CLINICAL TRIAL PROTOCOL

Full Title: 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

Trial Name: REDUCO

Trial Number: NGF0123

Compound Name: Recombinant Human Nerve Growth Factor (rhNGF)

Regulatory Agency Identifier Numbers:	EU-CT number: **2023-507561-26-00** IND number: 115892
Investigational Product:	Recombinant Human Nerve Growth Factor (rhNGF) CCI mg/vial in a CCI preparation
Trial phase:	II
Version:	**FINAL 3.0**
Amendment:	**Final 2.0**
Amendment Scope:	**Global**
Sponsor Approval Date:	11/March/2024

CONTACT INFORMATION

SPONSOR	Dompé farmaceutici S.p.A., Via Santa Lucia 6 - 20122 Milan Italy Phone: +39.02.583831 Fax: +39.02.58383324
Sponsor Medical Expert	PPD
	PPD
Sponsor Medical Monitor	PPD
Sponsor Development Director	PPD
Sponsor Clinical Trial Manager	PPD
Sponsor Coordinating Principal Investigator	PPD

Sponsor SAE Reporting	PPD
CRO Safety Reporting	PPD Pharmacovigilance 24-Hour Safety Hotline: +44 1223 374240 Safety Hotline Fax: +44 1223 374102 Email: **mailto:EMEAASIASafetyCentral.SM@ppd.com**
Sponsor Qualified Person	PPD

AMENDMENT DETAILS

History of Amendments
A total of 1 prior global amendment has occurred as shown in the table below:

Document	Sponsor Approval Date (dd/mmm/yyyy)	Approximate {(#/%)} Enrolled
Amendment 1	16 November 2023	0% enrolled globally
Original Protocol	13 September 2023	0

History of Amendments

The table below provides an overview of the current amendment to NGF0123 – REDUCO Protocol Final v. 3.0, Amendment 2.0 dated 11 March 2024.

Amendment Number:	2
Approximate {%/#} Enrolled:	6%/ 18 patients.

Reason(s) for Amendment:	Primary: Regulatory agency (AIFA) request to amend.	Secondary: Other (clarification of language)

Summary of the Amendment:	The primary purpose of the current amendment is to update the protocol for study NGF0123 reflecting AIFA's feedback on clarifying an exclusion criterion, update of a repealed Directive, rationale for placebo-controlled trial design, and to elaborate further on the selection of IMP dosing.
Is this amendment likely to have a substantial impact on safety or rights of the participants, or on the reliability and robustness of the data generated in the clinical trial?	No

Summary of Changes in the Current Amendment (Amendment 2.0):

Section # and Name	Description of Change	Brief Rationale for Change
1.1 Synopsis, Design	The following text was added: '*…and will receive commercially available preservative free AT, TID, provided by the Sponsor.*'	For further clarification of study procedures.

1.1 Synopsis, Criteria for Inclusion/Exclusion	The exclusion criterion around ocular surgery was further clarified by adding the following text (exclusion criterion #4): *'4. Possibility of the need for Ocular surgery at the time of inclusion in the study or anticipated ocular surgery expected during the participation in the study.'*	Following the request by AIFA
1.2 Schedule of Activities, Table 1. Schedule of Activities Planned During the Different Visits	The table was modified to include a circumflex accent against CCI and the legend of the table was modified to add the following accompanying text: '^ CCI *will be administered to all subjects but completed only by those that either have* CCI *or have filled this form before.'*	For further clarification of study procedures.
2. Introduction	The repealed Directive was deleted: '~~*Directive 2001/20/EC of the European Parliament and*~~…'	Following the request by AIFA
4.2 Rationale for Trial Design	The following text was added: *'…to provide the minimum amount of lubrication needed in DED patients while ensuring appropriate wash-out from any other previous topical treatment effect.'*	For further clarification of study design.
4.2 Rationale for Trial Design	The following text was added to clarify the rationale for a placebo-controlled trial design: *'…In addition to testing the two concentrations of rhNGF (Cmcg/mL and C mcg/mL), this study incorporates a vehicle control group. The vehicle control group has been incorporated in this study in order to demonstrate efficacy of our new formulation in improving symptoms and signs in patients with dry eye, a disease where it is known that a relevant placebo effect may be expected and the presence of an inactive control group favors the correct interpretation of any treatment effect.'*	Following the request by AIFA
5.2 Exclusion Criteria	The exclusion criterion around ocular surgery was further clarified by adding the following text (exclusion criterion #4): *'4. Possibility of the need for ocular surgery at the time of inclusion in the study or anticipated ocular surgery expected during the participation in the study.'*	Following the request by AIFA
5.4 Screen Failures	The following text was added to clarify re-screening criteria: *'Patients may be re-screened on a case-by-case basis after reviewing it with the Sponsor.'*	For further clarification of study procedures.

6.2 Rationale for Trial Intervention	The following text was added and paragraph was amended to provide further clarification on rationale for dose selection and dosing schedule (updated text in *Italic*): '…The rationale for *the selection of the two proposed* ~~*testing*~~ CCI concentrations of rhNGF stems from *both manufacturing and clinical rationales. Specifically, from a manufacturing standpoint, the development of the new* CCI *formulation opens the possibility to test a rhNGF concentration as* CCI *as* C *mcg/mL, which will provide the opportunity to test the minimally effective dose in the target population. From a clinical standpoint, both the* C *and* CC *mcg/mL proposed concentrations can reasonably be expected to be safe and effective in the target population based* on previous clinical trial *experience with different rhNGF concentrations. Specifically,*~~s where~~ *starting from the first phase 2 clinical trial performed in Europe (REPARO, NGF0212) in patients with Neurotrophic Keratitis, both the* C *and* C *mcg/mL were proven safe and effective on corneal healing. The following clinical trials in DED also tested* CCI concentrations of rhNGF *up to* ~~*was used (*~~C mcg/mL. *In the DED studies we observed efficacy on tear function but a suboptimal tolerability of the higher concentrations tested*~~*) and a large percentage of patients reported* CCI *(for example,* 48% *patients reported* CCI *in* NGF0121 - PROTEGO-1, up to 63.5% in NGF0118, *and* 42% in NGF0216). Therefore, *the*~~a~~ new formulation of rhNGF was prepared *to*~~hat~~ allow CCI concentration with the goal of consequently CCI CCI CCI TEAEs for patients, *while maintaining efficacy on DED signs*. The C mcg/mL dose group ~~should bear~~*has the potential to show clinical benefit* ~~efficacy~~ since an earlier open-label study (NGF0213) in dry eye patients demonstrated that with *doses* CCI *as* C mcg/mL'	Following the request by AIFA
6.3 Preparation, Handling, Storage and Accountability	The following text was added: '*…The vehicle is not to be instilled on the day of the Baseline visit (Visit 2).*'	For further clarification of study procedures
6.3 Preparation, Handling, Storage and Accountability	The following text was added (updated text in Italic): 'Patients will instill one drop in both eyes three	For further clarification of study procedures

	times a day (TID) (approximately every 6 hours), starting *on Day 1 (Baseline visit) and continue the treatment ~~in the morning when they wake up~~*, for the next 2 weeks. *Depending on the time of the subject's Baseline visit, it is reasonable and possible that all doses may not be administered on this day.'*	
7.1.1 Criteria for Permanent Discontinuation of Trial Intervention	Criterion #2 was amended to include the following text (added text in Italic): '2) Worsening of the clinical condition that requires treatment discontinuation per physician decision *or ocular surgery*.'	To align with other similar guidance provided by AIFA
7.1.1 Criteria for Permanent Discontinuation of Trial Intervention	The following text was added: '*…and will receive preservative free AT, TID, provided by the Sponsor*.'	For further clarification of study procedures
8.2 Trial Visits and Follow-Up Assessments	Definition of End of Trial (EoT)/ End of Study (EOS) was amended to remove ambiguity: '**End of Trial (EoT)/End of Study (EOS):** as per **article 2 (26)** of the European Clinical Trial Regulation (CTR), EoT/End of Study (EOS) is the last visit of the last patient globally ~~*or a later point as required by study activities*~~.	Following request by AIFA
8.3 Assessments and Clinical Definitions, Table 6. List of Assessments	The following text was added under CCI CCI '*…This form will be administered to all subjects but completed only by those that either have* CCI *or have filled this form before*.'	For further clarification of study procedures
11.1 Regulatory and Ethical Considerations	The repealed Directive was deleted under the section on Europe: '… ~~*and repealing Directive 2001/20/EC, where applicable*~~'	Following the request by AIFA
11.11 Serious Breaches Management	This section was amended to reflect the following changes: '~~*Process (as defined under art. 52 of European Regulation 536/2014):*~~ ~~*Institutions, CRO, and investigators that are involved in the clinical trial management are obliged to notify the Sponsor about any Serious Breaches and suspected Serious Breach within 24 hours from the identification of such Serious Breach at the following email address: seriousbreaches.gcp@dompe.com*~~ ~~*The Sponsor will manage the event according to the internal process for the management and*~~	For further clarification of study procedures

	~~*notification of the relevant competent authority.*~~ ~~*Process (as defined under art. 52 of European Regulation 536/2014):*~~ *Institutions, CRO, and investigators involved in the clinical trial management must notify the Sponsor about any Serious Breaches and suspected Serious Breach within 24 hours from the identification of such Serious Breach.* *If not otherwise specified in relevant study plans, manuals and agreements with the contracted CRO, the Sponsor will manage the event according to the internal process for the management and notification of the relevant competent authority*.'	

Table of Contents

LIST OF TABLES

LIST OF ABBREVIATIONS/ ACRONYMS/ DEFINITIONS OF TERMS

Abbreviation or Acronym	Definition
ADR	Adverse Drug Reaction
AE	Adverse Event
AT	Artificial Tears
BCDVA	Best Corrected Distance Visual Acuity
BID	Bis in die (twice a day)
°C	Temperature Degree Celsius
CFR	Code of Federal Regulations
CRA	Clinical Research Associate
CRO	Contract Research Organization
CsA	Cyclosporine A
CSR	Clinical Study Report
DB	Database
DE	Dry Eye
DED	Dry Eye Disease
DFE	Dilated Fundus Exam
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
DRM	Data Review Meeting
DSMB	Data Safe Monitoring Board
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
ENR	Enrolled Population
ETDRS	Early Treatment of Diabetic Retinopathy Study
EOS	End of Study
EOT	End of Treatment
EoT	End of Trial
ETV	Early Termination Visit
°F	Temperature degree Fahrenheit
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPI	First Patient In
FPFV	First Patient First Visit
fTBUT	Fluorescein Tear Break-Up Time
FU	Follow-up
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form

Abbreviation or Acronym	Definition
ICH	International Conference on Harmonisation
IEC/EC	Independent Ethics Committee
ICE	Intercurrent event
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRT	Interactive Response Technology
ITT	Intention-To-Treat
IUD	Intrauterine Device
LNGFR	Low-affinity Nerve Growth Factor Receptor
LogMAR	Logarithm of the Minimum Angle of Resolution (Chart)
LPLV	Last Patient Last Visit
MAR	Missing at Random
MedDRA	Medical Dictionary for Regulatory Activities
mcg/mL	micrograms/milliliter
mcL	microliter
MCMC	Markov Chain Monte Carlo
MI	Multiple Imputation
MMRM	Mixed Model for Repeated Measures
MNAR	Missing Not At Random
NEI	National Eye Institute
NGF	Nerve Growth Factor
NIKBUT	Non-Invasive Keratograph tear Break Up Time
NIMP	Non-Investigational Medicinal Product
NK	Neurotrophic Keratitis
CCI	CCI
pg/mL	picograms/milliliter
PI	Principal Investigator
PID	Patient Identification Number
PP	Per Protocol Population
PT	Preferred Term
p75(NTR)	p75 Neurotrophin Receptor
QoL	Quality of Life
Q1	first quartile (lower quartile)
Q3	third quartile (upper quartile)
RCT	Randomized Clinical Trial
rhNGF	recombinant human Nerve Growth Factor
RMP	Risk Mitigation Plan
RND	Randomized Population
RP	Retinitis Pigmentosa
SAE	Serious Adverse Event
SAF	Safety Population
SANDE	Symptom Assessment in Dry Eye
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDV	Source Data Verification

Abbreviation or Acronym	Definition
SLE	Slit-Lamp Examination
SMP	Safety Management Plan
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TID	Ter in die (three times a day)
TrkA	Tropomyosin receptor kinase A
USA	United States of America
VAS	Visual Analogue Scale
VBR	Validated Bulbar Redness scale
Vs	Versus
§	Section

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Information	Description
Study Number	NGF0123
Title of Study (Study Name)	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 (REDUCO study)
IND/EudraCT No.	115892/ EUCT# **2023-507561-26-00**
Centers (Country/Region)	10 to 15 sites in total (USA and Italy) Please refer to the list of approved centers in Trial Master File
Development Phase	**Phase II**
Background Information	Nerve growth factor (NGF) is a polypeptide essential for the survival and growth of sympathetic and sensory neurons, and for differentiation of neurons in the central nervous system (**Levi-Montalcini, R. 1987**). NGF and its receptors TrkA and p75 are expressed in the anterior segment of the eye (iris, ciliary body, lens, cornea, and conjunctiva), and NGF is released in the aqueous humor. Studies suggest that NGF affects all tissues of the anterior segment, hence playing a crucial role in the pathophysiology of several diseases of the anterior segment of the eye (Lambiase, A. et al. 2011). Dry eye disease (DED) is a multifactorial disease of the ocular surface, characterized by impairment of quantity of tears and/or quality of the tear film. It is also accompanied by tear film hyperosmolarity, ocular discomfort symptoms, ocular surface inflammation and damage, and neurosensory abnormalities (Craig, J.P. et al. 2017). Until now, treatment has been limited to the use of artificial tears or varenicline to temporarily improve lubrication of the ocular surface, or to the use of topical steroids, cyclosporine A or lifitegrast to decrease the inflammatory reaction. Experimental and clinical evidence suggests that NGF may affect all the pathogenic mechanisms of dry eye, potentially restoring ocular surface homeostasis (Lambiase, A. et al. 2012). Specifically, NGF has been shown to stimulate tear film production, promote corneal epithelial healing, and stimulate conjunctival epithelial differentiation and mucin secretion (Coassin, et al. 2005; Lee, et al. 2006; Bonini, et al. 2000; Rios, et al. 2007). OXERVATE® is a 0.002% ([redacted] mcg/mL) recombinant human nerve

