Dompé farmaceutici S.p.A.

REDUCO

NGF0123

NCT06244316

A 4-Week, Phase II, Multicenter, Randomized, Double-Masked, Vehicle-Controlled, Parallel Group Study With 4 Weeks of Follow-Up to Evaluate Safety and Efficacy of a new formulation of Recombinant Human Nerve Growth Factor (rhNGF) Eye Drop Solution at two different Concentrations in patients with Dry Eye Disease

25Feb2025

Statistical Analysis Plan

Version *2.0*

Prepared by:

PPD

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
AT	Artificial Tears
ATC	Anatomical Therapeutic Chemical
BCDVA	Best Corrected Distance Visual Acuity
BID	Bis in die (twice a day)
°C	Temperature Degree Celsius
CI	Confidence Interval
CSR	Clinical Study Report
DED	Dry Eye Disease
DFE	Dilated Fundus Exam
eCRF	Electronic Case Report Form
ENR	Enrolled Population
EOS	End of Study
EOT	End of Treatment
ET	Early Termination
EU	European Union
FAS	Full Analysis Set
FCS	Fully Conditional Specification
FTBUT	Fluorescein Tear Break-Up Time
IB	Investigator Brochure
ICE	Intercurrent Event
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IRT	Interactive Response Technology
LogMAR	Logarithm of the Minimum Angle of Resolution (Chart)
LS	Least Square
MAR	Missing at Random
MedDRA	Medical Dictionary for Regulatory Activities
mcg/mL	micrograms/milliliter
mcL	microliter

MI	Multiple Imputation
MMRM	Mixed Model for Repeated Measures
MNAR	Missing Not at Random
CC	[redacted]
CCI	[redacted]
NIMP	Non Investigational Medicinal Product
CCI	[redacted]
PD	Protocol Deviation
PP	Per Protocol Population
PT	Preferred Term
QoL	Quality of Life
Q1	first quartile (lower quartile)
Q3	third quartile (upper quartile)
RCT	Randomized Clinical Trial
rhNGF	recombinant human Nerve Growth Factor
RND	Randomized Population
SAE	Serious Adverse Event
SAF	Safety Population
SANDE	Symptom Assessment in Dry Eye
SAP	Statistical Analysis Plan
SCR	Screened Population
SD	Standard Deviation
SE	Standard Error
SLE	Slit-Lamp Examination
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
CCI	[redacted]
USA	United States of America
V	Visit
CCI	[redacted]
VBR	Validated Bulbar Redness scale
Vs	Versus
WHODrug	World Health Organization Drug Dictionary

Revision History

Version	Changes Made	Document Date
Final 1.0	First release.	CCI [redacted]
Final 2.0	CCI [redacted]	25Feb2025

1. Introduction

This statistical analysis plan (SAP) describes the analyses and data presentations for Dompé's protocol REDUCO "A 4-Week, Phase II, Multicenter, Randomized, Double-Masked, Vehicle-Controlled, Parallel Group Study With 4 Weeks of Follow-Up to Evaluate Safety and Efficacy of a new formulation of Recombinant Human Nerve Growth Factor (rhNGF) CCI Solution at two different Concentrations in patients with Dry Eye Disease" which was issued on 16Nov2023. This SAP is aligned with version 3.0 of the protocol, dated 11Mar2024. It contains definitions of analysis populations, derived variables, and statistical methods for the analyses of efficacy and safety.

The study plans only a final analysis: this analysis will be conducted when all randomized patients have completed the study (completed treatment at week 4 (visit 4) and completed follow-up at week 8 (visit 5)) and the study database has been locked and all personnel have been unmasked.

The purpose of the SAP is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of study data prior to database lock and final analyses. This SAP will be finalized and signed prior to the clinical database lock for the final analyses. Additional post-hoc analysis may be produced to allow further comparisons between treatment and control, according to the results obtained.

This SAP is to be interpreted in conjunction with the protocol. Should the SAP and protocol be inconsistent with respect to the planned analyses, the language of the SAP is governing. If the final clinical study report (CSR) contains changes to any planned statistical analyses (including unplanned analyses), the justification for any such differences will be fully documented in the CSR.

2. Objectives

2.1. Primary Objective

- To evaluate the efficacy of C mcg/mL and C mcg/mL concentrations of the new formulation of rhNGF ophthalmic solution versus vehicle, in order to demonstrate superiority of at least one of the concentrations over vehicle in the improvement of ocular symptoms of dry eye in patients with dry eye disease (DED) at Week 8.

2.2. Key Secondary Objectives

- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in increasing the number of patients with improved reflex tear production as compared to vehicle at week 4.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle at week 4.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving ocular surface integrity (corneal epitheliopathy) as compared to vehicle at week 4.

2.3. Secondary Objectives

- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in increasing the number of patients with improved reflex tear production as compared to vehicle at week 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle at week 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving tear film stability as compared to vehicle at weeks 4 and 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving ocular surface integrity (corneal and conjunctival epitheliopathy) as compared to vehicle at weeks 4 and 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving the severity and frequency of dry eye symptoms as compared to vehicle at weeks 4 and 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving dry eye symptoms as compared to vehicle at week 4.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving associated symptoms in DED as compared to vehicle at weeks 4 and 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving the quality of life in patients with DED as compared to vehicle at weeks 4 and 8.
- To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving best corrected visual acuity in DED as compared to vehicle at weeks 4 and 8.

2.4. Safety Objectives

- To evaluate safety of the new formulation of rhNGF ophthalmic solution.
- To evaluate tolerability of the new formulation of rhNGF ophthalmic solution.

CCI

- CCI

3. Investigational Plan

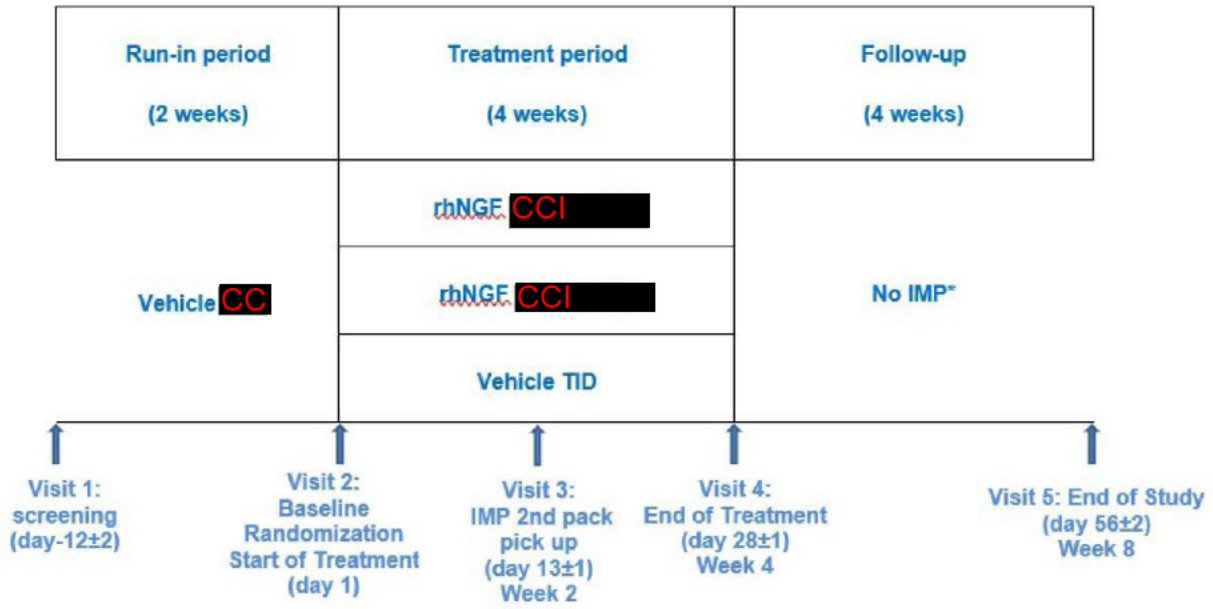
3.1. Overall Study Design and Plan

This trial is a 4-weeks Phase II, multi-centre, randomized, double-masked, vehicle-controlled, parallel-group, dose-finding clinical trial with 4 Weeks of Follow-Up to evaluate safety and efficacy of a new formulation of recombinant human Nerve Growth Factor (rhNGF) CCI solution at two different concentrations in patients with dry eye disease (DED). It is designed as a superiority clinical trial to assess the efficacy and safety of a new ophthalmic formulation of rhNGF as compared to a vehicle in patients with DED. This is a prospective Phase II trial for a new formulation of CCI (C mcg/mL and C mcg/mL) of rhNGF CCI .

Male or female dry eye disease patients aged ≥18 years of any race/ethnicity and eye color will be screened for enrollment to minimize bias in population sampling. All patients successfully enrolled will be randomized at Baseline – Day 1 visit in a 1:1:1 ratio to either of the CC CCI of rhNGF formulation (C mcg/mL or C mcg/mL) or vehicle.

The study is divided in 3 periods: Screening & Run-in Period (Day -12±2/Screening Visit 1 to Day 1/Baseline Visit 2); Treatment Period (day 1/Baseline Visit 2, Week 2/Day 13 ± 1 Visit 3, Week 4/Day 28 ± 1 Visit 4); Follow-up Period (End of Treatment Visit 4 at Week 4 to End of Follow-up - End of Study Visit 5 at Week 8, Day 56 ± 2). Procedures for patient inclusion in the study will be performed at both Visit 1 (Screening) and Visit 2 (Baseline). The End of Study Trial visit is expected at Week 8. The maximum total study duration will be about 10 weeks for each patient.

Figure 1: Study Schema



*artificial tears CCI provided by the sponsor.

