
- Change and percent change from baseline in mean diurnal IOP at each post-baseline visit
- Having a mean diurnal IOP reduction ≥ 20%, ≥ 25%, or ≥ 30% from Baseline (Visit 2) at each post-baseline visit
- Having a mean diurnal IOP ≤ 18mmHg at each post-baseline visit

For continuous secondary endpoints (change and percent change from baseline in IOP/mean diurnal IOP) at Week 1 (Visit 3), Week 6 (Visit 4), and Month 3 (Visit 5), both an analysis using a MMRM will be performed and descriptive summaries will be generated. The MMRM will be analogous to the MMRM used in the analysis of the primary endpoint. For both the analysis using MMRM and descriptive summaries of the IOP or mean diurnal IOP (raw) scores, IOP values obtained after using rescue therapy will be censored (i.e., treated as missing).

For the following binary secondary endpoints, the responder rates will be analyzed using the Pearson's chi-square test for a 2×2 contingency table; differences between the DE-117 and Timolol arms will be reported along with corresponding p-values:

- Having a mean diurnal IOP reduction ≥ 20%, ≥ 25%, or ≥ 30% from Baseline (Visit 2) at each post-baseline visit
- Having a mean diurnal IOP ≤ 18mmHg at each post-baseline visit

The above secondary efficacy endpoint analyses will be repeated on pediatric subjects.

8.1.3. Subgroup Analyses

The homogeneity of treatment effects among prospectively defined subgroups was assessed using MMRM analyses of IOP (at each visit and timepoint) and mean diurnal IOP (at each visit) for the following subgroups:

- Age (< 18, ≥ 18 and < 65, or ≥ 65 years)
- Sex (males or females)
- Race (White or Black)
- Primary ocular diagnosis [OAG (including pigmentary glaucoma and pseudoexfoliative glaucoma), OHT, or JOAG]
- Prior use of IOP-lowering medication (ß-adrenergic antagonist, prostamide or prostaglandin analogue, α-adrenergic agonist, carbonic anhydrase inhibitors, or none)
- Mean diurnal IOP at baseline (< 25 or ≥ 25 mmHg)
- Lens status (phakic or pseudophakic/aphakic)

Other subgroup analyses may be performed as deemed necessary.

8.2. Efficacy Analyses for Open-Label Period

For efficacy measures during open-label period, no inferential analysis will be performed. Summary statistics will be provided on the following variables for open-label and entire study periods separately for all subjects who entered the open-label treatment period.

- Raw scores, change from baseline, and percent change from baseline in IOP at each timepoint/post-baseline visit
- Raw scores, change from baseline, and percent change from baseline in mean diurnal IOP at each post-baseline visit

9. SAFETY ANALYSES

The safety-related measures collected in this study include AEs, best-corrected visual acuity, slit-lamp biomicroscopy (severity scores for 12 parameters), ophthalmoscopy (cup-to-disc ratio, glaucomatous optic nerve severity score, and assessments of retina, macula, choroid, and vitreous), and eyelid sulcus/eyelid/eyelash/iris color. In the event that a subject took both DE-117 and Timolol during double-masked period of the study, the subject will be summarized with those receiving DE-117.

All the safety-related measures will be summarized descriptively by actual treatment received on the Safety population.

9.1. Adverse Events

AEs, SAEs, SARs, serious SARs, and significant AEs (AEs leading to study drug discontinuation and AEs leading to death) will be tabulated by type of AEs by treatment arm using actual treatment received for all AEs, ocular AEs, and non-ocular AEs separately.

Besides the overall AE summary, AEs, SAEs, SARs, serious SARs will be tabulated by SOC and preferred term for all AEs, ocular AEs, and non-ocular AEs separately. A subject who experienced multiple AEs within a SOC or preferred term will be counted only once for that SOC or preferred term. Non-serious AEs (including number of events) and ESIs will also be summarized by SOC and preferred term.

All the AE summaries will be performed for double-masked, open-label, and entire study periods separately. For double-masked period summaries, overall AE summary, summary of AE by SOC and PT, SAEs, SARs, and serious SARs will also be tabulated on pediatric subjects separately. For open-label and entire study period summaries, tabulations by treatment received during double-masked period will be provided as well as overall summary for subjects exposed to DE-117. So, for subjects who exposed to the Timolol arm during the double-masked period, their AE summaries under the Overall DE-117 column will be those AEs that started during the open-label treatment period.

AEs, SAEs, SARs, serious SARs, ocular AEs, AEs leading to death, AEs leading to study drug discontinuation, non-TEAEs, and ESIs, if any, will be listed separately.

9.1.1. Ocular Inflammation

Subjects who developed AEs during the study with one of the following preferred terms will be included in the ocular inflammation summary:

- Anterior chamber cell
- Anterior chamber flare
- Eye inflammation
- Anterior chamber inflammation
- Iridocyclitis
- Iritis

- Uveitis

Other preferred terms might be added upon review of the AE data before database lock.

Ocular inflammation leading to study drug discontinuation, requiring steroid treatment, or requiring NSAIDs treatment will be summarized at the subject level by frequency and percentage separately.

Ocular inflammation start time (in terms of study day) and duration of the AE will be summarized at the eye level using mean, standard deviation, median, minimum, and maximum by study eye and fellow eye separately. To allow for the computation of simple descriptive statistics, only subjects who develop ocular inflammation during the study will be used for the ocular inflammation start time summary, and only subjects who develop these AEs during the study and later resolved will be used in the duration of AE analysis.

The aforementioned summaries will be performed at the eye level. All ocular inflammation AEs will be provided in a listing.