Information	Description
	growth factor (rhNGF) ophthalmic solution approved for clinical use by the EMA in July 2017 for treatment of moderate-to-severe neurotrophic keratitis (NK), and by the FDA in August 2018 for the treatment of NK. Treatment with rhNGF eye drops showed a good safety profile in two Phase I studies in healthy volunteers (studies NGF0112 and NGF0117), and in a Phase Ib/II study in patients with NK (study NGF0212-REPARO). The safety and efficacy of treatment with rhNGF eye drops in patients with NK has been demonstrated by two pivotal randomized clinical trials (RCT) in patients with NK at stage 2 and 3 (studies NGF0212 and NGF0214). Furthermore, rhNGF eye drops have also been evaluated in a Phase Ib/II study in patients with retinitis pigmentosa at concentrations of 60 mcg/mL and 180 mcg/mL (NGF0113), and in a Phase I/II study in patients with glaucoma at a concentration of 60 mcg/mL (NGF0314). The safety and efficacy of treatment with rhNGF eye drops in patients with dry eye was first evaluated in an open-label, uncontrolled study in which 4 weeks of treatment with rhNGF eye drops at concentrations of C mcg/mL and C mcg/mL (obtained by on-site dilution of the C mcg/mL solution) showed to be safe and effective in improving symptoms, corneal staining, and tear function as compared to baseline (NGF0213). These results prompted a Phase II RCT in patients with dry eye disease (NGF0216) and in patients with ocular discomfort symptoms following refractive surgery (NGF0116), which confirmed the favorable tolerability profile of rhNGF eye drops at a concentration of C mcg/mL when administered up to 6 times daily for 8 weeks. An additional Phase II RCT (study NGF0118) confirmed the safety profile of rhNGF eye drops at a concentration of C mcg/mL administered BID and TID for 4 weeks. Based on Schirmer test I data, a higher number of patients responded to treatment with rhNGF BID compared with vehicle. This study also showed that rhNGF TID treatment significantly improved fTBUT compared with vehicle and had clinically relevant but delayed beneficial effects in improving dry eye symptoms (based on SANDE questionnaire). A phase III RCT in patients with moderate to severe Sjogren's dry eye (NGF0121) demonstrated that rhNGF eye drops at a concentration of C mcg/mL instilled TID for 4 weeks significantly improved Schirmer test without anesthesia at completion of treatment as compared to vehicle. The effect was sustained until the end of follow-up at week 12. Symptoms (SANDE scores) also showed significant improvement 4 weeks post completion of treatment at week 8. Eye and eyelid pain were the most common adverse events reported with rhNGF treatment. Another Phase III clinical trial is currently ongoing to evaluate the efficacy of treatment with rhNGF eye drops at a concentration of C mcg/mL with 3 times daily dosing in patients with Sjogren's dry eye that already receive topical immunomodulatory treatment with cyclosporine

Information	Description
	A eye drops (NGF0221, PROTEGO-2). The results of the studies in patients with DED, as well as the results on reflex tear secretion obtained from the NK trials (NGF0212 and NGF0214), suggested that rhNGF may be effective for patients with aqueous deficient DED. In fact, in previous studies rhNGF ameliorated tear function and ocular surface epithelial damage, with CCI and discomfort being the most commonly observed ocular adverse events (AEs) during treatment. Therefore, in an effort to CCI experienced by patients during treatment, we propose to test two CCI concentrations of rhNGF. This is made possible by a new CCI rhNGF formulation that allows CCI concentrations of rhNGF ophthalmic solutions to be prepared. The CCI in rhNGF concentration may improve the tolerability of the treatment and, hence, symptoms of CCI while maintaining improvement in tear function and ocular surface epithelial healing. The aim of this study is to evaluate safety, efficacy and tolerability of a new CCI rhNGF-based ophthalmic solution at two different concentrations of Cmcg/mL and C mcg/mL in comparison with vehicle.
Objective	**Primary objective** To evaluate the efficacy of Cmcg/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). **Secondary Objectives** • To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle. • To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving tear film stability as compared to vehicle. • 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. • To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving best corrected distance visual acuity (BCDVA) as compared to vehicle. • To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving associated symptoms of dry eye as compared to vehicle. • To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving quality of life as compared to vehicle. **Safety Objectives** • To evaluate the safety of the new formulation of rhNGF ophthalmic solution

Information	Description
	• To evaluate the tolerability of the new formulation of rhNGF ophthalmic solution This study aims to demonstrate the efficacy of a new formulation of rhNGF in improving both symptoms and signs in patients with DED. Therefore, if at least one of the two concentrations of the new formulation of rhNGF shows superiority over vehicle in improving symptoms through the primary endpoint, the secondary objective will be to prove that the same concentration is also effective in improving clinical signs, as evaluated through the secondary endpoints. **Additional objective** • To validate an Italian version of the CCI CCI
Design	This is a phase II, multi-center, randomized, double-masked, parallel-arm, vehicle-controlled, prospective clinical trial to evaluate the safety and efficacy of two concentrations (Cmcg/mL and C mcg/mL) of a new formulation of rhNGF ophthalmic solution, administered as one (1) drop three (3) times a day in both eyes, for four (4) weeks in patients with DED. Patients will be evaluated at screening visit (day -12±2 - Visit 1), baseline (day 1 - Visit 2), week 2 (day 13±1- Visit 3), week 4 End of Treatment (EOT; day 28±1 - Visit 4), and week 8 end of follow-up and End of Study (EOS; day 56±2- Visit 5). An Early Exit visit could be performed in case of premature study discontinuation. The study duration per each patient will be of maximum 10 weeks, and it is divided into four phases as follows: **1. Screening & Start of Run-In (day -12±2, Visit 1):** Procedures for inclusion will be performed at both Visit 1 (Screening) and Visit 2 (Baseline). At Visit 1, eligible patients will be instructed to discontinue all topical ophthalmic medications and during the Run-In period they will only be allowed to use Study Product (vehicle) ophthalmic solution provided by the Sponsor. The first eye drop of the Study Product (vehicle) will be applied, in both eyes of patients, at the site by the investigator during Visit 1. Patients will be instructed on how to prepare the Study Product (vehicle) daily at home and then how to self-administer one drop 3 times a day in both eyes. A Patient Diary for the Run-In phase will be supplied to the patient. Investigators will document the first administration of the Study Product (vehicle) in the patient's medical record following Visit 1 to confirm the first administration of the Study Product (vehicle). **2. Run-In Period (day -12±2 Screening Visit 1 to day 1/Baseline Visit 2):**

Information	Description
	No topical treatment allowed except for the Study Product vehicle ophthalmic solution provided by the Sponsor, 3 times a day, at approx. 6-hour intervals, in both eyes. **3. Treatment Period (day 1 Baseline Visit 2 to week 4, day 28 ± 1 Visit 4):** At the end of Run-In period (Visit 2), patients fulfilling the inclusion/exclusion criteria will be randomized 1:1:1 and treated for 4 weeks with either Study Product (vehicle or IMP): ➢ new formulation of rhNGF ophthalmic solution at [redacted] mcg/mL 3 times a day, at approx. 6-hour intervals, in both eyes, for 4 weeks ➢ new formulation of rhNGF ophthalmic solution at [redacted] mcg/mL 3 times a day, at approx. 6-hour intervals, in both eyes, for 4 weeks. ➢ vehicle of the new formulation, 3 times a day, at approx. 6-hour intervals, in both eyes, for 4 weeks At the conclusion of the day 1 baseline visit (Visit 2) eligible patients will be dispensed a 2-week supply of Study Product (vehicle or IMP). Patients will be instructed to self-administer at home the Study Product (vehicle or IMP) one drop 3 times a day in both eyes. A 2-week supply of Study Product (vehicle or IMP) will additionally be dispensed to the patients during the treatment period at Visit 3. Starting from Visit 2, during the treatment period, only the Study Product (vehicle or IMP) TID at 4-8-hour intervals will be allowed for topical ocular treatment. Different administration frequencies will be recorded and considered as protocol deviation. Any concomitant ophthalmic treatment initiated during the treatment period must be documented on the concomitant medication log and represents a protocol deviation. A Patient Diary for recording the treatment phase will be supplied to the patient. At Visit 2 (baseline visit) a Study Eye will be determined. Namely, the Study Eye is the worst eye: assuming that all the inclusion/exclusion criteria are met in both eyes, the worse 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 worst NEI staining score for cornea and conjunctiva (total score for cornea staining followed by total score for conjunctiva). If both eyes are identical, then simply the right eye will be considered the Study Eye. At the investigator's discretion, any patient could be seen for an Unscheduled Visit for safety evaluation. In case of Adverse Events, patients may be withdrawn at the investigator's discretion. Patients who prematurely discontinued the treatment (for any reason) will be asked to complete the assessment planned by the protocol and

Information	Description
	will receive commercially available preservative free AT, TID, provided by the Sponsor. In case of withdrawal from the study, patients will be asked to complete the assessment expected for Visit 4 as Early Exit Visit. A flag will be inserted in the eCRF to define if the Visit 4 is a per protocol visit or if it is done as Early Exit 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):** Following the completion of the treatment period, patients will be followed up for an additional 4 weeks and will be evaluated at 8 weeks (visit 5) -end of the follow-up period. No treatment will be allowed except for commercially available preservative free artificial tears (AT) TID provided by the Sponsor. A Patient Diary for the Follow-Up period will be supplied to the patient. Different administration frequencies will be recorded and considered as protocol deviation. Any concomitant ophthalmic treatment initiated during the Follow-Up period must be documented on the concomitant medication log and represents a protocol deviation. At the investigator's discretion, the subject may be seen for an Unscheduled Visit to perform safety assessments. Patients who prematurely terminate the Follow-Up period (for any reason) will be asked to complete the assessment planned for Visit 5 (Week 8) as Early Exit Visit. A flag will be inserted in the eCRF to define if the Visit 5 is a per protocol visit or if it is done as Early Exit Visit. All enrolled patients will attend the following clinic visits and collect Study Products and AT in the following sequence: • Screening (Visit 1/Day -12±2): Run-In Study Product (vehicle) Pick-Up • Baseline (Visit 2/Day1): First 2 weeks of treatment Study Product Pick-Up (vehicle or IMP) • Mid-Treatment (Visit 3/Week 2): Second 2 weeks of treatment Study Product Pick-Up (vehicle or IMP) • End of 4-week Treatment (Visit 4/Week 4): Sponsor provided AT Pick-Up (NIMP) • End of Follow-Up Visit and End of Study Visit (Visit 5/Week 8)
Number of Patients	Two hundred and ninety-one (291, 97 per group) male or female patients need to be randomized in a 1:1:1 ratio to have at least 276 evaluable patients (92 per group). Patients randomized to the vehicle arm will be randomly assigned 1:1 to two different vehicle filling volumes, namely 1 mL or 2 mL of diluent, to preserve masking. The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrollment. Considering a screen failure rate of 20%, approximately 350 subjects need to be screened in order to have 291

Information	Description
	patients enrolled.
Criteria for Inclusion/Exclusion	**Inclusion criteria** 1. Male or female aged ≥18 years of any race/ethnicity and eye color. 2. A diagnosis of dry eye disease at least 6 months before enrollment (current use or recommended use of artificial tears for the treatment of dry eye). 3. Dry eye disease characterized by the following clinical features: a. Symptoms Assessment in Dry Eye (SANDE) questionnaire Global Score ≥50, and b. Schirmer-I test without anesthesia >2 mm and <10 mm/5 minutes, and c. Total corneal fluorescein staining grade ≥3 (NEI scale) and/or total conjunctival lissamine green staining score ≥3 assessed by the NEI grading system, and d. Fluorescein tear film break-up time (fTBUT) < 10 seconds The same eye must have fulfilled all the above criteria. 4. Best corrected distance visual acuity (BCDVA) score on ETDRS chart of ≥0.1 decimal units (≤1.0 logMAR) in each eye at the time of study enrollment 5. Negative pregnancy test in females of childbearing potential. 6. Only patients who satisfy all informed consent requirements will be included in the study; the patient and/or his/her legal representative must have read, signed, and dated the informed consent document before any study-related procedures are performed; the informed consent form signed by patients and/or legal representatives must have been approved by the IRB for the current study. 7. Have the ability and willingness to comply with study procedures. **Exclusion criteria** 1. Inability to speak and understand the local language sufficiently to understand the nature of the study, to provide written informed consent, and to allow the completion of all study assessments. 2. Evidence of an active ocular infection in either eye. 3. Presence of any other ocular disorder or condition requiring topical ocular medication during the entire duration of the study. 4. Possibility of the need for ocular surgery at the time of inclusion in the study or anticipated ocular surgery expected during the participation in the study. 5. History of severe systemic allergy or severe ocular allergy (including seasonal conjunctivitis, AKC, VKC) or chronic conjunctivitis and/or keratitis other than dry eye. 6. Ocular scarring due to irradiation, alkali burns, Stevens-Johnson syndrome and ocular cicatricial pemphigoid.