3.2. Study Endpoints

Objectives	Endpoints
Primary Objective	**Primary Efficacy Endpoint**
• To evaluate the efficacy of C mcg/mL and C mcg/mL concentrations of the new formulation of rhNGF ophthalmic solution versus vehicle, in order to demonstrate superiority of at least one of the concentrations over vehicle in the improvement of ocular symptoms of dry eye in patients with dry eye disease (DED).	• Mean change from baseline to Week 8 in symptoms of dry eye assessed by SANDE Global Score [Time Frame: week 8 (V5)]

Objectives	Endpoints
Key Secondary Objectives	**Key Secondary Efficacy Endpoints**
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in increasing the number of patients with improved reflex tear production as compared to vehicle at week 4	• Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye [Time Frame: at week 4 (V4)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle at week 4	• Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye [Time frame: at week 4 (V4)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving ocular surface integrity (corneal epitheliopathy) as compared to vehicle at week 4	• Mean change from baseline in total CCI [redacted] in the Study Eye as assessed by the investigator [Time Frame: at week 4 (V4)]
Secondary Objectives	**Secondary CCI [redacted] (*) Efficacy Endpoints**
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle at week 8	• Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye [Time frame: at week 8 (V5)] • Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye [Time Frame: at week 8 (V5)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving tear film stability as compared to vehicle at weeks 4 and 8	• Mean change from baseline in fluorescein tear break-up time (fTBUT)- in Study Eye [Time Frame: at weeks 4 (V4) and 8 (V5)] • CCI [redacted]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving ocular surface integrity (corneal and conjunctival epitheliopathy) as compared to vehicle at weeks 4 and 8	• Mean change from baseline in total CCI [redacted] in the Study Eye as assessed by the investigator [Time Frame: at week 8 (V5)] • CCI [redacted]

Objectives	Endpoints
	CCI [redacted]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving the severity and frequency of dry eye symptoms as compared to vehicle at weeks 4 and 8	• Mean change from baseline in symptoms questionnaire (SANDE) scores for severity and frequency [Time Frame: at weeks 4 (V4) and 8 (V5)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving dry eye symptoms as compared to vehicle at week 4	• Mean change from baseline in symptoms of dry eye assessed by SANDE Global Score [Time Frame: at week 4 (V4)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving associated symptoms in DED as compared to vehicle at weeks 4 and 8	• Mean change from baseline of the CCI [redacted] [Time Frame: at weeks 4 (V4) and 8 (V5)] • CCI [redacted]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving the quality of life in patients with DED as compared to vehicle at weeks 4 and 8	• Mean change from baseline as assessed by the CCI [redacted] QoL scores [Time Frame: at weeks 4 (V4) and 8 (V5)]
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving best corrected visual acuity in DED as compared to vehicle at weeks 4 and 8	• Mean change from baseline in BCDVA score [Time Frame: at weeks 4 (V4) and 8 (V5)]
Safety Objectives	**Safety Endpoints**
• To evaluate safety/tolerability of the new formulation of rhNGF ophthalmic solution	• Safety will be monitored by the incidence and frequency of treatment-emergent adverse events (TEAEs) assessed throughout the study including run-in period.
• To evaluate safety of the new formulation of rhNGF ophthalmic solution	• Mean change from baseline in corneal endothelial cell density in both eyes [Time Frame: at week 8 (V5)] • Change from baseline in the proportion of patients with vitritis, retinal or vitreal

Objectives	Endpoints
	hemorrhages, increase in cup-to-disc ratio, retinal or posterior vitreal detachment, retinal tears, or maculopathy on dilated fundus exam (DFE) in both eyes [Time Frame: at week 8 (V5)] • Mean change from baseline in bulbar conjunctival redness (VBR 10 score) in both eyes [Time Frame: at weeks 4 (V4) and 8 (V5)]
• To evaluate tolerability of the new formulation of rhNGF ophthalmic solution.	• Treatment discontinuation rate due to tolerability will also be evaluated.
CCI	CCI
• CCI CCI (**) Only in patients with CCI	• CCI

3.3. Estimands

3.3.1. Primary Estimand

The estimand for the primary objective is defined as follows:

- Treatment: 4 weeks follow up after 4 weeks of CC administration of rhNGF new formulation (CCI and vehicle).
- Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set.
- Variable: mean change from baseline to week 8 in symptoms of dry eye assessed by SANDE Global Score.
- Intercurrent events (ICEs) and strategies to handle them:
  - Treatment discontinuation (only IMP): a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.1.1 for further details).
  - Use of a prohibited medication, in the week before any assessment of SANDE Global Score: a hypothetical strategy will be applied where any data collected in the week after the occurrence of the ICE will be discarded. Missing data will be imputed by using a multiple imputation with copy-reference approach (see Section 8.1.1 for further details).
- Population-level summary: difference in mean change from baseline to week 8 in

symptoms of dry eye assessed by SANDE Global Score between each concentration group of Investigational Medicinal Product (IMP - groups IMP1 and IMP2) and the vehicle group as derived by the Mixed Model for repeated measures defined in Section 8.1.1.

In summary, the primary estimand is defined as the difference in mean change from baseline to week 8 in symptoms of dry eye assessed by SANDE Global Score between treatments in the targeted patient population regardless of study treatment discontinuation and had prohibited medications not been available to patients in the week before any study evaluation.

3.3.2. Key Secondary Estimands

Key secondary objective #1

The estimand for the key secondary objective #1 is defined as follows:

- Treatment: 4 weeks of CCI administration of rhNGF new formulation (CC CCI and vehicle).
- Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set.
- Variable: proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye at week 4 (V4).
- ICEs and strategies to handle them:
  - Treatment discontinuation (only IMP): a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.2.1 for further details).
  - Use of a prohibited medication, in the week before any assessment of Schirmer-I score: a hypothetical strategy will be applied where any data collected in the week after the occurrence of the ICE will be discarded. Missing data for Schirmer-I score will be imputed by using a multiple imputation with copy-reference approach (see Section 8.2.1 for further details), then the proportion of patients with an improvement ≥ 10 mm/5min in the Study Eye will be derived.
- Population-level summary: difference in proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye at week 4 (V4) between each concentration group of IMP (groups IMP1 and IMP2) and the vehicle group as derived by a logistic model defined in Section 8.2.1.

In summary, the estimand for the key secondary objective #1 is defined as the difference in proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye at week 4 (V4) between treatments in the targeted patient population regardless of study treatment discontinuation and had prohibited medications not been available to patients in the week before any study evaluation.

Key secondary objective #2

The estimand for the key secondary objective #2 is defined as follows:

- Treatment: 4 weeks of CC administration of rhNGF new formulation (CC

[redacted] and vehicle).

- Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set.
- Variable: mean change from baseline in Schirmer-I score without anesthesia in the Study Eye at week 4 (V4).
- ICEs and strategies to handle them:
  - Treatment discontinuation (only IMP): a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.2.1 for further details).
  - Use of a prohibited medication, in the week before any assessment of Schirmer-I score: a hypothetical strategy will be applied where any data collected in the week after the occurrence of the ICE will be discarded. Missing data will be imputed by using a multiple imputation with copy-reference approach (see Section 8.2.1 for further details).
- Population-level summary: difference in mean change from baseline to week 4 in Schirmer-I score without anesthesia in the Study Eye between each concentration group of IMP (groups IMP1 and IMP2) and the vehicle group as derived by an ANCOVA model defined in Section 8.2.1.

In summary, the estimand for the key secondary objective #2 is defined as the difference in mean change from baseline to week 4 in Schirmer-I score without anesthesia in the Study Eye between treatments in the targeted patient population regardless of study treatment discontinuation and had prohibited medications not been available to patients in the week before any study evaluation.

Key secondary objective #3

The estimand for the key secondary objective #3 is defined as follows:

- Treatment: 4 weeks of [redacted] administration of rhNGF new formulation ([redacted] and vehicle).
- Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set.
- Variable: mean change from baseline in total [redacted] in the Study Eye as assessed by the investigator at week 4 (V4).
- ICEs and strategies to handle them:
  - Treatment discontinuation (only IMP): a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.2.1 for further details).
  - Use of a prohibited medication, in the week before any assessment of [redacted] a hypothetical strategy will be applied where any data collected in the week after the occurrence of the ICE will be discarded. Missing data will be imputed by using a multiple imputation with copy-reference approach (see Section 8.2.1 for further details).

- Population-level summary: difference in mean change from baseline to week 4 in total CCI in the Study Eye as assessed by the investigator between each concentration group of IMP (groups IMP1 and IMP2) and the vehicle group as derived by an ANCOVA model defined in Section 8.2.1.

In summary, the estimand for the key secondary objective #3 is defined as the difference in mean change from baseline to week 4 in total CCI in the Study Eye as assessed by the investigator between treatments in the targeted patient population regardless of study treatment discontinuation and had prohibited medications not been available to patients in the week before any study evaluation.

3.4. Treatments

In the present document, the definition "study treatment" will always refer to the Investigational Medicinal Product (IMP) only (rhNGF or Vehicle).

3.4.1. Investigational Medicinal Product (IMP)

The IMP consists of:

- A sterile CCI containing vehicle or CCI mg/vial of rhNGF, packaged in a glass vial and administered after reconstitution with;
- CCI or 2mL of diluent contained into a separate glass vial.

CCI

CCI, the IMP is a sterile solution for ocular administration at the following concentrations as active ingredient:

- IMP 1: rhNGF C mcg/mL (C mL CCI
- IMP 2: rhNGF C mcg/mL (C mL CCI
- Vehicle IMP: rhNGF 0 mcg/mL (Vehicle in 1 mL or 2 mL diluent).

Details of IMPs are described in Table 1.

Table 1. Description of Treatments

Treatment Name	Vehicle IMP	IMP 1	IMP 2
Active Ingredient	none	rhNGF	rhNGF
Quality Control	Dompé Farmaceutici S.p.A. (Italy)		
Dose (daily)	CCI Vehicle	CCI	CCI mcg/mL/eye CC
Pharmaceutical form	CCI vehicle + CCI	CCI rhNGF + CCI	
Formulation	ophthalmic solution (CCI CCI)		
Regimen	CCI	CCI	CCI
Route of Administration	Ocular	Ocular	Ocular

Stability CCI of the CCI solution	CCI	CCI	CCI
Stability CCI of the pharmaceutical form	CCI	CCI	CCI

Investigational Medicinal Products will be dispensed to the patient as listed in the Schedule of Activities (Appendix 13.1).

3.4.2. Non Investigational Medicinal Product

The Non Investigational Medicinal Product (NIMP) will be used in the follow-up period only and will be dispensed at Visit 4 End of Treatment only. Details are shown in Table 2 below.

Table 2. Description of NIMP

Treatment Name during Follow-up Period	NIMP (preservative free artificial tears)
Dosage (daily)	CCI
Regimen	CCI , CC CCI
Formulation	Preservative free, sterile solution, single-use/multi-use
Route of administration	Topical ocular CCI

3.5. Dose Modification

Dose modification is not anticipated.

3.6. Definition of Study Eye

The Study Eye is the worst eye: assuming that all the inclusion/exclusion criteria are met in both eyes, the worst eye (Study Eye) will be determined at the baseline visit based on the lower Schirmer I (without anesthesia) score. If the Schirmer I score of both eyes is identical, the Study Eye will be determined based on the CCI If both eyes are identical even considering NEI, then simply the right eye will be considered as the Study Eye.