9.1.2. Macular Edema

Subjects who developed AEs during the study with one of the following preferred terms will be included in the macular edema summary:

- Macular edema
- Cystoid macular edema

Macular edema start time (in terms of study day) and duration of the AE will be summarized using mean, standard deviation, median, minimum, and maximum by study eye and fellow eye separately. To allow for the computation of simple descriptive statistics, only subjects who develop macular edema during the study will be used for the macular edema start time summary, and only subjects who develop these AEs during the study and later resolved will be used in the duration of AE analysis.

Change from baseline in BCVA at the onset of macular edema, when macular edema is resolved, and at the last visit will be summarized using mean, standard deviation, median, minimum, and maximum by study eye and fellow eye separately. Pseudophakic eyes or phakic eyes at baseline who developed macular edema during the study will be summarized by frequency and percentages separately.

The aforementioned summaries will be performed at the eye level. All macular edema AEs will be provided in a listing.

9.1.3. Cosmetic Change(s)

Subjects who developed AEs during the study with one of the following preferred terms will be included in the cosmetic change summary:

- Blepharal pigmentation
- Eyelash changes
- Eyelash hyperpigmentation

- Eyelash thickening
- Growth of eyelashes
- Lid sulcus deepened
- Trichiasis
- Iris hyperpigmentation

Other preferred terms might be added upon review of the AE data before database lock.

Cosmetic change(s) start time (in terms of study day) will be summarized using mean, standard deviation, median, minimum, and maximum by study eye and fellow eye separately. To allow for the computation of simple descriptive statistics, only subjects who develop cosmetic change(s) during the study will be used for the cosmetic change start time summary.

The aforementioned summaries will be performed at the eye level. All cosmetic change AEs will be provided in a listing.

9.2. Best-Corrected Visual Acuity

BCVA (logMAR scores) and changes from baseline will be summarized by treatment, analysis visit, and study period (double masked and open-label) for study eyes and fellow eyes, separately. In addition, any change (worsening or improvement) of ≥ 0.2 LogMAR (2 lines) from baseline will be summarized and listed.

9.3. Slit-lamp Biomicroscopy

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

9.4. Ophthalmoscopy

Cup-to-disc ratio will be summarized with n, mean, standard deviation, median, minimum and maximum by treatment, analysis visit, and study period (double masked and open-label) for study eyes and fellow eyes separately. In addition, subjects with at least 0.2 increase in cup/disc ratio from baseline will be listed.

Glaucomatous optic nerve findings will be assessed as 0 = none, 1 = mild, 2 = moderate, or 3 = severe. Frequency and percentage of rating scores will be summarized by treatment and analysis visit for study eyes and fellow eyes, separately. In addition, subjects with any worsening (increase) of ≥ 2 units from baseline will be listed.

Retina/Macula/Choroid and vitreous will be assessed as normal or abnormal. Shift from baseline at Month 3 will be summarized by treatment on study eyes and fellow eyes separately. In addition, subjects with change from baseline from normal to abnormal in these parameters will be listed.

9.5. Eyelid Sulcus, Eyelid, Eyelash, and Iris Color

For changes from baseline in eyelid pigmentation, eyelid hair growth, eyelash length, eyelash thickness, eyelash pigmentation, eyelash number, and iris color/pigmentation, frequency and percentage of subjects with No change, Increased, or Decreased will be summarized by treatment, analysis visit, and study period (double masked and open-label) for study eyes and for fellow eyes. For changes from baseline in eyelid sulcus, count and percentage of changes (Yes) will be summarized by treatment and analysis visit (Month 3, Month 6, Month 9, and Month 12) for study eyes and for fellow eyes. In addition, any changes from baseline in eyelid sulcus will be listed.

9.6. Vital Signs

Resting blood pressure (systolic and diastolic) and pulse rate and their change from baseline will be summarized by treatment, analysis visit, and study period (double masked and open-label).

A listing for vital signs will be provided.

10. ADDITIONS AND CHANGES TO THE PROTOCOL-SPECIFIED ANALYSES

10.1. Full Analysis Set Definition

The full analysis set definition has been updated to add the requirement that baseline IOP data has to be present:

The Full Analysis Set (FAS) will include all randomized subjects who received at least one dose of study medication and provided Baseline IOP data (at any timepoint) and at least one post-baseline IOP measurement (at any timepoint). Efficacy analyses will be performed using the FAS and summarized by treatment as randomized.

10.2. Key Secondary Endpoint

An additional key secondary endpoint (IOP at each scheduled timepoint [08:00, 10:00, and 16:00] at Week 1 [Visit 3], Week 6 [Visit 4], and Month 3 [Visit 5] in the study eyes with mean diurnal IOP < 25 mmHg at the Baseline Visit) was added based on analysis results of a recently completed study in Asia. This key secondary endpoint was not in the clinical study protocol, but instead it was added in the SAP. All endpoints for this study are presented in Section 2.2.

10.3. Sensitivity Analysis of the Primary and Secondary Efficacy Endpoints

Protocol Amendment 1 (Version 1.1) Section 14.5, Handling of Missing Values, specified a sensitivity analysis of the primary efficacy endpoints and the secondary binary IOP endpoints (Month 3) in which missing values would be imputed using the last-observation-carried-forward (LOCF) approach. The LOCF approach is removed from the sensitivity analyses because the PMM generalizes the MMRM to include mixed missing data mechanisms beyond the MAR assumption of the MMRM. Thus, the PMM is sufficient to evaluate the robustness of the results from the MMRM.

11. REFERENCES

1. Permutt, T., Li, F. (2017). Trimmed means for symptom trials with dropouts. Pharm Stat. 16(1):20-8. doi: 10.1002/pst.1768. PubMed PMID: 27523396.

12. APPENDICES