Information	Description
	7. Destruction of conjunctival goblet cells such as in Vitamin A deficiency. 8. Severe blepharitis or obvious inflammation of the lid margin. 9. Intraocular inflammation defined as Tyndall score >0. 10. Medical history of tumor malignancy in the previous 3 years 11. Systemic disease not stabilized within 1 month before the screening visit (e.g., diabetes with glycemia out of range, thyroid malfunction) or judged by the investigator to be incompatible with the study (e.g., current systemic infections) or with a condition incompatible with the frequent assessment required by the study. 12. History of a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or had a clinically significant allergy to drugs, foods, CCI eye drop or other local anesthetics or other materials, including ocular vital dyes, tropicamide eye drops, commercial artificial tears. 13. Known or suspected allergy to CCI CCI and/or any other component of the new rhNGF formulation. 14. Fertile patients (i.e., not surgically sterilized, or postmenopausal women for at least 1 year) are excluded from participation in the study if they do not practice abstinence from heterosexual intercourse as per usual and customary lifestyle, or are unwilling to use an acceptable form of contraception such as condom with spermicidal cream or jelly for males, or for females if they meet any one of the following conditions: a. Currently pregnant (positive urine pregnancy test at screening or baseline visits) or planning to become pregnant during the duration of the treatment phase of the clinical trial. b. Patient is breastfeeding. c. Unwilling to use birth control measures such as mechanical barrier methods (spermicide in conjunction with a barrier such as a condom or diaphragm or intrauterine device) during the entire course of and 30 days after the study treatment period, or, d. Unwilling to continue to use highly effective birth control measures such as hormonal contraceptives (oral, implanted, transdermal, or injected) during the entire course of and 30 days after the study treatment period. 15. Any concurrent medical condition that, in the judgment of the principal investigator, might interfere with the conduct of the study, confound the interpretation of the study results, or endanger the patient's well-being. 16. Contact lenses or punctum plug use in either eye during the Run-In, treatment, and follow-up phases of the study (previous use is

Information	Description
	not an exclusion criterion but must be removed and discontinued at the screening visit). 17. Medical history of drug addiction or alcohol abuse (>1 drink /day for women and >2 drinks /day for men following USDA dietary Guidelines 2020-2025). 18. Any prior ocular surgery including but not limited to amniotic membrane transplant, refractive (PTK/LASIK/Epi-LASIK/LASEK/SMILE), palpebral, cataract surgery, trabeculectomy, vitrectomy and pan-retinal photocoagulation (PRP) within 90 days before the screening visit. 19. Participation in a clinical trial with a new active substance, including medical devices, during the previous 60 days. 20. Participation in another clinical trial study at the same time as the present study.
Study Medication, Dosage and Mode of Administration	The route of administration for the study products is ophthalmic with the administration applied in both eyes. The IMPs consist in a new formulation to be reconstituted daily by the patients (kit containing CCI rhNGF + diluent for reconstitution) for topical ocular administration at the following concentrations of active ingredient: • IMP 1: rhNGF Cmcg/mL • IMP 2: rhNGF C mcg/mL • IMP (vehicle): rhNGF 0 mcg/mL During the treatment period, the IMPs are administered according to the following scheme: - IMP 1: One drop of rhNGF Cmcg/mL instilled in both eyes TID (every approx. 6 hours). - IMP 2: One drop of rhNGF C mcg/mL instilled in both eyes TID (every approx. 6 hours). - IMP (vehicle): One drop of Vehicle instilled in both eyes TID (every approx. 6 hours). A bi-weekly kit will be provided to the patient at the time of baseline (visit 2) and week 2 (visit 3) visits. An ancillary kit containing sterile pipettes for vials adapter and sterile wipes will be provided at the same time. In addition to that, for the Run-In period a bi-weekly kit of vehicle will be given to the patients, and for all the Follow-Up period the Sponsor will also supply preservative free artificial tears (AT).
Duration of Treatment	Four (4) weeks (28 Days±1) of treatment with IMPs according to the randomization list.
Primary Efficacy Endpoint	The primary efficacy endpoint is the mean change from baseline to Week 8 in symptoms of dry eye assessed by SANDE Global Score [Time Frame: week 8 (V5)].

Information	Description
Other Endpoint	*The key secondary efficacy endpoints are:* • Proportion of patients improving to Schirmer-I test without anesthesia ≥10mm/5min in the Study Eye [Time Frame: at week 4 (V4)] • Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye [Time frame: at week 4 (V4)] • Mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator [Time Frame: at week 4 (V4)] *The secondary efficacy endpoints are:* • 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 ≥10mm/5min in the Study Eye [Time Frame: at week 8 (V5)] • Mean change from baseline in fluorescein tear break-up time (fTBUT)- in the Study Eye [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in symptoms questionnaire (SANDE) scores for severity and frequency [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in symptoms of dry eye assessed by SANDE Global Score [Time Frame: at week 4 (V4)] • Mean change from baseline in total conjunctival lissamine green staining (NEI scale) in the Study eye as assessed by the investigator [Time Frame: at week 4 (V4)] • Mean change from baseline in CCI assessed by the CCI CCI scale [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in best corrected distance visual acuity (BCDVA) [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline of CCI CCI by the CCI CCI [Time Frame: at weeks 4 (V4) and 8 (V5)] *The exploratory endpoints are:* • Mean change from baseline in NIKBUT-first and in NIKBUT-

Information	Description
	average in the Study Eye only at selected sites that have the required equipment (Oculus keratograph 5M) [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in response to CCI [redacted] CCI [redacted] [Time Frame: week 4 (V4)] • Mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator [Time Frame: at week 8 (V5)] • Mean change from baseline in total conjunctival lissamine green staining (NEI scale) in Study Eye as assessed by the investigator [Time Frame: at week 8 (V5)] • Validate an Italian version of the CCI [redacted] [Time Frame: at baseline, week 4 (V4)]
Safety Endpoint	• Safety will be monitored by the incidence and frequency of Treatment-Emergent Adverse Events (TEAEs) assessed throughout the study including Run-In period. • Mean change from baseline in corneal endothelial cell density in both eyes performed at sites that have a specular microscope [Time Frame: at week 8 (V5)] • Change from baseline in the proportion of patients with vitritis, retinal or vitreal 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 in both eyes (VBR 10 score) [Time Frame: at weeks 4 (V4) and 8 (V5)] • Treatment discontinuation rate due to tolerability issues.
Methods, procedure and assessment	Site staff will conduct the following assessments and activities in the order outlined below during each visit. **Visit 1 (Day -12±2): SCREENING** 1. Informed Consent/HIPAA (HIPAA only for USA) 2. Demographics, Ocular and Medical History, Previous and Concomitant Ocular and Systemic Medications and Conditions 3. Pregnancy Test 4. SANDE questionnaire 5. Ophthalmic Examinations for each eye: A. Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS

Information	Description
	B. External Ocular Examination C. Schirmer-I test without anesthesia D. Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes) E. Assess bulbar redness (VBR 10) F. Instill fluorescein and wait 30 seconds. G. Assess fTBUT. H. After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale score range 0-15). I. Instill lissamine green. J. Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18) within 2 minutes of instillation of dye. 6. Inclusion/exclusion criteria assessment by the investigator 7. Dispense the Study Product (vehicle) eye drops to be instilled; the first eye drop will be instilled by the Investigator in both eyes and then the Study Product (vehicle) will be instilled by the patient at home 3 times a day in both eyes for 12 days ±2. *Depending on the time of the Subject's baseline visit, all doses may not be administered on this day.* 8. Deliver instructions to patient. 9. Dispense the Patient Diary to record the administration of Study Product (vehicle) during the Run-In period (vehicle accountability), any new or changes in concomitant medications, any unusual medical condition. 10. Investigator documentation of the first administration of the Study Product (vehicle) in the patient's medical record following Visit 1 to confirm the first administration of the Study Product (vehicle). 11. AE monitoring **RUN-IN period (from Day -12 to Day 1):** At home, patients will proceed with the Run-In of 12 days ± 2 using the dispensed Study Product (Vehicle) eye drops (1 drop in both eyes, 3 times a day). No other ophthalmic topical medications are permitted. **Visit 2 (Day 1): BASELINE** Patients will be randomized if they continue to meet eligibility criteria at Visit 2/Baseline. 1. Concomitant Ocular and Systemic Medications and Conditions 2. Record AEs 3. Pregnancy test 4. SANDE questionnaire 5. CCI

Information	Description
	6. Ophthalmic examinations in each eye: A. Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS B. External Ocular Examination C. Assess NIKBUT (at selected centers) D. Schirmer-I test without anesthesia. E. Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes) F. Assess bulbar redness (VBR 10) G. Instill fluorescein and wait 30 seconds. H. Assess fTBUT. I. After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15). J. Apply lissamine green. K. Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18) within 2 minutes of instillation of dye. L. Perform CCI eye drop CCI response test (only in patients with CCI). M. Perform specular microscopy to assess corneal endothelial cells density (at selected sites that have a specular microscope already available). N. Instill tropicamide eye drop solution 1% O. After 15 minutes or when pupils are adequately dilated perform fundus examination in both eyes 7. Inclusion/Exclusion: Patients must continue to meet the Screening/Enrollment Criteria. At the end of the Visit 2/Baseline (Day 1), it will be determined if the subject qualifies for continued participation and treatment in the study. 8. Collect used/unused Study Product (vehicle) of the Run-In phase (to be checked by independent personnel) 9. Review the Patient Diary of the Run-In phase. 10. Randomization 11. Study product dispensing: eligible patients will be supplied with a bi-weekly kit of Study Product (IMP) after randomization, sufficient for 2 weeks of treatment. 12. Deliver instructions to subject. 13. Dispense the Patient Diary to record adherence to the treatment administration of Study Products during the treatment period, any new or changes in concomitant medications, any unusual medical condition. 14. AE monitoring.

	Visit 3 - Week 2 (Day 13±1) STUDY PRODUCT PICK-UP VISIT 1. Concomitant Ocular and Systemic Medications Update 2. Record AEs 3. Check Patient Diary 4. Collect used/unused Study Product (to be checked by independent personnel), 5. Study product dispensing: Patients will be supplied with a biweekly kit of Study Product sufficient for 2 weeks of treatment. **Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT (OR EARLY EXIT VISIT)** 1. Concomitant Ocular and Systemic Medications Update 2. Record AEs 3. Check the Patient Diary for the treatment period. 4. SANDE questionnaire 5. CCI [redacted] 6. Ophthalmic Examinations: A. Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS B. External Ocular Examination C. Assess NIKBUT (at selected centers) D. Schirmer-I test without anesthesia. E. Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes) F. Assess bulbar redness (VBR 10) G. Instill fluorescein and wait 30 seconds. H. Assess fTBUT. I. After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15). J. Apply lissamine green. K. Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18) within 2 minutes of instillation of dye. L. Perform CCI [redacted] eye drop CCI [redacted] response test (only in patients with CCI [redacted]) 7. Collect used and unused Study Products (to be checked by independent personnel) 8. Retrieve Patient Diary for the treatment period. 9. Dispense preservative free artificial tears (AT) eye drops to be administered 3 times a day. 10. Deliver instructions to Subject. 11. Dispense the Patient Diary to record adherence to the preservative free artificial tears (AT) eye drops during the Follow-Up period, any new or changes in concomitant medications, any unusual medical condition.

Information	Description
	12. AE monitoring. **Visit 5 - Week 8 (day 56±2) FOLLOW-UP & END OF STUDY VISIT (OR EARLY EXIT VISIT)** 1. Concomitant Ocular and Systemic Medications Update 2. Record AEs 3. Check the Patient Diary for the Follow-Up period. 4. SANDE questionnaire 5. CCI 6. Ophthalmic Examinations: A. Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS B. External ocular examination C. Assess NIKBUT (at selected centers) D. Schirmer-I test without anesthesia. E. Slit lamp examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes) F. Assess bulbar redness (VBR 10) G. Instill fluorescein and wait 30 seconds. H. Assess fTBUT. I. After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15). J. Apply lissamine green. K. Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18). L. Perform specular microscopy to assess corneal endothelial cells density (at selected sites that have a specular microscope already available). M. Instill tropicamide eye drop solution 1% N. After 15 minutes/once pupils are adequately dilated perform fundus examination in both eyes 7. Retrieve the Patient Diary for the Follow-Up period
Statistical considerations	**Sample size and randomization.** The sample size of the study is calculated based on results from the previous study NGF0118. Expecting a mean difference of about 11 points in SANDE Global Score improvement from baseline to Week 8 in favor of rhNGF Eye Drops Solutions, a total sample size of 276 evaluable patients (92 per group) allows to achieve an overall power of 80% to show superiority of at least one rhNGF ophthalmic solution dose over Vehicle on the improvement from baseline of SANDE Global Score at week 8, considering an overall one-sided alpha of 0.025. The sample size calculation takes into consideration a Bonferroni correction for two