4. General Statistical Considerations

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed with SAS® software, version 9.4 or higher.

Descriptive statistics for continuous variables in summary tables will include the number of patients in the analysis (n), mean, standard deviation (SD), median, first quartile (Q1), third quartile (Q3) and range (minimum, maximum). Descriptive statistics for qualitative variables in summary tables will include counts and percentages per category. If appropriate, 95% confidence intervals (CIs) around the mean or the proportion will be presented. The number of patients with missing data will be presented under the "Missing" category. Missing values will not be included in the denominator count when computing percentages. When continuous data are summarized, only the non-missing values will be evaluated for computing summary statistics. Any exception will be declared.

In general, regardless the strategy of the analysis described in Section 8, a summary table with descriptive statistics will be produced for each efficacy endpoint. Summaries will be generated for observed cases, on absolute values and changes from baseline at each timepoint for continuous variables, on proportions at each timepoint for qualitative variables.

For the purpose of efficacy analyses in this trial, the statistical analysis (both descriptive and inferential) will be done on the Study Eye which is determined at baseline visit (visit 2). Details will be provided in the statistical analysis outputs as notes. In case the analysis involves both eyes, it will be clearly declared. This consideration only applies to the assessments that are done at individual eye level and does not apply to any assessment done at patient level (e.g., SANDE, CCI ...).

For summary precision, mean, median, Q1 and Q3 will have one more decimal place than the reported value, SD will have two more decimal places than the reported value, minimum and maximum will have the same decimal place as the reported value. Percentages and 95% CIs will have one decimal place. When count data are presented, the percentage will be suppressed when the count is zero in order to draw attention to the non-zero counts. A row denoted "Missing" will be included in count tabulations where specified on the shells to account for dropouts and missing values.

Data transformation (e.g. natural log transformation) might be used in order to satisfy the assumption of normality requested by parametric statistical tests and models. In case such assumptions are not met even on the transformed data, non-parametric counterpart tests will be used. In case the natural log transformation is used, a back transformation to the original scale will be also provided, in order to facilitate the clinical interpretation of the results.

All patient data collected on the eCRF will be listed by treatment group and patient and ordered by site, if applicable. If data is collected by eye, Study Eye will be flagged.

4.1. Sample Size

The sample size of the study is calculated based on results from the previous study CCI
Expecting a mean difference of about 11 points (with unequal standard deviations in the active and vehicle groups: observed mean changes (SD) were -28.2 (26.36) and -17.4 (20.29) for IMP and vehicle, respectively) in improvement of SANDE Global Score from baseline to Week 8 in favor of rhNGF ophthalmic CCI) formulation, a total sample size of 276 evaluable patients (92 per group) is needed. This will allow to achieve an overall power of CCI for demonstrating superiority of at least one concentration of rhNGF ophthalmic formulation over vehicle in the improvement of SANDE Global Score from baseline to week 8, with an overall one-sided alpha CCI .

CCI .

Assuming a CCI of patients not evaluable for primary analysis after enrollment, the total number of patients to be enrolled in the study will be about 291 (97 per group).

In Dompé previous studies, a screen failure rate of about 20% has been observed. This implies that approximately 350 patients will need to be screened in order to have 291

patients enrolled and at least 276 evaluable. In any case, the screen procedures should be interrupted when the expected sample size of 291 patients has been reached.

4.2. Randomization, Stratification, and Masking

Interaction Response Technology (IRT) will be used to randomize the patients in a 1:1:1 ratio to each of the three treatment arms.

Eligible patients will be randomized in a 1:1:1 ratio to rhNGF [redacted] mcg/mL [redacted] (about 97 patients), rhNGF [redacted] mcg/mL [redacted] (about 97 patients) or vehicle ophthalmic solution [redacted] (about 97 patients). Patients in the vehicle arm will be randomly assigned 1:1 to two different vehicle filling volumes to preserve masking. Nevertheless, all the patients randomized to the vehicle arm will be analyzed as a single treatment arm.

Each randomized patient will be allocated with a randomization number, according to the stratified randomization list. Dropouts after randomization will not be replaced.

Randomization will be stratified by site to ensure balanced assignment across treatment groups within each site. The stratified permuted block randomization list will be generated with a computer procedure by an independent statistician not involved in the conduct of the study.

This is a double-masked study. Appearance, including packaging and labeling, of the study products (IMP or vehicle) will not allow recognition of the actual treatment. Masked information on the identity of the assigned study products will be provided for each patient.

Unmasking can only occur in case of emergency, when knowledge of the treatment identity is essential for treating the patient. If the treatment code needs to be broken in the interest of patient safety for a medical emergency, the Investigator is allowed to break the treatment code for the specific patient, even before informing the Sponsor.

4.3. Analysis Set

The following analysis sets will be used in the statistical analyses.

4.3.1. Screened Population (SCR)

A patient will be defined as screened after the signature of the informed consent, the assignment of a Screening number and regardless of the completion of all the screening procedures. eCRF should be completed for each patient that signed the informed consent, including the screening failures.

4.3.2. Enrolled Population (ENR)

A patient in the SCR population will be defined as enrolled if meeting all the inclusion criteria and none of the exclusion criteria at the end of the screening procedure at Visit 1 (Day -12±2) and Visit 2 (Day 1). Patients in the SCR population but not included in the ENR population will be defined as screen failures.

4.3.3. Randomized Population (RND)

The Randomized population will consist of all patients in the SCR population who were assigned a randomization number.

4.3.4. Full Analysis Set (FAS)

The Full Analysis Set (FAS) will consist of all participants in the RND population who took at least one dose of IMP. The FAS will be analyzed according to the intention-to-treat principle, i.e. by treatment allocation. The FAS population will be used for the primary analysis of the study and to present results on efficacy data.

4.3.5. Per-Protocol Population (PP)

The Per Protocol (PP) population will consist of all patients in the FAS population who do not have major protocol deviations. Primary efficacy analysis will be conducted on the FAS population while SAF and PP populations will be used for safety and sensitivity analysis, respectively.

Reasons for the exclusion from the PP will be determined in the masked Data Review Meeting. A non-exhaustive list of possible reasons for exclusion from the PP can be found in Section 5.2.

4.3.6. Safety Population (SAF)

The Safety (SAF) population will consist of all patients in the RND population who received at least one dose of the IMP. The Safety population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.

4.4. Analysis Window/Visit

For safety and efficacy endpoints, data will be evaluated and reported according to the nominal visit at which they have been collected, even if performed out of the tolerance interval limits declared in Figure 1.

Efficacy analyses. Before applying any imputation methods, or any other strategy required by the handling of intercurrent events as detailed in Section 3.3, a remapping will be done for any missing assessment at each planned visit. Remapping is allowed only for missing total scores (i.e. missing data for each item will not be replaced). In case data are missing at a given nominal visit, data collected at any other unscheduled visit (or ETV if done within an acceptable time-window of a previous visit) will be remapped to that visit, if the date of collection is within the tolerance interval limits defined in table of activities in Section 13.1 (i.e. ±1 for V3 – Day 13, ±1 for V4 – Day 28, ±2 for V5 – Day 56). Data collected under a nominal visit at out of tolerance interval limits will be used in the analysis unless the patient is declared an early study withdrawal: in this latter case, the evaluations recorded under the nominal visit will be set to missing and the actual values will be moved to a new timepoint named “Early Termination Visit”. This new timepoint will be only listed.

Safety analyses. Data collected under a nominal visit at out of tolerance interval limits will be treated as described for the efficacy. Data collected at an “Early Termination Visit” will be tabulated, separately from nominal visit values (i.e. Week 4 and Week 8).

4.4.1. Baseline

For the purpose of the statistical analysis, Baseline is defined as the last visit prior to and including date of the randomization visit (V2 – Day 1). Unless otherwise specified, baseline values are defined as the measurements taken during this visit. In case of multiple measurements

during baseline visit, the last assessment will be considered as the baseline evaluation. In case of missing value at baseline visit, but availability of other values collected before the first administration of the IMP (e.g. at Screening), the last available value collected will be used as the baseline value.

4.5. Handling of Missing Data

Data will be presented in the listings as reported. For summaries and analysis, the conventions described in the following paragraphs apply.

4.5.1. SANDE Global Score

For the efficacy inferential analysis, missing SANDE Global Score data will be imputed using the imputation methods described in Section 8.1.1 and Section 8.1.2. Descriptive analyses will be based on observed data.

4.5.2. Key Secondary and Secondary Endpoints

For the efficacy inferential analysis, missing data on key secondary and secondary endpoints will be imputed using the imputation methods described in Section 8.2.1.2. or Section 8.2.1.3, depending on the nature of the data. Descriptive analyses will be based on observed data.

4.5.3. Adverse Event Data

Algorithm to impute partial or missing end dates is described in Appendix 13.2, but partially or missing AE start dates will not be imputed. The rule for the classification of AEs and TEAEs is also shown in Appendix 13.2 with details on the derivation of the period for TEAEs after date imputation.

In case one or more AE characteristics are missing (for example severity and relationship to study drug), the most conservative approach will be taken (in the example, the AE will be considered severe, and drug related).

In listings of AE data, the partial dates as collected will be displayed. Imputed dates with details of what has been imputed in brackets (e.g. D if day is imputed, M if month, DM if both) will be provided.

4.5.4. Prior/Concomitant Medication and Procedure Data

Algorithm to impute partial or missing end dates is described in Appendix 13.2, but partially or missing medication/procedure start dates will not be imputed. The rule for the classification in "prior" or "concomitant" medication/procedure is also shown in Appendix 13.2.

In listings of concomitant medication and procedure data, the partial dates as collected will be displayed. Imputed dates with details of what has been imputed in brackets (e.g. D if day is imputed, M if month, DM if both) will be provided.

5. Patient Disposition

5.1. Disposition

The number of patients who were screened, and the eligibility criteria met/failed will be summarized based on all screened patients. The number and percentage of screen failure patients will be summarized for each exclusion criteria met and/or inclusion criteria failed.

Disposition of all randomized patients will be summarized by treatment group including:

- Full Analysis Set
- Safety Set
- Per-Protocol Set
- Number of patients who completed the study treatment
- Number of patients who prematurely discontinued the study treatment
    - Number of patients who prematurely discontinued the study treatment but complete the study
    - Number of patients who prematurely discontinued the study treatment and discontinued the study
- Primary reason for treatment discontinuation
- Number of patients who completed the study
- Number of patients who prematurely discontinued the study (early termination)
- Primary reason for study discontinuation.
- Number of patients who were unblinded during the study and the reason (Emergency situation, Other).

Primary reasons for treatment discontinuation collected on the eCRF will be summarized with the following categories separately:

- Adverse Event
- Death
- Lost to Follow-up
- Physician Decision
- Protocol Deviation
- Withdrawal by Patient
- Pregnancy
- Treatment Discontinuation due to Tolerability Issues
- Other

Primary reasons for study discontinuation collected on the eCRF will be summarized with the following categories separately:

- Adverse Event
- Death
- Lost to Follow-up
- Physician Decision
- Protocol Deviation
- Screen Failure
- Study Terminated by Sponsor
- Withdrawal by Patient
- Other

The distribution of the time from randomization to study discontinuation will also be summarized using time-to-event analysis. A log-rank test will be used to compare distributions among the three groups (two IMP groups and the Vehicle group).

Screening data will be listed by patients as collected based on all screened patients (SCR). Disposition data will be listed by patients as collected based on all randomized patients (RND) by site.

Inclusion and exclusion criteria deviations will be presented for all screened patients in a data listing by site.

In addition, total study duration from the screening (date of informed consent) to the last visit of each patient will be summarized.

5.2. Protocol Deviations

Protocol deviations (PDs) that will be analyzed and reported in the TLFs come from two different sources: CTMS and SAS programming. All PDs will be reviewed in a masked manner before the hard lock of the database in order to check the ones that actually have an impact (e.g. bias) on the statistical analysis. PDs will be classified for analysis purposes in Major and Minor PDs according to the level of bias that they can produce on the primary and/or key secondary endpoints. Not all deviations reported in the CTMS will be analyzed (i.e. will not be classified as either major or minor PDs), if not relevant from an analysis perspective. Major PDs will define patients that will be excluded from the PP set.

<u>Major protocol deviations derived in SAS:</u>

The following PDs will be derived in SAS and classified as major. Any further change, if any, will be discussed and documented during the blind DRM.

- Lack of compliance with IMP (compliance <80% or compliance >120%).
- Exposure to IMP lower than expected (exposure in days < 24 days).
- Missing all post-baseline assessments for one or more of the primary and key secondary endpoints (subjects should have at least one post-baseline assessment for each of the primary and key secondary endpoints).
- Intake of prohibited medications.

Minor protocol deviations derived in SAS:

- Lack of compliance with NIMP (compliance <80% or compliance >120%).

Finally, number of patients with at least one major/minor PD will be summarized. Major PD will be tabulated, while all PDs (major and minor) will be listed, including full description and the GCP severity classification, as reported in the PD rule document (significant / not significant). Tabulation and listing will be based on all randomized patients.

6. Demographics and Baseline Characteristics

6.1. Demographics and Baseline Characteristics

Demographic and baseline characteristics data to be analyzed will include age, sex, race, ethnicity, childbearing potential, based on the FAS accordingly to the planned treatment.

Descriptive statistics will be calculated for the following continuous variables:

- Age (years)

Number and percentage of patients will be provided for the following categorical variables:

- Sex (Male, Female)
- Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Other, Multiple, Not Reported, Unknown)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported, Unknown)
- Childbearing Potential (Yes, No, Not Applicable)
- Region (US, EU)
- Study Eye (Right Eye, Left Eye).

6.2. Medical History

Medical history will be summarized based on the SAF according to the actual treatment received by condition type (history condition / concomitant condition). Medical history will be coded according to latest version of Medical Drug Regulatory Activities (MedDRA) and will be summarized by body system code and preferred term (PT), with body system code sorted alphabetically and PTs within each body system code in descending order of frequency.

Patients experiencing more than one disease will be counted only once within each SOC and PT.

Ophthalmic medical history will be summarized separately from non-ophthalmic medical history. Ophthalmic medical history will be summarized for the Study Eye and fellow eye separately.

A by-patient listing of ophthalmic and non-ophthalmic medical histories will be presented and ordered by site. Ongoing conditions will be considered as “concomitant conditions” and will be denoted in the listing. Any conditions with end dates on or after screening visit or with missing end dates will also be considered as “concomitant conditions”.

6.3. Dry Eye History

Dry eye history will be summarized by eye (study eye and fellow eye) for time from diagnosis (years), cause of dry eye, receiving of prior treatment, and eye affected by moderate to severe dry eye. Time from diagnosis will be calculated by date of informed consent – date of diagnosis for each eye + 1, then divided by 365.25. Partial dates are treated according to below rules:

- if month and day are missing, se to 01-July of that year;
- if only day is missing, set to 15th of that month.

Dry eye history will be presented in a by-patient listing by site as collected per eCRF.

7. Treatments, Medications, and Procedures

7.1. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the latest version of World Health Organization Drug Dictionary (WHODrug) during the study.

Ophthalmic and non-ophthalmic medications (both prior and concomitant) will be summarized based on the FAS, sorted by alphabetical order of Anatomical Therapeutic Chemical 4 (ATC4) level, and then descending frequencies of preferred term. If ATC4 level is missing, the last available level will be used.

By-patient listings of prior and concomitant medications will be presented.

Partial missing start or end dates will be imputed as described in Appendix 13.2 for medications analysis.

7.1.1. Prior Medications

Prior medications are defined as medications that stop before the first dose of study drug.

Ophthalmic prior medications will be summarized separately from non-ophthalmic prior medications. Ophthalmic prior medications will be summarized for the study eye and fellow eye separately. Medications will be sorted by alphabetical order of ATC4 level, and then descending frequencies of preferred term. If ATC4 level is missing, the last available level will be used.

7.1.2. Concomitant Medications

Concomitant medications are defined as medications first received at or after the first dose of study drug, medications received before first dose of study drug and continued after first dose, or medications with missing stop date.

Ophthalmic concomitant medications will be summarized separately from non-ophthalmic concomitant medications. Ophthalmic concomitant medications will be summarized for the study eye and fellow eye separately.

7.1.3. Prohibited Medications

During the whole period of the participation to the trial (about 10 weeks) patients should not use ophthalmic steroids, ophthalmic immunomodulators such as cyclosporine A and lifitegrast, intranasal tear-secretagogues such as varenicline, ophthalmic topical anti-histamine, any derivatives of nerve growth factor, or any medications as part of another clinical trial. The use of

artificial tears - lubricating CCI not provided by the Sponsor is also forbidden. Patients should not use punctal plugs, contact lenses (including scleral lenses), moisture goggles, warm compresses or electro/magnetic stimulation devices on the eye, face or scalp.

The algorithm for the identification of the prohibited medications taken during the study has been reported in Appendix 13.4.

Before the study unmasking, a masked review of the concomitant medications taken by the patients during the study will be done in order to verify the presence of prohibited medications; their frequency and dosage will also be considered in order to define the severity of the deviation.

Prohibited medications will be summarized based on FAS according to the planned treatment. Ophthalmic prohibited medications will be summarized separately from non-ophthalmic prohibited medications. Ophthalmic prohibited medications will be summarized for the study eye and fellow eye separately.

A by-patient listing of prohibited medications will be presented by treatment.

7.2. Prior and Concomitant Procedures

Prior and concomitant procedures will be coded using the latest version of MedDRA during the study. Ophthalmic and non-ophthalmic procedures will be summarized based on the FAS according to the planned treatment, sorted by system organ class (SOC) and PT, with SOCs sorted alphabetically and PTs within each SOC in descending order of frequency.

By-patient listings of prior and concomitant ophthalmic and non-ophthalmic procedures will be presented by site.

Partial missing start or end dates will be imputed as described in Appendix 13.2 for medications analysis.

7.2.1. Prior Procedures

Prior procedures are defined as procedures that stop before the first dose of study drug.

Ophthalmic prior procedures will be summarized separately from non-ophthalmic prior procedures. Ophthalmic prior procedures will be summarized for the study eye and fellow eye separately.

7.2.2. Concomitant Procedures

Concomitant procedures are defined as procedures first received at or after the first dose of study drug, procedures received before first dose of study drug and continued after first dose or procedures with missing stop date.

Ophthalmic concomitant procedures will be summarized separately from non-ophthalmic concomitant procedures. Ophthalmic concomitant procedures will be summarized for the study eye and fellow eye separately.

7.3. Study Treatments

The study product vehicle, IMP and NIMP will be dispensed as detailed in the Schedule of Activities (Appendix 13.1).

A by-patient listing of study treatments will be presented and ordered by site.

7.3.1. Exposure and Compliance

The compliance for both the IMP and NIMP will be assessed by number CCI collected in eDiary.

Exposure and compliance to IMP

In treatment period, starting from the first IMP administration done at the baseline visit until the last expected administration, the IMP should be administered CCI (1 drop each time and three times per day). Compliance calculation is based on the number of administrations, C CI .

The duration (days) of exposure to the IMP will be calculated by using data collected in the eCRF as:

$$exposure\ IMP\ (days)_{eCRF} = last\ date\ of\ treatment_{eCRF} - first\ date\ of\ treatment_{eCRF} + 1$$

where first date of treatment and last date of treatment are derived from the dates collected in the eCRF in the Randomization and End of Treatment forms, respectively.

Compliance for the study eye will be derived on the period defined by the exposure (i.e. by using only data collected in the interval defined by first and last date / timepoint collected in the eCRF) as the proportion (%) between the actual number of administrations done in the study eye and the expected number of administrations done in the study eye. CCI

CCI

CCI

CCI

on

CCI

The actual number of administrations done in the study eye is derived by using data collected in the eDiary. CCI

Extra-administrations recorded in the eDiary between first and last administration date (timepoint not recorded in the eDiary) will be also included in the count if administered in the study eye. CCI

The compliance will then be derived as:

$$compliance\ IMP = 100 * \frac{number\ of\ actual\ administration_{eDiary}}{expected\ number\ of\ administration_{eCRF}}$$

Compliance will also be analyzed weekly from baseline to Week 4.

Gross non-compliance will be defined as accountability lower than 80% or greater than 120% and in case of gross non-compliance the patient will be excluded from the Per Protocol dataset.

Exposure and compliance to NIMP (ATs) in the follow-up period.

In follow-up period, the NIMP should be administered CCI [redacted] CCI [redacted] each time and CCI [redacted] per day) from V4 / Week 4 to V5 / Week 8. Compliance calculation is based on the number of administrations, CCI [redacted].