Information	Description
	comparisons (each active vs. vehicle). Assuming a 5% rate of patients not evaluable after enrollment, the total number of patients to be enrolled in the study will be about 291. Considering a screen failure rate of 20%, approximately 350 subjects need to be screened in order to have 291 subjects enrolled. Patients fulfilling all the inclusion criteria and none of the exclusion criteria will be randomized in a 1:1:1 fashion among the three treatment groups (rhNGF C mcg/mL, rhNGF C mcg/mL and vehicle) through interactive response technology (IRT). Patients randomized to the vehicle arm will be randomly assigned 1:1 to two different vehicle filling volumes, namely 1 mL or 2 mL of diluent, to preserve masking. All patients randomized to the vehicle arm will be analyzed as a single treatment arm regardless of the two different diluent volumes assigned. **Statistical methods** Summary statistics are defined for quantitative variables (number of observations, mean, standard deviation, median, Q1-Q3, minimum and maximum) and qualitative variables (number and percentage per category). If appropriate, confidence intervals around the means or the proportions will be presented. The change from baseline in SANDE global score at week 8 will be analyzed by means of a mixed model for repeated measures (MMRM). The analyses will include the fixed effects of treatment (rhNGF C mcg/mL, rhNGF C mcg/mL and vehicle), visit (2 levels: Weeks 4, 8), baseline value, and treatment by visit interaction. The covariance matrix used will be "unstructured". Each NGF concentration group will be compared versus vehicle at Week 8 using the least square mean differences. 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. Each key secondary endpoint will be analyzed in the same manner described for the primary endpoint, with the only difference that ANCOVA will replace the MMRM for continuous endpoints and logistic model will replace MMRM for the binary endpoint. Independently of the results on primary endpoints, all secondary endpoints will be analyzed at each available time point by means of descriptive statistics and by appropriate statistical tests. Change from baseline values and shift tables versus baseline may be summarized for all available post-baseline visits. AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA). Individual AEs will be listed in patient data listings. TEAEs will be presented in terms of the number of AEs and incidence by treatment,

Information	Description
	seriousness, relationship to treatment, severity, and study period (treatment or follow-up). Other safety parameters will be summarized by treatment at each available time point by means of descriptive statistics. The Safety (SAF) and the Full Analysis Set (FAS) population will consist of all patients who will be randomized and receive at least one dose of the IMP. Safety population will be analyzed according to the actual treatment received; Full Analysis Set population will be analyzed according to Intention-To-Treat (ITT) principle, i.e., by treatment allocation. The Per Protocol (PP) population will consist of all patients in the FAS population who do not have Major Protocol Deviations. Primary and secondary efficacy analyses will be conducted on the FAS population while SAF and PP populations will be used for safety and sensitivity analyses, respectively. The Statistical Analysis Plan (SAP) will be issued before database lock with more technical and detailed elaboration of the principal features of statistical analyses. Any deviation from the original statistical plan will be described in the Clinical Study Report.

1.2 SCHEDULE OF ACTIVITIES

Table 1: Schedule of activities planned during the different visits

	Run-In period		Treatment period		FU 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 eye drops 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 [redacted]		X		X	X

	Run-In period		Treatment period		FU 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**
BCDVA by ETDRS	X	X		X	X
External Ocular Examination	X	X		X	X
NIKBUT (in selected centers)		X		X	X
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
Corneal fluorescein staining NEI scale	X	X		X	X
Apply lissamine green	X	X		X	X
Conjunctival lissamine green Staining NEI scale.	X	X		X	X
CCI CCI (CCI CCI before and after CCI eyedrop) ***		X		X	
Specular microscopy (Corneal endothelial cell density) (in selected centers)		X			X
Instill tropicamide eye drops		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

	Run-In period		Treatment period		FU 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**
Adverse events evaluation		X	X	X	X
LABORATORY TESTS					
Pregnancy Test	X	X		X	

*In case of premature discontinuation of the study, the subject 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 ancillary kit will be also provided, see §6.3

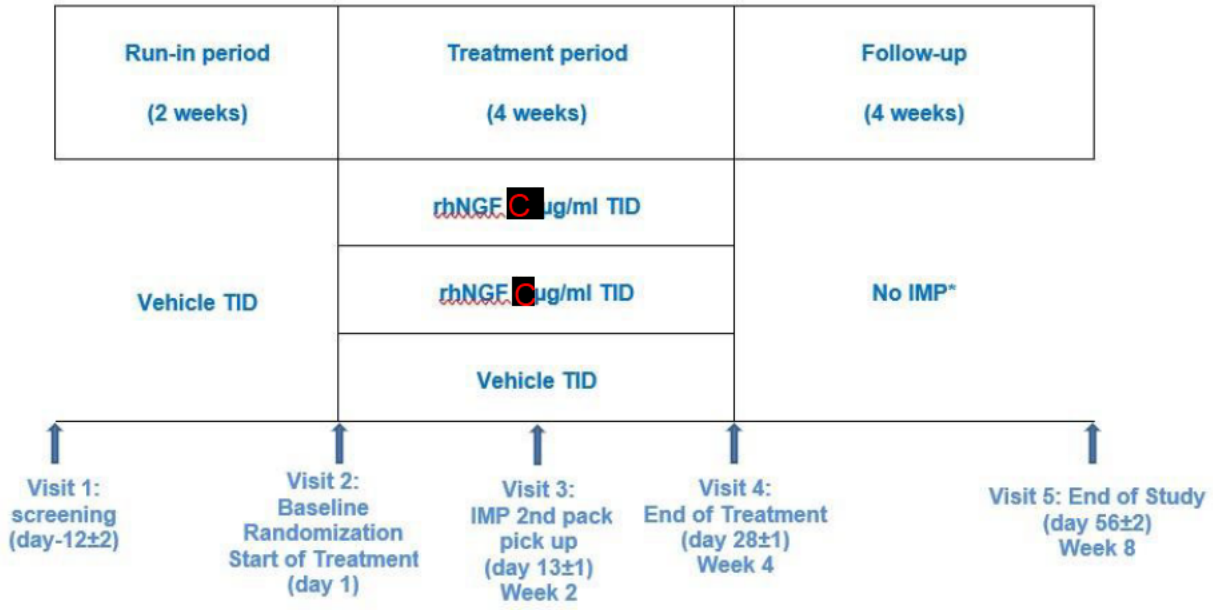
^ CCI will be administered to all subjects but completed only by those that either have CCI or have filled this form before.

*** Only in patients with CCI

**** 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.

1.3 SCHEMA

The study schema is shown below.



*artificial tears 3 times daily provided by the sponsor

2 INTRODUCTION

The trial will be conducted according to the protocol and its amendments, in compliance with GCP and the applicable regulatory requirements in USA and Europe as indicated in the ICH-GCP E6 (R2) guideline for good clinical practice and the other relevant guidelines for the conduct of clinical trials. The clinical study is regulated by the EU CTR Regulation no. 536/2014.

Recombinant human NGF (rhNGF) is the active principle of OXERVATE® 0.002% ([C] mcg/mL) rhNGF ophthalmic solution a marketed medicinal product already approved for clinical use by the EMA in July 2017 for treatment of moderate-to-severe neurotrophic keratitis (NK), and by the FDA in August 2018 for the treatment of NK. Over 16000 NK patients have been already exposed to OXERVATE® 0.002% rhNGF eye drop formulation at [C] mcg/mL at the recommended dose regimen of 1 drop/ eye every 2h (6 times a day within a 12-hour period) for 8 weeks (OXERVATE® U.S. Package Insert). A summary of the most relevant clinical observations is included in this section and a list of selected scientific references is provided at the end of this document §14.

2.1 PURPOSE OF TRIAL

The aim of the study is to investigate the safety and the efficacy of a new ophthalmic formulation (eye drops) of two [CCI] concentrations of recombinant human nerve growth factor (rhNGF) [C] and [C] mcg/mL in patients with dry eye. The treatment is 4 weeks long and the primary endpoint is efficacy in improving dry eye symptoms, measured 4 weeks after the completion of the study treatment (week 8).

2.2 NAME AND DESCRIPTION OF THE INVESTIGATIONAL MEDICINAL PRODUCT

Recombinant hNGF, the active principle, has the same sequence of native hNGF. The cGMP grade investigational medicinal product (IMP) consists of a [CCI] product comprising recombinant human nerve growth factor (rhNGF, [C] mcg/vial), [CCI] dissolved in 1 mL (IMP2) or 2 mL (IMP1) of a diluent containing [CCI] The vehicle solution contains the same [CCI] preparation without rhNGF, and it is dissolved in 1 mL or 2 mL of the same diluent to ensure the study masking. The reconstitution of the [CCI] product with the diluent yields in all cases a sterile aqueous solution suitable for ophthalmic use (eye drops). For more information see §6.1.

The vehicle solution is used during the Run-In period by all eligible patients for 12±2 days, as described in §6, and the same vehicle is administered to the IMP (vehicle) group for 4 weeks between visit 2 and visit 4. IMP1 is the ophthalmic solution containing [C] mcg/mL of rhNGF while IMP2 is the ophthalmic solution containing [C] mcg/mL of rhNGF. These solutions (and the components) are indicated in this study with the general name of Study Product(s).

2.3 RELEVANT NON-CLINICAL STUDIES

Nonclinical studies were performed to assess the safety and efficacy of the proposed new ophthalmic formulation of rhNGF and the vehicle.

2.3.1 Efficacy Studies

The efficacy of the new formulation of rhNGF was investigated in a mouse model of CCI CC (CCI model of CCI . The results showed CCI efficacy of the new formulation of rhNGF eye drops and the reference one. The topical ophthalmic administration of rhNGF C mcg/mL new formulation was well tolerated in this model (4 eye drops / day for 10 days) with no alterations in corneal sensitivity or epithelial integrity in treated mice.

2.3.2 Toxicology Studies

The ocular tolerance and potential toxicity of the new formulation containing the test item rhNGF (recombinant human nerve growth factor) and the new excipient, CCI was assessed following daily administrations by the ocular topical route (eye drops, 3 times a day in both eyes, 4-hour apart) for at least 13 weeks in the New-Zealand White rabbits and the reversibility of any findings was evaluated during a 2-week recovery period. In addition, a proof of exposure of the compounds was determined.

Animals were administered with rhNGF at C or at C µg/day or vehicle (Reconstitution Solvent containing the new excipient CCI eye drops, three times a day, in both eyes for at least 13 weeks. The following parameters and end points were evaluated: Mortality, clinical observations, body weights and body weight gains, food consumption, ophthalmology evaluation, including modified Mac Donald - Shadduck examination with detailed ophthalmic examination, tonometry and esthesiometry, clinical pathology parameters (hematology and clinical chemistry), proof of exposure, organ weights, macroscopic and microscopic examinations.

No rhNGF or CCI level was determined in the respective analyzed serum samples. This lack of systemic absorption after the topical ocular administration of rhNGF was expected.

During the in-life phase, rhNGF, formulated in CCI did not cause any mortality or clinical signs. There were neither test item-related changes in body weight nor in food consumption. There were no rhNGF formulation-related ophthalmic findings for both dose levels at all evaluated examinations or measurements during the dosing period. No ophthalmic findings were detected in the control animals treated with the formulation vehicle containing CCI No treatment-related changes in clinical pathology parameters were observed.

The pathology evaluation did not reveal any test item-related changes in organ weight or test item-related findings at macroscopic and microscopic examinations.

In conclusion, under the study experimental conditions, daily ocular topical administration of

rhNGF at C or C µg/day to the New-Zealand White rabbit for at least 13 weeks was well tolerated during the in-life phase and did not cause clinical signs, clinical pathology and organ weights changes, gross and microscopic findings. As expected after ocular administration by eye drops, no systemic exposure to rhNGF or CCI was detected.

2.4 PHARMACOKINETICS AND PRODUCT METABOLISM

2.4.1 Biodistribution in Non-clinical Studies

Biodistribution studies after single CCI and repeated topical ocular administration in rabbits showed that when administered CCI rhNGF reached both the retina and optic nerve and its uptake persisted even after 60 days from exposure, while using eye drops, there was only a short time of residence and accumulation level, and a negligible uptake was observed for rhNGF in both retina and optic nerve.

Eye drops. A distribution study was performed using tritium [^{3}H]rhNGF administered by eye drops in rats. Micro-autoradiography of the eyes showed that [^{3}H]rhNGF can reach the retina and the optic nerve in rats. The distribution of radioactivity in the eyeballs of male and female albino rats was determined, subjectively, following ocular administration of [^{3}H]rhNGF at nominal dose level of 0.25 or 1.0 mcg/eye/dose (actual dose levels 0.24 and 1.36 mcg/eye/dose) using micro-autoradiography techniques. Tissues were harvested at 2, 4, 8, 12 and 24 h post-dose. At low dose, levels were detected in the cornea, lens and sclera. At high dose, radioactivity was present in the optic nerve, iris, ciliary body, retina and choroid albeit at lower levels than in the sclera and cornea. The sclera and cornea contained moderate levels at all sampling time.

CCI In the CCI administration of rhNGF in the rabbit at ascending doses, high amounts of rhNGF were measured in the vitreous and retina at the 120-h sampling point. In a GLP study, rhNGF quantification in the serum and tissue biodistribution in vitreous, retina and optic nerve were carried out in rabbits following 7 CCI administrations over 3 months. The level of rhNGF in serum was mostly under the lower limit of quantification (C pg/mL) when the CCI concentration was administered (C mcg/mL) but rhNGF was measured dose-proportionally in serum in the mid and high concentration groups (50 and 250 mcg/mL). The level of rhNGF in vitreous, optic nerve and retina was measured with a certain variability but rather dose-proportionally. After the recovery period, rhNGF was still detectable in the retina of the 250 mcg/mL group.

2.4.2 Clinical Pharmacokinetics

In humans, pharmacokinetics has been studied in phase I (NGF0112, NGF0117) and Phase II (NGF0212-REPARO) studies after eye drops administration. Minor changes (not related to rhNGF dose) were observed in serum NGF values, but the changes from baseline were sporadic and variable from positive to negative, thus suggesting an individual physiological fluctuation of the natural basal NGF levels rather than treatment- related absorption of rhNGF eye drops.