The duration (days) of exposure to the NIMP will be calculated by using data collected in the eCRF as:

$$exposure\ NIMP\ (days)_{eCRF} = last\ administration\ date\ of\ NIMP_{eCRF} - date\ of\ first\ dispensing\ of\ NIMP_{eCRF} + 1$$

where:

- last administration date of NIMP is derived from the date collected in the eCRF in the End of Study form
- date of first dispensing of NIMP is derived as the minimum among the dates of ATs dispensation that are recorded in Artificial Tears Dispensation form and Additional Dispensation form of the eCRF (since for subjects who discontinued IMP, ATs can be dispensed in advance respect to V4 / Week 4).

Compliance for the study eye will be derived for the follow-up period only as the proportion (%) between the actual number of administrations done in the study eye and the expected number of administrations done in the study eye. CCI [redacted]

The expected number of CCI [redacted] in the study eye is derived as follows:

CCI [redacted]

The actual number of administrations in the study eye is derived by using data collected in the eDiary, planned for the follow-up phase. Only administrations done in the period defined by the exposure to NIMP (extremes included) will be used in the calculation. Extra-administrations recorded in the eDiary in the same period will be also included in the count if administered in the study eye. Any extra-administration is collected in the study eDiary at the following question "How many extra doses of the artificial tears today?". (e.g. if the answer is "4", then 4 additional administrations will be added in the count of actual administrations).

The compliance will then be derived as:

$$compliance\ NIMP = 100 * \frac{number\ of\ actual\ administration_{eDiary}}{expected\ number\ of\ administration_{eCRF}}.$$

7.3.2. Accountability

Accountability for the IMP will be made by determining the number of Study Product vials dispensed to the patient at Day1/Baseline/V2 and at Week 2/V3 and the number of unused study medication vials returned at Week 2/V3, Week 4/V4 (EOT) Week 8/Follow-up (EOS)/V5. Accountability will be evaluated according to the following formula:

$$Accountability = 100 * \frac{(number\ of\ vials\ dispensed) - (number\ of\ unused\ vials\ returned)}{total\ number\ of\ vials\ dispensed}$$

Below calculations will be done for treatment period and follow-up period separately to assess the accountability for IMP.

Number of vials dispensed refers to the total dispensed vials of Study Product as per the protocol up to the last date of specific study product administration. The number of vials of Study Product in each kit dispensed is C per protocol. Thus, the total number of vials dispensed would be calculated by number of kits dispensed multiplied by C

Number of unused vials returned refers to the total unused vials of Study Product returned, over the duration for which the patient is on the treatment period.

Patient level listings of all study products administered along with reasons not dispensed study products will be provided.

The SAF, where treatment assignment is according to the actual treatment received, will be used for all summaries and listings of exposure and compliance to the study treatment. Accountability will be listed based on the SAF only.

8. Efficacy Analysis

Unless otherwise specified, all efficacy analyses with statistical hypothesis testing performed and descriptive analyses will be analyzed for the study eye only. Assessments collected for the fellow eye will be presented in listings only.

Unless otherwise specified, descriptive statistics will be used to summarize efficacy assessments for study eye at each scheduled visit. Change from baseline values for quantitative variables and shift from baseline for qualitative variables will be summarized for all available post-baseline visits by means of descriptive statistics.

CCI

All collected assessments will be presented in by-patient listings by treatment arm and ordered by site.

8.1. Primary Efficacy Endpoint

The primary endpoint is the mean change from baseline at Week 8 in symptoms of dry eye assessed by SANDE Global Score.

SANDE questionnaire includes two VAS-based questions that assess:

- DED symptom frequency (from 0 to 100)
- DED symptom severity (from 0 to 100)

compiled by the patients.

The global SANDE score (from 0 to 100) is calculated by multiplying the frequency score by the severity score and obtaining the square root. Results will be rounded to integer.

8.1.1. Primary Analysis

The overall null hypothesis H_0 is that the change from baseline in the SANDE Global Score for both of the two concentrations of rhNGF ophthalmic solution (μ_{DOSE5} and μ_{DOSE10}) are greater or equal (worse and no improvement, respectively) than the vehicle one ($\mu_{VEHICLE}$):

$$H_0 = \mu_{DOSE5} \geq \mu_{VEHICLE}\ and\ \mu_{DOSE10} \geq \mu_{VEHICLE}$$

$$H_1 = \mu_{DOSE5} < \mu_{VEHICLE}\ or\ \mu_{DOSE10} < \mu_{VEHICLE}$$

where μ denotes the mean change from baseline at week 8 in SANDE global score. For SANDE global Score, lower score is better.

Considering the multiple comparisons (rhNGF [C] mcg/mL versus vhicle, and rhNGF [C] mcg/mL versus vehicle) to claim overall superiority, a Bonferroni multiplicity correction of type I error will be applied on multiple comparisons. Each comparison will be consequently tested at one-sided alpha = 0.0125, in order to preserve the overall one-sided alpha of 0.025. The overall null hypothesis H_0 will be rejected if the associated primary analysis one-sided p-value will be lower than 0.0125 for one of two comparisons or both comparisons.

The primary analysis will be performed via Mixed-effects Model for Repeated Measures (MMRM) in the FAS according to the planned treatment. The analyses will include the change from baseline in SANDE global score as the dependent variable; the fixed effects of treatment (3 levels: rhNGF [C] mcg/mL, rhNGF [C] mcg/mL, and vehicle), visit (2 levels: Week 4, Week 8), baseline value, and treatment by visit interaction. An "unstructured" covariance matrix will be used. If the model fails to converge by using unstructured covariance matrix, compound symmetry matrix will be used instead. Each treatment group (IMP1 and IMP2) will be compared with the vehicle group at Week 8 using the difference in least square means (LS means) (i.e. difference between each concentration group and vehicle in mean change from baseline). The model can be summarized as follows:

$$change\ from\ baseline = \beta_0 + \beta_1 treatment + \beta_2 base + \beta_3 visit + \beta_4 treatment * visit + \varepsilon$$

where $\varepsilon \sim N(0, R)$ and R is the "unstructured" covariance matrix with blocks defined by the patients.

Based on this modeling, LS Mean, SE, and two-sided 95% CI at Week 4 and Week 8 will be provided for each treatment arm (rhNGF [redacted] mcg/mL, rhNGF [redacted] mcg/mL, and vehicle); difference in LS means, two-sided 95% CI, and corresponding one-sided p-value will be provided for each comparison at each timepoint. P-values for the comparisons at Week 8 will be used to declare the superiority of at least one of the [redacted] over vehicle.

LS means change from baseline and corresponding 95% CI at Week 8 will be plotted in a forest plot by treatment arm for all main and sensitivity analysis.

Descriptive statistics for SANDE Global Score at each visit will be presented based on observed data and data after applying hypothetical strategy.

Primary Estimand:

Missing data that may be obtained notwithstanding the intent to apply the treatment policy strategy and after having applied the hypothetical strategy when needed (see Section 3.3.1), will be addressed by using multiple imputation (MI) based on copy-reference approach. This approach does not assume benefits for the rhNGF in case of discontinuation and limits a post-discontinuation clinical effect to that of the vehicle. Specifically, the copy-reference approach will be implemented in this way:

- Intermittent and monotone missing data for SANDE Global Score at baseline, Week 4 and Week 8 will be imputed by using the Fully Conditional Specification (FCS) method under the missing not at random (MNAR) assumption where only the subset of observations that come from the Vehicle group are used to derive the imputation model. The missing pattern is assumed to be arbitrary. The PROC MI procedure and FCS REGRESSION statement will be used with the seed of 47292748 and 50 imputed datasets. The minimum and maximum imputed values will be restrained to 0 and 100, respectively, via MIN and MAX options. In case of convergence issues, after imputation, imputed values lower than 0 or greater than 100 will be set to 0 and 100, respectively. The imputed values will be rounded to integers.
- For each imputed dataset, the change from baseline at Week 4 and Week 8 is derived.
- The change from baseline at Week 4 and Week 8 will then be used in a MMRM as described above with change from baseline as dependent variable and baseline value, treatment arm, visit, and treatment by visit interaction as independent variable. Patient will be considered as cluster variable and an “unstructured” covariance matrix will be set to model the within-patient correlation. If convergence fails, compound symmetry matrix will be used to model the within-patient correlation.
- Results obtained on each imputed dataset will be combined using Rubin’s rule to draw inference via PROC MIANALYZE procedure. A statistical significance level of 0.0125 will be used to declare the significance of the comparisons of each treatment arm vs vehicle.

Appendix 13.3 includes the SAS code for the primary analysis illustrating the key elements for the analysis.

8.1.2. Sensitivity Analyses

In case at least one of the two primary comparisons is statistically significant, a sensitivity analysis will be conducted (on the statistically significant comparison(s)).

The following sensitivity analyses are defined to assess the robustness of results on the primary endpoint versus assumptions used in the statistical model for the main estimators. Before applying the analyses described below, when applicable, any datapoint collected in the week after intake of prohibited medications will be set to missing (applying the hypothetical strategy for ICEs defined in Section 3.3.1).

- The comparison between treatment and control will be performed in the FAS population by means of MI under missing at random (MAR) instead of MNAR assumption. The MI approach described in Section 8.1.1 will then be repeated by changing Step 1 MNAR assumption to MAR assumption. Treatment and SANDE Global Score at baseline, Week 4 and Week 8 will be used in the FCS method. The seeds will remain the same.
- Tipping point will assess how departures from MI under MNAR assumptions must be in order to overturn conclusions from the primary superiority analysis. Tipping point will be based on iterative application of MI approach described in Section 8.1.1, where the imputed values for each rhNGF arms are shifted at a constant Δ to represent a worse effect in each iteration. The tipping points are the smallest Δs at which no statistical significance is shown (one-sided p≥0.0125) for each arm. Tipping point analysis will be run only if the superiority has been proven for at least one of the CCI of the rhNGF. The tipping point will be firstly searched in the values defined by ±SD of the SANDE Global Score observed at baseline in the overall sample. If not found, the range will be enlarged to ±2SD.
- Analysis on complete cases not having taken prohibited medications in the week before assessment. Patients in FAS without any missing SANDE global score (i.e. evaluable at Baseline, Week 4 and Week 8) will be fitted into MMRM model described in Section 8.1.1.

The following additional sensitivity analyses will be performed:

- Analysis on all observed cases. Patients with missing primary endpoint (i.e. change from Baseline at Week 8 missing) will be excluded. All observed data will be fitted into MMRM model described in Section 8.1.1.
- MMRM described in Section 8.1.1 will be applied to Per-Protocol Set based on the same imputed dataset described in Section 8.1.1.

8.1.3. Supportive Analyses

Moreover, the following two supportive analyses by changing the strategies used in the primary estimand (therefore changing the estimand) will be done:

- Modified Strategy #1 to handle ICEs for primary endpoint will be modified as below:
    - Treatment discontinuation: a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.1.1).
    - Use of a pre-defined prohibited medication at any time during the study: a hypothetical strategy will be applied where any data collected after the first administration of a prohibited medication will be set to missing. Missing data will

be imputed by using a multiple imputation with copy-reference approach (see Section 8.1.1).

- Modified Strategy #2 to handle ICEs for primary endpoint will be modified as below:
    - Treatment discontinuation: a treatment policy strategy will be applied where all the observed values will be used regardless of the occurrence of the ICEs. Missing data due to any reason will be handled by using a multiple imputation with a copy-reference approach (see Section 8.1.1).
    - Use of a pre-defined prohibited medication in the week before any assessment of SANDE: a composite strategy will be applied where any data collected in the week after the occurrence of the ICE (if present) will be replaced by a value worse than all values observed for SANDE Global Score at week 8.

The modified strategy #1 will be analyzed as described for the primary estimand.

For the modified strategy #2, the following approach will be followed to combine the two different types of imputation and to prevent the bias related to the use of prohibited medications from spreading to all imputed values:

- Any datapoint collected in the week after intake of prohibited medications will be set to missing and imputed as done for any other missing data by using the copy-reference approach as described in Section 8.1.1. In this way, all data can be imputed without letting the bias related to use of prohibited medications spread to other imputed values. Fifty datasets are obtained.

- Modified strategy #2 is applied in any dataset to data collected at Week 8: if the value for SANDE Global Score at Week 8 was observed (and then replaced by imputation in the previous step) in the week after an intake of a prohibited medication, this value is now replaced by the worst possible value (i.e. 100). In this way, the relationship among the variables inserted in the FCS regression is not affected by the replacement with the worst value.

  Change from baseline at Week 8 is calculated and used in the ANCOVA model with the trimmed approach described by Permutt and Li (Permutt T and Feng L. 2017). Only Baseline value and Treatment are used as covariates. Fifty models are run, one for each imputed dataset. This method provides an exact test that does not require any modeling assumption. The estimand of the primary analysis is the difference between treatment groups in mean SANDE change from baseline to week 8 in the FAS population, including all patients who have no SANDE measurement at week 8 for any reason. The exact imputed value does not matter, as long as it is worse than all values observed in patients with SANDE measurements at week 8. The only requirement for this method is that it must be reasonable to consider the outcome of dropouts as worse than the highest SANDE Global Score of the completers. The estimand is the between-group difference in mean change from baseline in the percentage k subpopulation with the lowest SANDE values at Week 8. For this analysis, k=50%, 70%, and 90% will be used. The principle is to exclude all imputed data. Thus, if the missing rate is higher than 10%, k will be adjusted accordingly. The method is based on an exact permutation test for the null hypothesis that the treatment distributions for the considered outcome at week 8 are equal.

From each dataset, the observed mean difference between each active arm and the vehicle is derived. Then, a permutation distribution is calculated in order to derive the SE of each difference by using the permutation standard deviation. Finally, observed mean differences and their SEs are used to combine the results in the PROC MIANALYZE by using the Rubin's rule.

In case no data at Week 8 are collected after having taken a prohibited medication in the previous week, this sensitivity analysis is skipped.

8.2. Secondary Efficacy Endpoints

8.2.1. Key Secondary Endpoints

If the primary analysis of primary endpoint leads to rejection of the null hypotheses, key secondary endpoints will be tested by using the Hochberg approach (Hochberg Y. 1988) to show superiority of each concentration for which superiority vs. vehicle has been proven in the primary analysis.

Hochberg approach will be achieved by the following procedure:

1. Put all p values in descending order and assign ranks to those p values according to their ranks.
2. Calculate each individual adjusted p-value using Hochberg approach.
3. Compare the adjusted p-values from Step 2 with the alpha level for the comparison vs. vehicle for which superiority has been shown for the primary endpoint.
4. All the tests corresponding to those p values ranking ahead of the p value selected in Step 3, including the test corresponding to the p value selected in Step 3, will be considered as significant. The null hypothesis will be rejected.

The Hochberg approach will be implemented by SAS PROC MULTTEST procedure and HOCHBERG option.

This hierarchical test strategy protects the family-wise false positive error rate at the overall one-sided 0.0125 level for each concentration vs. vehicle. With 92 patients evaluable per treatment group, the study would have approximately a power of 67% to test the endpoint with the smallest p-value in the worst-case scenario where the first 2 endpoints tested are not statistically significant (i.e. with an adjusted alpha level of 0.00417).

In case of not rejection of the null hypothesis for primary endpoint (or for the concentration for which the superiority is not shown), the above testing strategy for key secondary endpoints will not be performed. Instead, independently of results on primary endpoint, descriptive in nature analyses will be performed on all key secondary endpoints at each available timepoint by means of descriptive statistics.

Descriptive statistics for key secondary endpoints assessments at each visit will be presented based on observed data and data after applying hypothetical strategy.

8.2.1.1. Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye at week 4

Missing data will be imputed by using a multiple imputation with copy-reference approach (see Section 8.1.1). For each imputed dataset, the change from baseline at Week 4 is derived. Patients with change from baseline at Week 4 ≥ 10 mm/5min will be considered as having improvement while patients with change from baseline at Week 4 < 10 mm/5mins will be considered as not having improvement. 95% CI on proportion of patients having improvement will be implemented by using Clopper Pearson method.

The derived binary variable described in the previous step will then be used in a logistic model as dependent variable; treatment (3 levels: rhNGF C mcg/mL, rhNGF C mcg/mL, and vehicle) and baseline Schirmer-I scores in the Study Eye as covariates.

Based on this modeling, the estimated proportions and corresponding two-sided 95% CI will be provided for each treatment arm; the estimated mean proportion differences, two-sided 95% CI, and corresponding one-sided p-value will be provided for each IMP group versus vehicle at Week 4.

8.2.1.2. Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye at Week 4

The MI procedure described in Section 8.2.1.1 will be applied to impute missing Schirmer-I scores at Week 4. Change from baseline at Week 4 will be fitted in the ANCOVA model with change from baseline at Week 4 as dependent variable, treatment (3 levels: rhNGF C mcg/mL, rhNGF C mcg/mL, and vehicle) and baseline Schirmer-I scores in the Study Eye as covariates.

Based on this modeling, a point estimate (the adjusted estimated treatment effect), associate SE, two-sided 95% CI, and corresponding one-sided p-value will be provided for each treatment group; a point estimate (the adjusted estimated treatment difference), associate SE, two-sided 95% CI, and corresponding one-sided p-value will be provided for each least squares (LS) mean treatment difference versus placebo at Week 4.

8.2.1.3. Mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator at week 4

Corneal fluorescein staining has 5 zones and each zone has a score range of 0-3. The total score is determined by simply adding up the scores of the 5 individual zones. The range of total corneal fluorescein staining in NEI scale is 0-15. If any of the 5 individual zones has a missing score, the total score would be missing.

The analysis method described in Section 8.2.1.2 will be repeated with change from baseline at Week 4 of total corneal fluorescein staining (NEI scale) in the Study Eye as dependent variable.

8.2.2. Secondary CCI Analyses

The secondary endpoints will be analyzed following the indications provided in the protocol for the key secondary endpoints, but actual observed data will be used regardless the onset of Intercurrent events. CCI

8.2.2.1. Secondary Endpoints

Although the method of analysis for the secondary endpoints is the same as the one established for the key secondary endpoints and that for SANDE at week 4 is the same of the one used for the primary endpoint, the results of the analyses on the secondary endpoints are to be interpreted as descriptive in nature, since no adjustment of the overall alpha level has been applied for taking care of these endpoints.

Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye at Week 8

Actual values and changes from baseline to Week 8 in Schirmer-I score without anesthesia will be summarized by means of descriptive statistics for the Study Eye. The comparisons between each active treatment and vehicle at week 8 will be performed using the same ANCOVA model and imputation strategy described in Section 8.2.1.2 for the comparisons at week 4; consequently, the point estimates, corresponding 95% CIs and p-values described in that section will be provided also for the week 8 timepoint.

Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye at week 8

Patients will be categorized into two levels: result ≥ 10 mm/5min in the Study Eye and result < 10 mm/5min in the Study Eye. The derived binary variable will then be summarized at Week 8 in a frequency table.

The comparisons between each active treatment and vehicle at week 8 will be performed using the same logistic model and imputation strategy described in section 8.2.1.1 for the comparisons at week 4; consequently, the point estimates, corresponding 95% CIs and p-values described in that section will be provided also for the week 8 timepoint.

Mean change from baseline in fluorescein tear break-up time (fTBUT) in Study Eye at weeks 4 and 8

The fTBUT will be measured twice during the first minute after the instillation of the fluorescein. If the 2 readings differ by more than 2 seconds, then a third reading is taken. The fTBUT value (in seconds) will be set to the average of the 2 or 3 measurements.

Actual values and changes from baseline to Week 4 and Week 8 in fTBUT will be summarized by means of descriptive statistics for the Study Eye.

The ANCOVA model described in Section 8.2.1.2 and the imputation strategy described in Section 8.1.1 will be applied to fTBUT in Study Eye at Week 4 and Week 8 separately.

Mean change from baseline in total CCI [redacted] in the Study Eye as assessed by the investigator at week 8

Actual values and changes from baseline to Week 8 in total CCI [redacted] will be summarized by means of descriptive statistics for the Study Eye.

The ANCOVA model and the imputation strategy described in Section 8.2.1.2 will be applied to the change from baseline of total CCI [redacted] scale in Study Eye at Week 8.

Mean change from baseline in symptoms questionnaire (SANDE) scores for severity and frequency at weeks 4 and 8

Actual observed values and changes from baseline to Week 4 and Week 8 in SANDE scores for severity and frequency will be summarized by means of descriptive statistics.

The ANCOVA model described in Section 8.2.1.2 and the imputation strategy described in Section 8.1.1 will be applied to SANDE symptoms questionnaire severity scores and frequency scores in Study Eye at Week 4 and Week 8 separately.

Mean change from baseline in symptoms of dry eye assessed by SANDE Global Score at week 4

Actual observed values and changes from baseline to Week 4 in SANDE Global Score will be summarized by means of descriptive statistics.