During the NGF0112 study in healthy volunteers, serial blood samples were collected pre-dose and after rhNGF eye drops. NGF serum concentrations were measured using a validated

ELISA method.

Serum concentrations of NGF at all blood sampling times for all dosed subjects were found not significantly different from basal levels (33.758 pg/mL), with the exception of six subjects. Specifically, NGF serum levels were detectable in one subject dosed with the C mcg/mL concentration, one subject dosed with a 60 mcg/mL concentration, 2 subjects dosed with 180 mcg/mL concentration, and two subjects under vehicle.

In these subjects with quantifiable serum levels of NGF the changes from baseline (Day-1) were sporadic and varied from positive to negative values thus suggesting an individual physiological fluctuation of the basal levels, rather than a treatment-related absorption.

The concentration versus time profiles of NGF were also assessed from individual serum samples of Japanese subjects (NGF0117 Study). In this study validated ELISA tests were used for PK measurements. The original validated method was the same applied to the NGF determination in serum samples taken from studies NGF0112 and NGF0212 with a LLOQ <32pg/mL. The new additional validated ELISA method improved the sensitivity in the determination of NGF serum levels at <15 pg/mL.

In this study five subjects showed serum levels of NGF detectable at Baseline (Day -1): 3 (15.0%) in the rhNGF-treated group and 2 (20.0%) in the vehicle group. Eight subjects showed serum levels of NGF detectable during the study period: 5 treated with rhNGF and 3 treated with vehicle.

The study NGF0117 also demonstrated that there were no pharmacokinetics differences due to ethnicity.

Pharmacokinetics parameters were also studied in patients with moderate (Stage 2) and severe (Stage 3) NK (NGF0212).

Blood samples were collected at several time points from Baseline (Day 0) to Week 8 during the NGF0212 study to determine the PK profile of rhNGF and to provide data concerning the possible systemic exposure of rhNGF in patients with Stage 2 and 3 NK treated with rhNGF via local application to the eye.

Pharmacokinetic profile of the patients participating demonstrated that there was no accumulation effect of rhNGF. Most patients had an rhNGF concentration below the LLOQ at all measured time points. Five patients (3 patients in the rhNGF C mcg/mL group and 2 patients in the rhNGF C mcg/mL group) had rhNGF levels that were above the LLOQ. These observations were likely due to fluctuations around a constitutive serum level, independent from the study treatment.

The results of the phase II study were consistent with the results in healthy volunteers. Due to the low incidence of subjects with detectable NGF in serum samples in previous studies and the low magnitude of observed NGF serum levels, no further pharmacokinetic evaluations were performed in preparation for this clinical study and no serum samples will be collected during this study.

2.4.3 Metabolism

rhNGF is a polypeptide. The activated rhNGF/TrkA complex can be internalized and rhNGF redistributed in the tissue and inactivated by proteolytic pathways.

2.5 RELEVANT CLINICAL TRIALS

Treatment with rhNGF eye drops showed a good safety profile in two Phase I studies in healthy volunteers (studies NGF0112 and NGF0117), and in a Phase I/II study in patients with NK (study NGF0212- REPARO). The safety and efficacy of treatment with rhNGF eye drops in patients with NK has been demonstrated by two pivotal randomized clinical trials (RCT) in patients with NK at stage 2 and 3 (studies NGF0212 and NGF0214). Furthermore, rhNGF eye drops have also been evaluated in a Phase I/II study in patients with retinitis pigmentosa at concentrations of 60 mcg/mL and 180 mcg/mL (NGF0113), and in a Phase I/II study in patients with glaucoma at a concentration of 60 mcg/mL (NGF0314).

The safety and efficacy of treatment with rhNGF eye drops in patients with dry eye was first evaluated in an open-label, uncontrolled study in which 4 weeks of treatment with rhNGF eye drops at concentrations of C mcg/mL and C mcg/mL showed to be safe and effective in improving symptoms, corneal staining, and tear function as compared to baseline (NGF0213). These results prompted a Phase II RCT in patients with dry eye disease (NGF0216) and in patients with ocular discomfort symptoms following refractive surgery (NGF0116), which confirmed the favorable tolerability profile of rhNGF eye drops at a concentration of C mcg/mL up to 6 times daily for 8 weeks. An additional Phase II RCT (study NGF0118) confirmed the safety profile of rhNGF eye drops at a concentration of C mcg/mL BID and TID for 4 weeks (obtained by on-site dilution of the C mcg/mL solution). Based on Schirmer test I data, it showed that a higher number of patients responded to treatment with rhNGF BID compared with vehicle. This study also showed that rhNGF TID treatment significantly improved fTBUT compared with vehicle and had clinically relevant but delayed and sustained beneficial effects in improving dry eye symptoms (based on SANDE questionnaire). A phase III RCT in patients with moderate to severe Sjogren's dry eye (NGF0121 - PROTEGO-1) demonstrated that rhNGF eye drops at a concentration of C mcg/mL instilled TID for 4 weeks CCI patients' aqueous tear production at completion of treatment as compared to vehicle, which was sustained up till the end of follow-up at week 12 (8 weeks post completion of treatment). Symptoms (SANDE scores) also showed CCI CCI at 4 weeks post completion of treatment (week 8), which is consistent with well-known CCI following an earlier improvement in signs in dry eye disease. However, this CCI in symptoms was not sustained until the end of follow-up. Eye and eyelid pain were the most common adverse events reported with rhNGF treatment. Another Phase III clinical trial is currently ongoing to evaluate the efficacy of treatment with rhNGF eye drops at a concentration of C mcg/mL with 3 times daily dosing in patients with Sjogren's Syndrome dry eye and autoimmune disease-associated dry eye that already receive immunomodulatory treatment (NGF0221, PROTEGO-2).

2.6 DISEASE REVIEW AND TRIAL RATIONALE

Dry eye disease (DED) is a multifactorial disease of the ocular surface, characterized by impairment of quantity of tears and/or quality of the tear film. It is also accompanied by tear film hyperosmolarity, ocular discomfort symptoms, ocular surface inflammation and damage, and neurosensory abnormalities. DED can become a chronic progressive disease with chronic discomfort, visual disturbance, tear film instability with potential damage to the ocular surface and consequences on quality of life (Craig JP, et al. 2017). Until now, treatment has been limited to the use of artificial tears or varenicline to temporarily improve lubrication of the ocular surface, or the use of topical steroids, cyclosporine A or lifitegrast to decrease the inflammatory reaction. These treatments have been associated with patient satisfaction issues such as a non-favorable tolerability profile and a slow onset of action.

Nerve growth factor (NGF) binds to two neurotrophin receptors p75(NTR) and p140(Trk) (TrkA) to induce auto-phosphorylation of the neurotrophin receptor, leading to the activation of various signaling pathways, like phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), mitogen activated protein kinase (MAPK), extracellular signal-regulated kinase (Erk). These pathways help in regulating all aspects of ocular surface biology including trophic support to corneal cells. rhNGF stimulates both ocular surface wound healing and cornea repair processes (**Lambiase et al 2012**).

Experimental and clinical evidence suggests that rhNGF may affect all the pathogenic mechanisms of dry eye, potentially restoring ocular surface homeostasis (**Lambiase et al 2012**). Specifically, NGF has been shown to stimulate tear film production, promote corneal epithelial healing, and stimulate conjunctival epithelial differentiation and mucin secretion (**Coassin et al 2005**; **Lee et al 2006**; **Bonini et al 2000**; **Rios et al 2007**).

The results of the studies in patients with DED discussed earlier (NGF0118, NGF0121), as well as the results on reflex tear secretion obtained from the NK trials (NGF0212 and NGF0214), suggested that rhNGF may be effective for patients with aqueous deficient DED. In fact, in previous studies rhNGF ameliorated tear function and ocular surface epithelial damage, but CCI and discomfort were commonly observed ocular adverse events (AEs) during treatment. Therefore, due to the highly symptomatic nature of this condition, in an effort to CCI experienced by DED patients during treatment, we propose to test two CCI concentrations of rhNGF using a new CCI rhNGF formulation that allows CCI concentrations of rhNGF ophthalmic solutions to be prepared.

Details on the non-clinical studies with this new formulation of rhNGF can be found in §2.3.

The proposed clinical trial is conducted in patients with dry eye because four previous clinical studies (NGF0118, NGF0121, NGF0212 and NGF0214) indicate some benefit in this population. The indication "dry eye" is not approved as of today by regulatory authorities.

2.6.1 Alternative treatments

Currently, there are a few commercially available treatments for DED that are

immunomodulatory, reduce inflammation and/or improve tear secretion from lacrimal glands, but do not address the CCI component of DED. These treatments include RESTASIS™ (cyclosporine), XIIDRA™ (lifitegrast), TYRVAYA™ (varenicline), and topical corticosteroids such as LOTEMAX™ and PREDFORTE™. MIEBO™ (perfluorohexyloctane) is instead reducing tear evaporation. In addition, there are numerous artificial tears that help lubricate the ocular surface. Other treatment options include punctal plugs to reduce drainage of tears so that the tear lake is maintained in an effort to increase wetting of the ocular surface.

2.7 SUMMARY OF BENEFITS AND RISK

Dry eye disease (DED) is a multifactorial disease and rhNGF (new formulation – CC dose) may represent a great benefit for all patients affected, because of the unique mechanism of action based on the physiological role of NGF in the biology of cornea and lacrimal glands. The new formulation allows the administration of a CC dose of rhNGF as eye drops, that can be kept at CCI °C (CCI °F) with improved accessibility to the medication. The study is conducted in moderate to severe dry eye because four previous clinical studies suggest some benefit in this population. Therefore, some patients with dry eye may also receive a benefit from the application of rhNGF for 28 days during this study.

The study is considered a low-risk interventional trial. It is conducted with concentrations (and doses) of rhNGF CCI than those already tested in previous clinical studies in dry eye condition and already authorized in some European countries and in the USA for neurotrophic keratitis (NK) treatment.

Four previous clinical studies were already conducted in patients with dry eye demonstrating that rhNGF is well tolerated in this condition.

The risk of this study is foreseen as comparable to the risk of the standard of care, in fact the dose regimens proposed in this protocol are C mcg/mL and C mcg/mL three drops per day (both eyes), which is CCI the dose approved for the treatment of NK (rhNGF C mcg/mL six drops/eye per day).

No particular safety risks are foreseen with respect to the safety profile of the marketed product (OXERVATE® C mcg/mL) and CC adverse drug reactions (discomfort pain) are expected because of the CCI doses used in this study.

The following potential study risks were evaluated:

1) Participant well-being e.g.- risk-benefit balance - burden of study visits
2) Lifestyle requirements- Study specific procedures which carry risk -additional to standard care (es: 12±2 days of Run-In, discontinuation of previous ophthalmic treatments)
3) Complexity of study procedures
4) Education, training, experience, and resources of all investigator site staff in GCP and study procedures

5) Manufacture and distribution of the product(s), storage at the study site, reconstitution of the solution by the patient (CCI + diluent), sterile conditions.

Table 2: Summary of potential risks for the patients and mitigation strategy

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
This is the first study with a new formulation of the rhNGF eye drops	rhNGF eye drop formulation at C mcg/mL has been already tested in patients and it is currently marketed (over 16000 patients exposed so far). In this study a new formulation at C mcg/mL and at C mcg/mL will be tested to evaluate safety, efficacy, and tolerability.	In previous studies rhNGF was used at the concentration of C mcg/mL. No difference in safety and possibly a reduction of CCI and CCI is expected with the use of the new formulation, especially considering the CCI in rhNGF concentration. Patients with known allergy to any component of the new formulation will be excluded from participating in the study. AE information and VBR10 data will be collected throughout the study including the Run-In and follow-up period (Visit 2).
Adherence to treatment with vehicle/IMPs/NIMP in the Run-In period, treatment period and Follow-Up period, respectively	During the different phases of the study (Run-In, treatment, Follow-Up), the accountability and the adherence to the specific treatments that will be supplied by the Sponsor should be registered and evaluated	Adherence to the use of the treatments is to be monitored via Patient Diaries implemented for each study period to register the administration of the vehicle (Run-In period), the IMPs/vehicle (treatment period) and preservative free AT (NIMP in Follow-Up period). The Patient Diary will be evaluated to define the adherence to the treatments. A site visit (Visit 3) is programmed after 2 weeks of treatment to check the Patient Diary for medication dosing compliance. The Study Products and AT will be dispensed in controlled amounts at specific visits and used/unused study products will be collected during the successive visits. A check of the returned Study Product vehicle from the Run-In period and IMP or vehicle will be done at every visit starting from visit 2 by independent personnel not directly involved in study activities.
IMP Preparation and storage	New formulation of Study Products to be reconstituted by the Patients (kit containing CCI + diluent) Storage temperature	Instruction for reconstitution will be provided to patients. First vehicle administration during the Run-In period will be performed at the site with the support of clinical staff to show patient reconstitution and administration system. A

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		document with the instructions will be provided to the patients. Storage temperature risk has been CCI with the new formulation since it no longer needs to be kept at CCI °C or CCI The Study Products can be stored at C C C, kept out of direct sunlight and high heat.
Project-specific training	Education, training, experience, and resources of all investigator site staff in GCP and study procedures	All pharmacy and clinical staff involved in the clinical trial will be trained according to the study specific procedure.

In conclusion this study is considered to be a low-risk interventional trial. Furthermore, patients participating in this study that are randomized to the IMP 1 and IMP 2 groups may potentially benefit from the ophthalmic application of rhNGF for 28 days.