Comparisons between each active treatment and vehicle will be performed within the MMRM model described for the primary endpoint (see Section 8.1) to maintain the results consistent. For this reason, the same strategy used for the ICEs established for the primary endpoint will also be applied to this endpoint regardless of being secondary only.

Mean change from baseline of the CCI [redacted] at weeks 4 and 8

CCI [redacted] The score for each subscale is defined as sum of scores divided by number of questions answered within each subscale. The percentage values will be divided by 10 before applying the algorithm. Symptom relief is not assessed at Baseline.

CCI [redacted] will be calculated as the sum of the scores for CCI [redacted] intensity at 24 hours and 2 weeks (questions 4-9) divided by the number of questions answered for questions 4-9. CCI [redacted] has a score range of 0-10.

Actual values and changes from baseline to Week 4 and Week 8 in Ocular Pain Score (assessed by OPAS questionnaire) will be summarized by means of descriptive statistics.

The ANCOVA model and the imputation strategy described in Section 8.2.1.2 will be applied to the CCI [redacted] at Weeks 4 and 8 separately.

The score of each domain will also be shown and summarized by means of descriptive statistics on the actual observed values (i.e. no imputation needed). Comparisons between each active treatment and vehicle will be performed using two-sample t-test or, if the required assumptions are not met, the two-sample Mann–Whitney U test will be used.

Mean change from baseline as assessed by the CCI [redacted] QoL score at weeks 4 and 8

QoL score is calculated as the sum of the scores for QoL subscale (questions 13-19) divided by the number of questions answered for questions 13-19.

Actual values and changes from baseline to Week 4 and Week 8 in CCI QoL score will be summarized by means of descriptive statistics.

The ANCOVA model and the imputation strategy described in Section 8.2.1.2 will be repeated for the CCI QoL subscale scores (score range: 0-10) in the Study Eye at Weeks 4 and 8, separately.

Mean change from baseline in BCDVA score in Study Eye at weeks 4 and 8

The BCDVA score is measured by ETDRS letter score. The score is given by the total number of letters read at 4-m distance. If 20 or more letters are read, then the score is given by the total number of letters read + 30. If less than 20 letters are read at 4-m distance, the score is given by the sum of the letters read at 4-m distance and the letters read at 1-m distance. The equivalent logMAR score is given by (Beck RW et al 2003):

$$logMAR = 1.7 - (0.02) * (ETDRS\ letter\ score)$$

Actual values and changes from baseline to Week 4 and Week 8 in BCDVA score will be summarized by means of descriptive statistics for the Study Eye. The summary table will be presented by using both scores (ETDRS and logMAR).

The ANCOVA model and the imputation strategy described in Section 8.2.1.2 will be repeated for change from baseline in BCDVA score in Study Eye at Weeks 4 and 8, separately.

8.2.2.2. CCI

Descriptive in nature analyses will be performed CCI at each available timepoint by means of descriptive statistics. Changes from baseline or shift tables versus baseline will be summarized for all available post-baseline visits. Parametric or non-parametric tests, depending on the distribution of each parameter, will be applied. For continuous data, the normality assumption will be assessed by a visual inspection of the distributions. Additional analyses, as deemed appropriate, may be performed CCI without any need to change the present SAP.

CCI

CCI

CCI

CCI

8.3 Additional Efficacy Endpoints

CCI

8.4 Subgroup Analysis

Subgroup analyses of primary and key secondary endpoints will be performed on the following subgroups of baseline characteristics:

- Age class (≤Median, >Median)
- Race (White, Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino)
- Gender (Male, Female)
- Region (EU, US)
- Certain etiology of dry eye (Yes/No)
- Certain medical history (Yes/No)
- Certain concomitant medications (Yes/No)

CCI [redacted]

9. Safety Analysis

All safety analyses will be based on the SAF according to the actual treatment received. No formal statistical testing will be performed for safety data.

9.1. Adverse Events

An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation patient administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Treatment-emergent AEs (TEAEs) are defined as AEs that are reported or worsened on or after the first dose of IMP.

Adverse events will be coded according to latest version of MedDRA. Unless otherwise specified, AEs will be summarized by treatment arm and by SOC and PT, with SOCs sorted in descending order of patient incidence and PTs within each SOC in descending order of patient incidence in Total group, for each treatment period ("On Treatment period", "On Follow-up period" and "Overall"). If the total frequency for any 2 or more PTs is equal, the PTs will be presented in alphabetical order.

When summarizing the number and percentage of patients with an event, patients with multiple occurrences of the same AE or a continuing AE will be counted only once. Patients will be presented according to the highest severity (the strongest relationship) in the summaries by severity (of related AEs), if patients reported multiple events under the same SOC and/or PT.

Ocular TEAEs will be summarized separately from non-ocular TEAEs. Ocular TEAEs will be summarized for the Study Eye and the fellow eye. Non-ocular TEAEs will be summarized regardless of the Study Eye or the fellow eye.

By-patient listings of ocular and non-ocular AEs will be presented by treatment and ordered by site.

9.1.1. Incidence of Adverse Events

An overall summary of TEAEs including the number and percentage of patients who experience the following will be presented:

- Any TEAE
- Any TEAE Related to Study Drug
- Any Ocular TEAE
- Any Ocular TEAE Related to Study Drug
- Any Non-ocular TEAE
- Any Non-ocular TEAE Related to Study Drug
- Any AESI
- Any Ocular AESI
- Any Non-ocular AESI
- Any Serious TEAE
- Any Serious TEAE Related to Study Drug
- Any Serious Ocular TEAE
- Any Serious Ocular TEAE Related to Study Drug
- Any Serious Non-ocular TEAE
- Any Serious Non-ocular TEAE Related to Study Drug
- Any TEAE Leading to Treatment Discontinuation
- Any Ocular TEAE Leading to Treatment Discontinuation
- Any Non-ocular TEAE Leading to Treatment Discontinuation
- Any TEAE Leading to Study Discontinuation
- Any Ocular TEAE Leading to Study Discontinuation
- Any Non-ocular TEAE Leading to Study Discontinuation
- Any TEAE Leading to Death
- Any Ocular TEAE Leading to Death
- Any Non-ocular TEAE Leading to Death

A by-patient listing of all AEs will be presented by site.

9.1.2. Relationship of Adverse Events to Study Drug

Study treatment TEAEs will be summarized by SOC and PT. Study treatment related AEs are those with a relationship to study treatment of 'Possible', 'Probable', and "Highly Probable' relationship based on the eCRF page. Any TEAE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an adverse drug reaction (ADR). AEs assessed as 'None' and 'Unlikely' will be considered unrelated for reporting purpose. Missing relationship will be counted as related to study treatment.

A by-patient listing of adverse drug reactions will be presented and ordered by site.

9.1.3. Unexpected Adverse Events

The classification of the TEAEs in expected/unexpected done by the Investigators and collected in the eCRF will not be analyzed (i.e. not reported in any TLFs). The Sponsor's classification will be reported in the CSR for SAEs only, based on the Reference Safety Information presented in the current version of the IB.

9.1.4. Severity of Adverse Event

TEAEs will be rated as Mild, Moderate, or Severe based on the following criteria:

- Mild: These events do not interfere with the patient's usual function.
- Moderate: These events interfere to some extent with the patient's usual function.
- Severe: These events interfere significantly with the patient's usual function. Severe events are usually incapacitating.

The adverse events with missing severity grade will be imputed as 'Severe'. Any TEAEs will be summarized by severity and by SOC and PT. In the TEAE severity table, if a patient reports multiple occurrences of the same TEAE, only the most severe (mild < moderate < severe) occurrence will be presented.

9.1.5. Serious Adverse Events

A Serious Adverse Event (SAE) is defined as any adverse experience that, in the view of either the Investigator or Sponsor, meets any of the following criteria:

- results in death,
- is life-threatening (i.e. the patient was at immediate risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event.

Serious TEAEs will be summarized by SOC and PT.

A by-patient listing will also be provided for all SAEs and ordered by site.

9.1.6. Adverse Events (AEs) of Special Interest

The following adverse events are considered to be Adverse Events of Special Interests (AESIs) and by default shall be reported as SAEs (medically important criteria):

- AEs that caused a decrease in visual acuity of >30 ETDRS letters or > +0.6 LogMAR (compared with the last assessment of visual acuity at the last visit) lasting >1 hour
- AEs that caused a decrease in visual acuity to the level of light perception or worse lasting >1 hour
- AEs that required surgical intervention (e.g., conventional surgery, vitreous tap or biopsy with intravitreal injection of anti-infectives, or laser or retinal cryopexy with gas) to prevent permanent loss of sight
- AEs associated with severe intraocular inflammation (i.e., 4+ anterior chamber cell/flare or 4+ vitritis)
- AEs that can cause retinal vascular occlusion such as intraocular pressure > 40 mmHg
- AEs that, in the opinion of the Investigator, may require medical intervention to prevent permanent loss of sight.

AESIs will be summarized by SOC and PT.

A by-patient listing will be provided for all AESIs and ordered by site.

9.1.7. Adverse Events Leading to Treatment Discontinuation

TEAEs with a study treatment action taken of 'Drug Withdrawn' will be considered as TEAEs leading to treatment discontinuation and will be summarized by SOC and PT.

A by-patient listing will also be provided for all adverse events leading to treatment discontinuation and ordered by site.

9.1.8. Adverse Events Leading to Study Discontinuation

TEAEs leading to study discontinuation as per recorded on the eCRF 'Adverse Event' page will be summarized by SOC and PT.

A by-patient listing will also be provided for all adverse events leading to study discontinuation and ordered by site.

9.1.9. Adverse Events Leading to Death

A by-patient listing will also be provided for all adverse events leading to death and ordered by site.

9.1.10. Adverse Drug Reactions (ADRs)

ADRs will be summarize by SOC, PT and by severity. Serious ADRs will be summarized by SOC and PT.

A by-subject listing will also be provided for all ADRs and ordered by site.

9.2. Pregnancy Test

For women of child-bearing potential, pregnancy test will be performed at screening, Baseline, and Visit 4/Week 4 (EOT)Visits.

Pregnancy test results will be presented in a by-patient listing and ordered by site.

9.3. Treatment Discontinuation due to Tolerability

Number and percentage of patients discontinued study treatment due to tolerability will be summarized as per recorded in the eCRF End of Treatment page.

9.4. Ophthalmological assessments

Relevant ophthalmological examinations will be performed at indicated visits in the Schedule of Activities (Appendix 13.1). Summary of ophthalmological examinations will be based on the SAF and summarized by Eye.