3 TRIAL OBJECTIVES AND ENDPOINTS

The aim of this study is to evaluate the safety, efficacy, and tolerability of a new formulation of rhNGF ophthalmic solution at two different concentrations of Cmcg/mL and C mcg/mL in comparison with vehicle in patients with dry eye disease (DED). This study aims to demonstrate the efficacy of a new formulation of rhNGF in improving both symptoms and signs in patients with DED. Therefore, if at least one of the two concentrations of the new formulation of rhNGF shows superiority over the vehicle group in improving symptoms through the primary endpoint, the secondary objective will be to assess if the same concentration is also effective in improving a clinical sign of DED, as evaluated through the secondary endpoints.

3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS

Table 3: Trial objectives and 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)]
Key Secondary Objectives	**Key Secondary Objectives Efficacy endpoints**
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle.	• 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.	• 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	• Mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator [Time Frame: at week 4 (V4)]
Secondary Objectives	**Secondary and Exploratory (*) Efficacy Endpoints**
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle	• 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	• Mean change from baseline in

Objectives	Endpoints
formulation of rhNGF ophthalmic solution in improving tear film stability as compared to vehicle	fluorescein tear break-up time (fTBUT)- in Study Eye [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in NIKBUT-first and NIKBUT-average in the Study Eye only at selected sites that have the required equipment (Oculus keratograph 5M) [Time Frame: at weeks 4 (V4) and 8 (V5)] (*)
• 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	• Mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator [Time Frame: at week 8 (V5) (*)] • Mean change from baseline in total conjunctival lissamine green staining (NEI scale) in Study Eye as assessed by the investigator [Time Frame: at weeks 4 (V4) and 8 (V5) (*)]
• 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	• 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	• 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	• Mean change from baseline of the CCI CCI CCI [Time Frame: at weeks 4 (V4) and 8 (V5)] • Mean change from baseline in response CCI [Time Frame: week 4 (V4)] (*)
• 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	• Mean change from baseline as assessed by the CCI [Time Frame: at weeks 4 (V4) and 8 (V5)]

Objectives	Endpoints
• To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving best corrected visual acuity in DED as compared to vehicle	• 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 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.
Additional Objective	**Additional Endpoint**
• Validate an Italian version of the CCI [redacted] CCI	• CCI [redacted] CCI [redacted] [Time Frame: Baseline, weeks 4 (V4)]

3.2 PRIMARY AND KEY SECONDARY ESTIMANDS

3.2.1 Primary Estimand

The estimand for the primary objective is defined as follows:

- Treatment: 4 weeks follow up after 4 weeks of TID administration of rhNGF new formulation (two concentrations 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: 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 § 10.6.2 for further details).
  - Use of a prohibited medication, as listed in § 6.6.1, 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 § 10.6.2 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 IMP (groups IMP1 and IMP2) and the vehicle group as derived by the Mixed Model for repeated measures defined in § 10.6.2.

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 subjects in the week before any study evaluation.

More details are provided in the statistical § 10.

3.2.2 Key Secondary Estimands

Key secondary objective #1

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

- Treatment: 4 weeks of TID administration of rhNGF new formulation (two concentrations 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).
- Intercurrent events (ICEs) and strategies to handle them:
  - 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 § 10.6.3 for further details).
    - Use of a prohibited medication, as listed in § 6.6.1, 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 § 10.6.3 for further details), then the proportion of subjects 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 § 10.6.3.

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 subjects 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 TID administration of rhNGF new formulation (two concentrations 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).
- Intercurrent events (ICEs) and strategies to handle them:
    - 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 § 10.6.3 for further details).
    - Use of a prohibited medication, as listed in § 6.6.1, 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 § 10.6.3 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 § 10.6.3.

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 subjects 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 TID administration of rhNGF new formulation (two concentrations and vehicle).
- Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set.
- Variable: mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as assessed by the investigator at week 4 (V4).
- Intercurrent events (ICEs) and strategies to handle them:
  - 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 § 10.6.3 for further details).
  - Use of a prohibited medication listed in § 6.6.1 in the week before any assessment of corneal fluorescein staining: 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 § 10.6.3 for further details).
- Population-level summary: difference in mean change from baseline to week 4 in total corneal fluorescein staining (NEI scale) 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 § 10.6.3.

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 corneal fluorescein staining (NEI scale) 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 subjects in the week before any study evaluation.

3.3 ASSESSMENT OF EFFICACY

- Specification of the efficacy parameters: please see § 1.1 and 3.1.
- Methods and timing for assessing, recording, and analyzing efficacy parameters: Please see § 1.2, 3.1, 8.1, 8.2 and 8.3.

The efficacy endpoints included in this clinical study were selected to optimize the characterization of the effects of rhNGF in moderate to severe DED in order to obtain robust evidence of the clinical efficacy of the treatment and its relevance for the patients. For this reason, subjective and objective measures are collected after 4 weeks of treatment and at the end of the Follow-Up period (week 8, V5). This study is looking at the effects of rhNGF on multiple markers of DED because it is expected to see efficacy in measures of tear production, in measures of CCI and CCI general DED symptoms and visual acuity. The prediction of efficacy, the study design and the associated SAP are based on previous clinical data obtained by the Sponsor that have enabled the preparation of this trial and the proposed assessments. In particular, the primary endpoint definition at week 8 (V5) (after 4 weeks of treatment and 4 weeks Follow-Up period) is based on clinical observations made during the studies NGF0118 and NGF0121 that showed an effect on the symptoms after the end of the treatment period. A combination of secondary and exploratory efficacy endpoints is making

sure the effect of the treatment is well characterized at week 4 (V4; 28±1 days from Baseline visit) and at week 8 (V5; 56±2 days from the Baseline visit - end of 4 weeks of Follow-Up).

4 TRIAL DESIGN

4.1 DESCRIPTION OF TRIAL DESIGN

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) eye drop 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 rhNGF as compared to a vehicle in patients with dry eye disease (DED). This is a prospective Phase II trial for a new formulation of two concentrations (C mcg/mL and C mcg/mL) of rhNGF eye drops. The new formulation is specifically prepared to allow the administration of CCI concentrations of rhNGF eye drop solution to CCI rhNGF induced CCI experience in patients with DED. The new formulation can be stored at CCI °C further improving the conditions of storage and manipulation of the drug.

- The study is carried out in multiple centers (USA and Italy).
- Male or female 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 by Interactive Response Technology (IRT); both the investigator and the patient will be masked to the treatment arm assigned. Patients will be randomized 1:1:1 to either of the two concentrations of rhNGF formulation (C mcg/mL or C mcg/mL) or vehicle. The total number of patients to be enrolled in the study will be about 291, 97 per group to target 276 evaluable patients (92 per group). The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrolment. Considering a screen failure rate of 20%, approximately 350 subjects need to be screened in order to have 291 patients enrolled and at least 276 patients evaluable.
- The study is divided in 4 phases: Screening & Start of Run-In period (day -12±2, Visit 1); Run-in Period (day -12±2/Screening Visit 1 to day 1/Baseline Visit 2); Treatment Period (day 1/Baseline Visit 2 to 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 inclusion will be performed at both Visit1 (Screening) and Visit 2 (Baseline).
- At Screening visit (Visit 1, day -12±2), eligible patients will be instructed to discontinue all topical ophthalmic medications and during the Run-In period they will only be allowed to use the Study Product (vehicle) ophthalmic solution provided by the Sponsor. This solution needs to be prepared daily and administered by the patient in both eyes as eye drops solution TID (at approximately 6-hours intervals).
- At Visit 2 (baseline visit) a Study Eye will be determined. Namely, the Study Eye is the worst eye: assuming that all the inclusion/exclusion criteria are met in both eyes, the worse 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 worse NEI staining score for cornea and conjunctiva (first NEI score for cornea then for conjunctiva). If both eyes are identical,

then simply the right eye will be considered the Study Eye.

- The treatment period will start at Baseline visit (Visit 2, day 1) and the IMP will be administered as an eye drops solution to be prepared daily and administered by the patient TID at approximately 6-h intervals in both eyes for a treatment period of 4 weeks. During the 4 weeks of masked treatment only the administration of IMP is allowed. The use (number of drops/day) of IMP will be clearly documented in a Patient Diary and in the eCRF. Different administration frequencies will be recorded and considered as protocol deviation. The treatment period will be completed at Visit 4 (day 28 ±1) and it will be followed by a 4 weeks Follow-Up period.
- During this Follow-Up period patients will be requested to administer preservative free artificial tears (AT) (NIMP) provided by the Sponsor TID at 6-h interval in both eyes for a 4-week period until Visit 5 (day 56 ±2, week 8). During the 4 weeks of Follow-Up period only the administration of AT provided by the Sponsor is allowed. The use of AT will be clearly documented in a Patient Diary and in the eCRF. Different administration frequencies will be recorded and considered as protocol deviation. Any topical ophthalmic concomitant treatment initiated during the Study period (between Visit 1 and Visit 5) must be documented on the concomitant medication log and represents a protocol deviation. The End of Study Trial visit (EOS) is expected at Week 8. The maximum total study duration will be about 10 weeks.
- Patients will be evaluated at Screening visit (day -12±2, V1) after which they will enter a Run-In period with the Study Product vehicle (from day -12±2 to day 1). Evaluations will continue at Baseline visit (day 1, V2), week 2 (day 13±1, V3), week 4 (day 28±1, EOT (V4)), and at week 8 (day 56±2 follow-up and EOS (V5)). At the investigator's discretion, any patient could be seen for an Unscheduled Visit for safety evaluation. An Early Exit Visit could be done in case of premature study discontinuation. In case of treatment discontinuation, patients will be asked to complete the assessments at the planned visits as per Protocol. In case of premature discontinuation of the study, the subject will be required to participate at the Early Exit Visit that will have the same assessments of Visit 4 (in case of early treatment and study discontinuation before Visit 4) or the same assessments 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.
- The trial design follows the principles of Quality by Design (QbD) with respect to eligibility criteria, randomization, masking, data quantity, endpoints, data integrity, IMP handling and administration, site feasibility, safety reporting, data monitoring and delegation of responsibilities. Detailed evaluations and timing are presented in § 1.2, 1.3 and 8.

4.2 RATIONALE FOR TRIAL DESIGN

The study has been designed as a dose-finding, superiority clinical trial with a Run-In period using vehicle since this is a new formulation of rhNGF. During the Run-In period of 12±2 days, patients will only use the new vehicle formulation (three times a day) in both eyes to provide the minimum amount of lubrication needed in DED patients while ensuring appropriate wash-out from any other previous topical treatment effect. This trial tests two CC concentrations of a new CCI rhNGF formulation (C mcg/mL or C mcg/mL) in patients with DED. This new formulation allows the

administration of [redacted] doses of rhNGF in eye drops. This new formulation simplifies the conditions of drug manipulation and drug storage. The storage of the new formulation of ophthalmic solution containing the active principle rhNGF is now at [redacted] C. This is reducing patient burden with respect to storage and handling of IMP in this study and provides an important advantage for the accessibility to the drug.

The rationale for testing [redacted] concentrations of rhNGF stems from previous clinical trials where a [redacted] concentration of rhNGF was used ([redacted] mcg/mL) and up to 48% of patients reported eye pain and 21% eyelid pain (NGF0121 - PROTEGO-1). Therefore, a new formulation of rhNGF was prepared that allowed [redacted] concentration with the goal of consequently [redacted] TEAEs for patients. Hence, this trial aims to determine the safety and efficacy of 2 [redacted] concentrations of rhNGF in dry eye patients.

Furthermore, a shorter duration of treatment was selected (4 weeks) with TID dosing instead of an 8-week long treatment with dosing six times a day (NGF0216).

The trial design is based on clinical observations made during previous clinical studies. Namely NGF0118, a 4-week, Phase II, multicenter, randomized, double-masked, vehicle-controlled, parallel group study with 12 weeks of follow-up to evaluate safety and efficacy of recombinant human Nerve Growth Factor (rhNGF) eye drops solution versus vehicle in patients with moderate to severe dry eye (DED) that was completed in 2021. In this study there were significant improvements in the rhNGF TID group when compared to vehicle on Schirmer test II and on assessments of TFBUT. In addition, rhNGF 4 weeks treatment had clinically relevant delayed beneficial effects compared with vehicle on SANDE scores (and other scores) which showed significant improvement during the follow-up period but not during the treatment period. This clinical observation is in line with the proposed mechanism of action for rhNGF on DED and DED symptoms and requires a specific demonstration during this clinical study with a primary endpoint on SANDE Global score after 4 weeks of Follow-Up period. SANDE score repeated measures will be collected 4 weeks apart: at Baseline, after 4 weeks of treatment and after 4 weeks of Follow-Up.

NGF0118 also demonstrated that TID dosing had better durability of sustained improvement in symptoms and signs in DED as compared to BID dosing prompting the definition of a TID regimen of administration at approximately 6h-intervals during this study.

In addition to testing the two concentrations of rhNGF ([redacted]mcg/mL and [redacted] mcg/mL), this study incorporates a vehicle control group. The vehicle control group has been incorporated in this study in order to demonstrate efficacy of our new formulation in improving symptoms and signs in patients with dry eye, a disease where it is known that a relevant placebo effect may be expected and the presence of an inactive control group favors the correct interpretation of any treatment effect.

5 TRIAL POPULATION

Male and female patients with diagnosis of dry eye who fulfill the inclusion/exclusion criteria will be enrolled in the study.

5.1 INCLUSION CRITERIA

1) Male or female aged ≥18 years of any race/ethnicity and eye color.
2) A diagnosis of dry eye disease at least 6 months before enrollment (current use or recommended use of artificial tears for the treatment of dry eye).
3) Moderate-to-severe dry eye characterized by the following clinical features:

a) Symptoms Assessment in Dry Eye (SANDE) questionnaire global score ≥50, and
b) Schirmer-I test without anesthesia >2 mm and <10 mm/5 minutes, and
c) Total corneal fluorescein staining grade ≥3 (NEI scale) and/or total conjunctival lissamine green staining score ≥3 assessed by the National Eye Institute (NEI) grading system, and
d) Fluorescein Tear Film Break-Up Time (fTBUT) < 10 seconds. The same eye must have fulfilled all the above criteria.

4) Best corrected distance visual acuity (BCDVA) score on ETDRS chart of ≥0.1 decimal units (≤1.0 logMAR) in each eye at the time of study enrollment
5) Negative pregnancy test in females of childbearing potential.
6) Only patients who satisfy all informed consent requirements will be included in the study; the patient and/or his/her legal representative must have read, signed, and dated the informed consent document before any study-related procedures are performed; the informed consent form signed by patients and/or legal representatives must have been approved by the IRB for the current study.
7) Have the ability and willingness to comply with study procedures.

5.2 EXCLUSION CRITERIA

1) Inability to speak and understand the local language sufficiently to understand the nature of the study, to provide written informed consent, and to allow the completion of all study assessments.
2) Evidence of an active ocular infection in either eye.
3) Presence of any other ocular disorder or condition requiring topical medication during the entire duration of the study.
4) Possibility of the need for ocular surgery at the time of inclusion in the study or anticipated ocular surgery expected during the participation in the study.
5) History of severe systemic allergy or severe ocular allergy (including seasonal conjunctivitis, AKC, VKC) or chronic conjunctivitis and/or keratitis other than dry eye.
6) Ocular scarring due to irradiation, alkali burns, Stevens-Johnson syndrome and ocular cicatricial pemphigoid.
7) Destruction of conjunctival goblet cells such as in Vitamin A deficiency.
8) Severe blepharitis or obvious inflammation of the lid margin.
9) Intraocular inflammation defined as Tyndall score >0.
10) Medical history of tumor malignancy in the previous 3 years
11) Systemic disease not stabilized within 1 month before the Screening visit (e.g., diabetes with glycemia out of range, thyroid malfunction) or judged by the investigator to be incompatible with the study (e.g., current systemic infections) or with a condition incompatible with the frequent assessment required by the study.
12) History of a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or had a clinically significant allergy to drugs, foods, CCI eyedrop or other local anesthetics or other materials, including ocular vital dyes, tropicamide eye drops, commercial artificial tears.
13) Known or suspected allergy to CCI and/or any other component of the new rhNGF formulation.
14) Fertile patients (i.e., not surgically sterilized, or postmenopausal women for at least 1 year) are excluded from participation in the study if they do not practice abstinence from heterosexual intercourse as per usual and customary lifestyle, or are unwilling to use an acceptable form of contraception such as condom with spermicidal cream or jelly for males, or for females if they meet any one of the following conditions:
    a) Currently pregnant (positive urine pregnancy test at screening or baseline visits) or planning to become pregnant during the duration of the treatment phase of the clinical

trial.
b) Patient is breastfeeding.
c) Unwilling to use birth control measures such as mechanical barrier methods (spermicide in conjunction with a barrier such as a condom or diaphragm or intrauterine device) during the entire course of and 30 days after the study treatment periods, or,
d) Unwilling to continue to use highly effective birth control measures such as hormonal contraceptives (oral, implanted, transdermal, or injected) during the entire course of and 30 days after the study treatment periods.

15) Any concurrent medical condition that, in the judgment of the principal investigator, might interfere with the conduct of the study, confound the interpretation of the study results, or endanger the patient's well-being.
16) Contact lenses or punctum plug use in either eye during the washout, treatment and follow-up phases of the study (previous use is not an exclusion criteria but must be removed and discontinued at the Screening visit)
17) Medical history of drug addiction or alcohol abuse (>1 drink /day for women and >2 drinks /day for men following USDA dietary Guidelines 2020-2025).
18) Any prior ocular surgery including but not limited to amniotic membrane transplant, refractive (PTK/LASIK/Epi-LASIK/LASEK/SMILE), palpebral, cataract surgery, trabeculectomy, vitrectomy and pan-retinal photocoagulation (PRP) within 90 days before the Screening visit.
19) Participation in a clinical trial with a new active substance, including medical devices, during the previous 60 days.
20) Participation in another clinical trial study at the same time as the present study.

5.3 SELECTION OF STUDY EYE

At Visit 2 (Baseline visit) a Study Eye will be determined. Namely, the Study Eye is the worst eye: assuming that all the inclusion/exclusion criteria are met in both eyes, the worse 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 worse NEI staining score for cornea and conjunctiva (first NEI score for cornea then NEI score for conjunctiva). If both eyes are identical, then simply the right eye will be considered the Study Eye.

5.4 SCREEN FAILURES

A patient will be defined as screened after the signature of the informed consent and the assignment of a Screening Number. The preparation of an eCRF will be done regardless of the completion of all the screening procedures. Patients that will go through screening but will not be enrolled will be considered a screen failure. Patients may be re-screened on a case-by-case basis after reviewing it with the Sponsor.
In this study a screen failure rate of about 20% was considered on the basis of the previous clinical experience obtained with the NGF0121 - PROTEGO-1 study, which also enrolled highly symptomatic dry eye patients. We expect to screen approximately 350 subjects in order to have 291 subjects enrolled.

The sample size remains at 291 subjects independent of screen failures.

5.5 LIFESTYLE CONSIDERATIONS

5.5.1 Meals and Dietary Restriction

No new drastic changes to diet adding fish oil supplements, flaxseed supplements, omega-3 supplements since they could potentially alter fTBUT.

5.5.2 Caffeine, Alcohol, Tobacco, and Other Habits

Patients should limit smoking tobacco since tobacco smoke is a known eye irritant, it may worsen dry eye disease and confound results. Patients should also avoid exposure to second-hand smoke for prolonged periods.

5.5.3 Physical Activity

No particular restrictions are required.

5.5.4 Other Activities

Avoid heavy use of eye make-up along lash lines like eyeliner and mascara since those could artificially alter fTBUT.

6 TRIAL INTERVENTION AND CONCOMITANT THERAPY

6.1 DESCRIPTION OF TRIAL INTERVENTION, DOSING AND ADMINISTRATION

Treatments are described in the table below.

Table 4: 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)	0 mcg/mL Vehicle	C mcg / eye/day 1 drop of C mcg/mL /eye TID	C mcg/ eye/day 1 drop of C mcg/mL/eye TID
Pharmaceutical form (see §2.2)	CCI vehicle + diluent	CCI rhNGF + diluent	
Formulation	ophthalmic solution (reconstituted eye drops)		
Regimen	1 drop (40mcL) both eyes TID (Every 6 h)	1 drop (40mcL) both eyes TID (Every 6 h)	1 drop (40mcL) both eyes TID (Every 6 h)
Route of Administration	Ocular	Ocular	Ocular
Stability & storage of the eye drop solution	CCI °C Keep it protected from sunlight and heat	CCI °C Keep it protected from sunlight and heat	CCI °C Keep it protected from sunlight and heat
Stability & storage of the pharmaceutical form	CCI °C Keep it protected from sunlight and heat	CCI °C Keep it protected from sunlight and heat	CCI °C Keep it protected from sunlight and heat

6.1.1 Non-Investigational Medicinal Product

The Non-Investigational Medicinal Product (NIMP) that should be used in the follow-up period is shown in the table below.

Table 5: Description of NIMP

Treatment Name during Follow-up Period	NIMP (Preservative free artificial tears)
Dosage (daily)	3 drops / eye
Regimen	One drop in each eye, TID (Every 6 h)
Formulation	Preservative free, sterile solution, single use/multi-use
Route of administration	Topical ocular (eye drops)

6.1.2 Trial Intervention Dose Modification

No dose modification is anticipated at this time.

6.2 RATIONALE FOR TRIAL INTERVENTION

The trial tests two CCI concentrations of a new CCI rhNGF formulation (C mcg/mL or C mcg/mL) in patients with DED. This new formulation not only allows to CCI the concentration of rhNGF down to C mcg/mL, but also allows the storage at CCI °C of the ophthalmic solution containing the active principle, hence reducing patient burden with respect to storage and handling of IMP. The rationale for the selection of the two proposed CCI concentrations of rhNGF stems from both manufacturing and clinical rationales. Specifically, from a manufacturing standpoint, the development of the new CCI formulation opens the possibility to test a rhNGF concentration as CC as C mcg/mL, which will provide the opportunity to test the minimally effective dose in the target population. From a clinical standpoint, both the C and C mcg/mL proposed concentrations can reasonably be expected to be safe and effective in the target population based on previous clinical trial experience with different rhNGF concentrations. Specifically, starting from the first phase 2 clinical trial performed in Europe (REPARO, NGF0212) in patients with Neurotrophic Keratitis, both the C and C mcg/mL were proven safe and effective on corneal healing. The following clinical trials in DED also tested concentrations of rhNGF up to C mcg/mL. In the DED studies we observed efficacy on tear function but a suboptimal tolerability of the higher concentrations tested (for example, 48% patients reported eye pain in NGF0121 - PROTEGO-1, up to 63.5% in NGF0118, and 42% in NGF0216). Therefore, the new formulation of rhNGF was prepared to allow a CCI concentration with the goal of consequently CCI

CCI TEAEs for patients, while maintaining efficacy on DED signs. The C mcg/mL dose group has the potential to show clinical benefit since an earlier open-label study (NGF0213) in dry eye patients demonstrated that with doses as CC as C mcg/mL (obtained by on-site dilution of the C mcg/mL formulation) treatment (BID, 4 weeks), patients showed improvement in both SANDE and Schirmer scores up to 4 weeks post-completion of treatment (day 56). Hence, this trial aims to determine the safety and efficacy of 2 CCI concentrations of rhNGF in dry eye patients.

TID and not BID dosing was selected based on evidence that TID dosing demonstrated sustained improvement in symptoms (SANDE scores) for up to 12 weeks after completion of treatment (week 16), unlike BID dosing, which was unable to maintain improved symptoms beyond the week 4 end-of-treatment time point (NGF0118).

Furthermore, a shorter duration of treatment was selected (4 weeks) with TID dosing based on recent studies with 4-week long treatment using TID dosing (NGF0118, NGF0121 - PROTEGO-1) that demonstrated efficacy in patients with moderate to severe dry eye. This is a reduction in both duration and dosing in comparison to an earlier study where dry eye patients were treated for 8 weeks with six times a day dosing (NGF0216) at an rhNGF concentration of C mcg/mL.

Topical ophthalmic route through eye drops was selected since PK studies demonstrated that there is minimal systemic absorption of rhNGF through this route of drug delivery.

6.3 PREPARATION, HANDLING, STORAGE AND ACCOUNTABILITY

The Study Products consists of:

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

The Study Products are reconstituted using an adapter and a sterile syringe and administered using a sterile pipette.

After reconstitution the Study Product is a sterile solution for ocular administration at the following concentrations as active ingredient:

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

Store the products before and after reconstitution at CCI °C protected from sunlight and heat. Once the CCI product is reconstituted in diluent, the solution should not be shaken since agitation may cause foaming. The eye drop solution should be prepared daily and used only for 1 day, kept at CCI °C sheltered from sunlight and heat.

The clinical labeling of the investigational medical product (IMP and vehicle) will be done in a manner that protects the masking of the study medications. It will include a statement of

caution and describe particulars applicable to regulatory requirements (e.g.: kit number, protocol number, storage conditions, name and address of the sponsor, statement that the product is for investigational use only, etc.). A clinical labeling with similar statements will be also provided for the Study Product vehicle to be used during the Run-In Period.

The Pharmacist and/or Investigator will be responsible for receipt, proper storage, dispensation reconciliation, usage, and final disposal of remaining Study Products (where applicable). The Study Products must be stored at CCI °C sheltered from sunlight and heat at the investigational sites, in an appropriate locked room accessible only to the pharmacist, the Investigator, or a duly designated person. A data logger will accompany the Study Products on shipment. It is essential that the investigational sites verify the temperature excursion during shipment vs. the acceptable storage conditions (CCI °C), in order to identify potential stability concerns during shipment. These must be immediately communicated to the Sponsor that will decide upon appropriate actions to be taken. Any deviation from the recommended preparation, storage and handling conditions of the Study Products should be immediately reported by the pharmacist to the Sponsor and to the Investigator, and the use of the Study Product should be suspended until the Sponsor has given authorization for its continued use. The Study Product supplies are to be used only in accordance with this protocol. The Investigator will not use any Study Product samples for other purposes (e.g., treating patients or deviating from the protocol with regard to dose regimen, duration of treatment, etc.). Under no circumstances will the Investigator give any Study Product samples to a third party.

Upon receipt of the Study Products (including Vehicle) and NIMP at the site, the investigator will log the receipt of the Study Products (including Vehicle) and NIMP and store as per the storage instructions provided with the Study Products (including Vehicle) and NIMP kits. Study Products (including Vehicle) and NIMP will be dispensed to the patients by qualified and delegated site staff according to the study requirements and as listed in the Schedule of Activities (Table 1). The site personnel will maintain an accountability log for Study Products (including Vehicle), documenting the receipt, dispensation, return, and destruction of all Study Products (including Vehicle). The site personnel will also maintain an accountability log for NIMP, documenting the receipt and dispensation of NIMP.

To establish and maintain accountability of Study Products (including Vehicle) and NIMP, the Pharmacist and/or Investigator will confirm the receipt of the Study Products (including Vehicle) and NIMP supply in writing by signing and dating standard drug accountability forms. The Pharmacist and/or Investigator will keep a cumulative inventory and dispensing records and will maintain all supplies under adequate security. An accurate drug disposition record will be kept, specifying the date and amount dispensed to each patient.