9.4.1. Slit Lamp Biomicroscopy

CCI [redacted]

A by-patient listing will also be provided by site.

9.4.2. Specular Microscopy

Specular microscopy will be performed to assess corneal endothelial cells density (cells/mm^2) at selected sites that have a specular microscope already available.

Results will be summarized using descriptive statistics at each scheduled visit for both eyes (Study Eye and fellow). Mean change from baseline at each post-baseline visit will also be summarized.

A by-patient listing will be provided by site.

9.4.3. Dilated Fundus Exam

Dilated fundus exam includes evaluation of vitreous, retina and optic nerve for vitritis, vitreal or retinal hemorrhage, maculopathy, retinal tears or detachment, posterior vitreous detachment, optic nerve appearance, and optic nerve cup-to-disc ratio. CCI [redacted]

The results of dilated fundus examination will be summarized using descriptive statistics at each visit for both eyes (Study Eye and Fellow Eye). Numbers and proportions of patients with vitritis, retinal or vitreal hemorrhages, retinal or posterior vitreal detachment, retinal tears, or maculopathy will be summarized at each visit. Shift from baseline of proportions will also be summarized for each parameter. Actual observed values and change from baseline at each post-baseline visit will be summarized for cup-to-disc ratio.

A by-patient listing will be provided by site.

9.4.4. Bulbar Conjunctival Redness

Bulbar conjunctival redness will be assessed at the slit-lamp using white light prior to vital dye instillation and graded according to the Validate Bulbar Redness (VBR 10) scale that has 10 reference images with increasing bulbar redness. The scale starts at grade 10 and has 10-point steps between reference images (score range 10-100). CCI [redacted]

The results of bulbar conjunctival redness score will be summarized by conjunctiva (nasal and temporal) using descriptive statistics at each scheduled visit and applicable time point for both eyes (Study Eye and Fellow Eye). Mean change from baseline at each post-baseline visit will be summarized.

A by-patient listing will be provided for both eyes by site.

9.4.5. External Ocular Examination

External ocular examination will be performed on both eyes at Screening, Baseline, Week4 and Week8 or at the Early Termination Visit.

A by-patient listing will be provided for both eyes by site.

10. Interim Analysis

No interim analyses are foreseen.

11. Changes in the Planned Analysis

The Mean change from baseline in total CCI [redacted] in the Study Eye at week 8 will be considered a secondary endpoint CCI [redacted]. This was a typo in the protocol.

12. References

OXERVATE ™ US package insert. Boston, MA: Dompe' U.S. Inc; 2018

Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. Biometrika 1988;75:800-2.

Permutt T and Feng L. Trimmed means for symptom trials with dropouts. Pharmaceuticals Statistics. 2017;16:20-28.

13. Appendices

13.1. Schedule of Activities

	Run-In period		Treatment period		Follow-up period
	V1 Screening (Day –12)	V2 Baseline (Day 1)	V3 Week 2 (Day 13)	V4 Week 4 (EOT) (Days 28)*	V5 Week 8 (EOS) (Days 56)*
Interval Tolerance (days)	±2		±1	±1	±2
Site Visit	X	X	X	X	X
ELIGIBILITY					
Informed consent	X				
Inclusion / Exclusion criteria	X	X			
Demographics, Medical History, Medications	X				
TRIAL INTERVENTIONS					
Randomization		X			
Trial Study Product dispensation**	X	X	X		
Artificial tears dispensation				X	
Patient Instruction	X	X		X	
Application of CCI by the investigator	X				
Patient's Diary dispensation	X	X		X	
Documentation in patient's medical record of first dose administration by Investigator	X				
Medication dosing compliance verification (diaries)		X	X	X	X

TRIAL PROCEDURES					
Check the Patient Diary		X	X	X	X
SANDE questionnaire	X	X		X	X
CCI	CCI				
BCDVA by ETDRS	X	X		X	X
External Ocular Examination	X	X		X	X
CCI	CCI				
Schirmer-I test without anesthesia	X	X		X	X
Slit Lamp Examination	X	X		X	X
Redness score (VBR 10)	X	X		X	X
Instill fluorescein	X	X		X	X
fTBUT	X	X		X	X
CCI	CCI				
CCI	CCI				
Conjunctival lissamine green Staining NEI scale.	X	X		X	X
CCI before and after CCI ***	CCI				
Specular microscopy (Corneal endothelial cell density) (in selected centers)		X			X
Instill tropicamide CCI		X			X
Dilated fundus examination		X			X
Collect used/unused Study Product (to be checked by independent personnel)		X	X	X	

Retrieve Patient Diary		X		X	X
Concomitant medications****		X	X	X	X
Adverse events evaluation		X	X	X	X
LABORATORY TESTS					
Pregnancy Test	X	X		X	

*In case of premature discontinuation of the study, the patient will be required to participate at the Early Exit Visit that will have the same assessment of Visit 4 (in case of early treatment discontinuation before Visit 4) or the same assessment of Visit 5 (in case the discontinuation from the study will occur after the ending of the treatment but before the expected Visit 5). A flag will be inserted in the eCRF to define if the Visit 4 or 5 is a per protocol visit or if it is done as Early Exit Visit.
** An CCI kit will be also provided
^ CCI will be administered to all subjects but completed only by those that either have CCI or have filled this form before.
*** Only in patients with CCI >0
**** All ocular and systemic medications, over-the-counter painkillers, herbal products, vitamins, and antacids taken within 4 weeks prior to the start of and throughout the study will be clearly documented on the Concomitant Medications eCRF page.

13.2. Missing Date Imputation for Missing or Partial Dates

Table 3: Algorithm for Treatment Emergence of Adverse Events.

AE start date	AE end date	RULE for TEAE definition	RULE for definition of study period for TEAEs only: "On Treatment" / "On follow-up"
Known	Known, Partial or Missing	If start date < IMP start date, then not TEAE If start date ≥ IMP start date, then TEAE	If start date > date of last IMP administration, then "On Follow-up. Otherwise, "On Treatment"
Partial, but known components show that it cannot be on or after IMP start date	Known, Partial or Missing	Not TEAE	Not applicable
Partial, could be on or after IMP start date OR Missing	Known	If stop date < IMP start date, then not TEAE If stop date ≥ IMP start date, then TEAE	If TEAE, then "On Treatment"
	Partial	Impute stop date as latest possible date (i.e. last day of month if only day is missing, Dec if only month is missing, 31st Dec if day and month are both missing, then: If stop date < IMP start date, then not TEAE If stop date ≥ IMP start date, then TEAE	
	Missing	Assumed TEAE	

Table 4: Algorithm for Prior / Concomitant medications.		
MEDICATION start date	**MEDICATION end date**	**RULE for Prior / Concomitant definition**
Known	Known	If stop date < IMP start date, then "prior" If stop date ≥ IMP start date, then "concomitant"
	Partial	Impute stop date as latest possible date (i.e. last day of month if only day is missing, Dec if only month is missing, 31st Dec if day and month are both missing, then: If stop date < IMP start date, then "prior" If stop date ≥ IMP start date, then "concomitant"
	Missing	Assign as concomitant
Partial OR Missing	Known	If stop date < IMP start date, then "prior" If stop date ≥ IMP start date, then "concomitant"
	Partial	Impute stop date as latest possible date (i.e. last day of month if only day is missing, Dec if only month is missing, 31st Dec if day and month are both missing, then: If stop date < IMP start date, then "prior" If stop date ≥ IMP start date, then "concomitant"
	Missing	Assign as concomitant

13.3. SAS Code for Primary Analysis

Step 1: Multiple Imputation

```
PROC SORT data=efficacy;
    by USUBJID;
RUN;

PROC MI data=efficacy nimpute=50 out=mi_data min=0 max=100 seed= 47292748; *score range: 0-100;
    class TRTPN;
    var V2 V4 V5; *V2=baseline, V4=Week 4, V5=Week 8;
    FCS regression;
    mnar model(V2 V4 V5/ modelobs=(TRTPN='3')); *Vehicle group has TRTPN=3;
RUN;
```

Data will be transformed to short format by AVISITN before applying multiple imputation.
The MI method will be used for both primary and secondary analyses after applying appropriate ICE handling strategies.

Step 2: MMRM Model

```
PROC MIXED data= mi_data_long;
    by _imputation;
    class TRTPN AVISITN USUBJID;
    model CHG=BASE TRTPN AVISITN TRTPN*AVISITN / DDFM=KR CL;
    repeated AVISITN/subject=USUBJID type=UN;
    lsmeans TRTPN*AVISITN/diff cl;
    ods output lsmeans=lsM diffs=diffM;
RUN;
```

Data will be transformed back to long format by combining visits before applying MMRM model.
If model fails to converge, the “type=UN” will be replaced by “type=CS”.
This model is the essential model for primary endpoint. Appropriate adjustments may be made based on modified Estimand strategies and sensitivity analyses.

13.4. Algorithm for classification of Prohibited medications

Table 5: Algorithm for Prohibited medications.

1st condition: ATC2 code	2nd condition: Route of administration	3rd condition: Taken during the study (i.e. that ends on or after the IC signature date)		
		MEDICATION start date	MEDICATION end date	RULE for classification in Prohibited Medications / Concomitant d
ATC2=S01 ("OPHTHALMOLOGICALS")	"Topical" OR "Intraocular" OR "Nasal" OR "Other"	Known	Known	If stop date ≤ IC date, then PROHIBITED=No If stop date > IC date, then PROHIBITED=Yes
			Partial	Impute stop date as latest possible date (i.e. last day of month if only day is missing, Dec if only month is missing, 31st Dec if day and month are both missing, then: If stop date ≤ IC date, then PROHIBITED=No If stop date > IC date, then PROHIBITED=Yes
			Missing	PROHIBITED=Yes
		Partial OR Missing	Known	If stop date ≤ IC date, then PROHIBITED=No If stop date > IC date, then PROHIBITED=Yes
			Partial	Impute stop date as latest possible date (i.e. last day of month if only day is missing, Dec if only month is missing, 31st Dec if day and month are both missing, then: If stop date ≤ IC date, then PROHIBITED=No If stop date > IC date, then PROHIBITED=Yes
			Missing	PROHIBITED=Yes

Note 1: All three conditions need to be satisfied to classify the medication as PROHIBITED

Note 2: To identify the impacted measurements taken within one week after taking these prohibited medications, if the medication start date is partial, the earliest possible date (i.e. first day of month if only day is missing, Jan if only month is missing, 1st Jan if day and month are both missing) will be imputed.