Adequate record of receipt and use or loss of drug will be retained. This inventory record kit must be available for inspection by the Sponsor and regulatory inspection at any time. Copies of this record will be provided to the Sponsor by the CRO throughout the duration of the study.

During the course of the study and until its conclusion, the Investigator will complete the drug accountability forms. Partially used or unused study drug boxes will be verified by the CRO

delegated Monitor. The partially used and unused study medication will be shipped to the Sponsor or will be destroyed after authorization by the Sponsor or by an authorized company according to GCP regulations.

Study Products' preparation and administration instructions will be provided separately to the site and to the patients.

Study products will be provided to the patients as a biweekly kit containing 14 daily boxes. Each daily box will contain one vial of CCI Study Product and one vial of diluent solution. In the bi-weekly kit will be also included an appropriate number of vial adapter, sterile wipes and empty luer lock syringes to ensure product reconstitution before use and disinfection. At time of dispensing, patients will be also provided with a separate biweekly ancillary kit containing sterile wipes and sterile pipettes for drug product administration. Patients will be provided with detailed instruction reporting the kit composition and procedures for product reconstitution and administration.

At visit 1 (Screening visit) eligible patients will be instructed by study personnel how to prepare and administer the Study Product (vehicle) eye drop solution and the first eye drop of the Study Product (vehicle) will be applied in both eyes at the site by the Investigator. The Investigator will document the first administration of the Study Product (vehicle) in the patient's medical record following Visit 1 to confirm the first administration of the Study Product (vehicle). Depending on the time of the subject's Screening visit, all doses may not be administered on this day. At visit 1, the study personnel will give the patient the 2-weekly boxed supply of the Study Product (vehicle) (CCI vehicle with diluent); patients will also receive a separate ancillary administration kit containing sterile pipettes and sterile disinfectant wipes for product reconstitution. Patients will be instructed to then self-administer at home the Study Product (vehicle) one drop 3 times a day in both eyes, approximately every 6 hours. The patient should bring the 2-weekly supply box home as soon as possible and store it at CCI °C, keeping it away from direct sunlight/heat.

During the Run-In period, patients will instill one drop of the solution (vehicle) in both eyes TID (approximately every 6 hours), starting in the morning when they wake up, for a total duration of 12±2 days. The contents of each vial are for daily administration to both eyes only for a given day. Agitation of the reconstituted product may cause foaming, hence should be avoided at any time. After the last administration for the day, the used vial should be returned to the original box and is not to be reused. The used/unused Study Product vehicle vials should be returned to the site at the end of the Run-In period (V2). The patient will bring them to the site during the Visit 2 and they will be checked by independent personnel not directly involved in study activities. The vehicle is not to be instilled on the day of the Baseline visit (Visit 2).

At Visit 2 (baseline visit- Day 1), the study personnel will give to the randomized patient the biweekly boxed supply of the Study Product (IMP or vehicle, CCI Study Product with diluent). The patient should bring the IMP, a biweekly supply box, home as soon as possible and store it at CCI °C, keeping it away from direct sunlight/heat. At Visit 2 (Baseline) patients will also receive a separate ancillary kit containing sterile pipettes and sterile disinfectant wipes. Further spare ancillary kit components could be provided to ensure product administration. IMP ophthalmic solution preparation and instillation instructions will

be provided to the patients.

Patients will instill one drop in both eyes three times a day (TID) (approximately every 6 hours), starting on Day 1 (Baseline visit) and continue the treatment for the next 2 weeks. Depending on the time of the subject's Baseline visit, it is reasonable and possible that all doses may not be administered on this day. The contents of each vial are for daily administration to both eyes only for a given day. After the last administration for the day, the used vial should be returned to the original box and is not to be reused.

After 2 weeks during Visit 3 (Study Product pick-up visit - Day 13±1) patients will return the used/unused study product vials to the site to be checked by independent personnel not directly involved in study activities. During the same Visit 3 patients will collect a second IMP 2-weekly kit and a separate ancillary kit.

At visit 4 the used/unused study product vials will be returned to the site by the patients to be checked by independent personnel not directly involved in study activities.

In summary, patients will return the used/unused study boxes/vials to the site personnel in this order: Study Product (vehicle) from the Run-In period at the baseline visit (Visit 2), the 2-weeks study product (IMP or vehicle) at mid-treatment visit (Visit 3), the second 2-weeks study product (IMP or vehicle) treatment at end-of-treatment visit (Visit 4).

At the scheduled visits (Baseline, weeks 2, week 4 and week 8), the Patient Diary will be reviewed by the Investigator with the patient for completeness and adherence to treatment. Missing information should not be provided during the Patient Diary check but reported as missing.

6.4 PARTICIPANT ASSIGNMENT, RANDOMISATION AND MASKING

6.4.1 Participant Assignment

Each patient who provides written consent to participate in this study will be assigned a unique 5-digit PID number (e.g., 01-001) consisting of a 2-digit study center number followed by the 3-digit screening number assigned sequentially by each study center, from 001 to 100. eCRF should be completed for each patient that signed the informed consent, including the screening failures.

6.4.2 Randomization

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

Eligible patients will be randomized in a 1:1:1 ratio to rhNGF C mcg/mL TID (about 97 patients), rhNGF C mcg/mL TID (about 97 patients) or vehicle ophthalmic solution TID (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. Drop-outs 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.

Each Patient kit number will be randomly associated with a treatment group. The randomization list will be provided to the facility responsible for IMP packaging/labeling for the purpose of IMP preparation.

The enrollment of patients will be scheduled in order to assure an inclusion of approximately 291 patients to ensure at least 276 evaluable patients assuming up to 5% dropout rate.

6.4.3 Masking and Unmasking Procedure

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 subject. 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. The Investigator must always notify the Sponsor, so that the reason for any premature unmasking can be documented, by means of a communication to CRO/Dompé Global Pharmacovigilance, Safety and Surveillance to the contact details in the section "CONTACT INFORMATION" and to Dompé Medical Expert. The Investigator will inform the Dompé representative (Dompé Medical Expert) if an emergency unmasking was performed without revealing the treatment identity, in order to avoid a dissemination of unmasked information.

Dompé shall be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities in the countries concerned, as per applicable requirements.

Additionally, Dompé Global Pharmacovigilance, Safety and Surveillance shall unmask the patient's treatment if a reported SAE meets criteria of a Suspected Unexpected Serious Adverse Reaction (SUSAR) in order to fulfill expedited regulatory reporting requirements. Unmasked information shall not be disclosed to Investigators or to Dompé and CRO Clinical Research staff. Specifically, the identity of the IMP treatments will remain unknown to the patient, Investigator, site staff, Dompé's clinical research personnel and CRO staff (apart from pharmacovigilance).

Unmasking events will be recorded and reported in the final study report.

6.5 TRIAL INTERVENTION COMPLIANCE

All patients enrolled in the trial will receive a Patient Diary at the screening visit (day -12±2), at the baseline visit (day 1), and at the end-of-treatment visit (week 4). The patients will be required to fill these diaries logging their use of the supplied drug (Study Product vehicle/IMP/NIMP). These Patient Diaries will be reviewed by the Investigator with the patient for completeness at the scheduled visits (baseline, weeks 2, 4 and 8). The data from the Patient's Diary will be entered in the eCRF as well.

Starting from the first IMP administration done at the baseline visit until the last expected administration (considering 1 drop TID, i.e. 3 drops per day), compliance for the study eye will be derived as the proportion (%) between the actual number of drops administered in the study eye and the expected number of drops in the study eye as if the subjects completed the study (up to 84 drops depending on the date of treatment completion status collected in the eCRF; for patients who prematurely discontinue the treatment, the expected number is set to 84). Further details will be inserted in the SAP.

Assessment of the IMP accountability will be made by determining the number of Study Medication vials (CCI product) dispensed to the patient at Baseline/V2 and at Week 2/V3 and the number of unused Study Medication vials returned at Week 4/V4 (EOT). The number of used and unused vials of diluent will also be collected.

6.6 CONCOMITANT THERAPY

As a general rule, the only ophthalmic medications given to the patients are Study Products or AT, from the Screening Visit, Day 1 and until all the final study evaluations have been completed at Follow-Up Visit, Day 56 ±2, including the vehicle during the Run-In phase and the preservative free artificial tears (AT) provided by the Sponsor during the Follow-Up period.

The use (number of drops/day) of Study Products (Including vehicle) and AT (NIMP) will be clearly documented in the Patient Diary and eCRF.

6.6.1 Prohibited Concomitant Therapy

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

6.6.2 Permitted Concomitant Therapy

During the Run-In period, no ocular topical treatment is allowed, except for Study Product vehicle ophthalmic solution, 3 times daily, provided by the Sponsor.

During the treatment period, only the study products (IMP or vehicle) will be allowed for topical ocular treatment.

During Follow-Up period, no topical treatment will be allowed except for commercially available preservative free AT, TID, provided by the Sponsor. Different administration frequencies of study products (including vehicle) and NIMP will be recorded and considered as protocol deviation.

During the entire duration of the study, any use of AT not provided by the sponsor will be recorded as concomitant medication and considered as protocol deviation.

Patients are encouraged to continue to take any systemic medications that they take as part of chronic disease management and not change their medical management as prescribed by their treating physician.

Patients are allowed to take over-the-counter painkillers, herbal products, vitamins and antacids. All 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.

Medication entries should be specific to drug product name and brand name (especially if containing a combination of active principles) and spelled correctly. The dose, unit, frequency, route of administration, start date, discontinuation date, and indication should also be recorded. For medications administered only one time, the frequency column may reflect "once."

6.6.3 Rescue Therapy

If a patient’s ocular health worsens to an extent that is considered beyond reasonable risk to their vision or maintenance of an intact ocular surface, they will be asked to discontinue the treatment but to remain in the study for the assessment planned by the protocol until the expected end of study. An appropriate treatment could be given by their physician. There will be no predefined rescue therapy. It should be checked if the patient still meets all the criteria expected to remain in the study. All details should be recorded in the eCRF.

6.6.4 Other Therapy

No other therapy outside of what is outlined in permitted concomitant therapy is allowed during the course of this study.

7 DISCONTINUATION OF TRIAL INTERVENTION AND PARTICIPANT WITHDRAWAL FROM TRIAL

7.1 CRITERIA FOR DISCONTINUATION OF TRIAL INTERVENTION

7.1.1 Criteria for Permanent Discontinuation of Trial Intervention

Treatment discontinuation

Patients with tolerability issues, or worsening of symptoms and/or signs may be discontinued at any time during the study for the following reasons:

1) AEs that at Investigator discretion require treatment discontinuation.
2) Worsening of the clinical condition that requires treatment discontinuation per physician decision or ocular surgery.
3) Violation of study procedures that represent a protocol deviation deemed by the Investigator to require treatment discontinuation.
4) Withdrawal of patient's consent for any reason.
5) Patient is lost-to-follow-up notwithstanding all efforts to contact him/her (at least three documented attempts by phone and/or email).
6) Other (e.g., death, pregnancy, planned operation).

Except for items from #4 to #6 of the list reported above that imply also a study discontinuation, in case one of the events from #1 to #3 occurs, patients will be asked to complete the assessments planned by the protocol regardless the discontinuation of the study treatment and will receive preservative free AT, TID, provided by the Sponsor. In case of more than one reason, the main one will be reported in eCRF.

Study discontinuation

A premature discontinuation will occur when a patient who signed the ICF ceases participation in the study, regardless of circumstances, before the completion of the study protocol procedures.

Patients should be discontinued from the study for one of the following reasons:

1) Withdrawal of patient's consent for any reason.
2) AEs, protocol deviations and any other condition for which study procedures may compromise the safety and well-being of the patient, as per physician decision.
3) Patient is lost-to-follow-up notwithstanding all efforts to contact him/her (at least three documented attempts by phone and/or email).
4) Other (e.g., death, pregnancy, planned operation).

In all other situations, including cases where patients should be discontinued from study treatment, all efforts will be made to complete study visits and procedures as per protocol.

Before removal, each case should first be discussed with Dompé.

The reasons for premature discontinuation from the study will be reflected on the Study Termination Record of the eCRF. Unless the patient has withdrawn consent, patients who discontinue the treatment will not be withdrawn from the study by default, but it will be asked

to complete safety and efficacy assessments as per protocol.

The investigator will advise patients who prematurely discontinue any therapies or treatments for their condition and refer them for further treatment as appropriate.

7.1.2 Temporary Discontinuation or Interruption of Trial Intervention

Any patient that chooses to discontinue the trial will not be allowed to re-enter or be replaced. It will be documented whether or not each patient completed the clinical study. If, for a patient, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

For any patient discontinuing the study following randomization, the Investigator will:

- Ask the patient to undergo, as far as possible, the following assessment as planned in this protocol. In case the patient withdraws from the study, the patient will be asked to undergo, as far as possible, a final medical visit (Early Exit Visit) to examine the patient's health conditions. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e., not clinically significant changes compared to screening).
- Arrange for alternative medical care of the withdrawn patient, if necessary.
- Report in the eCRF date and time of the last dose administration, and date and primary reason for study discontinuation.
- Record in the eCRF any follow-up if the patient is withdrawn for an AE. AE's should be followed until resolution.

7.1.3 Rechallenge

Rechallenge is not permitted in this trial.

7.2 PARTICIPANT WITHDRAWAL FROM THE TRIAL

There are no criteria for temporary withdrawal from the trial. Any discontinuation or withdrawal will result in a permanent discontinuation. The criteria for permanent discontinuation from the trial are listed in § 7.1.1.

7.3 LOST TO FOLLOW-UP

Every effort will be made by the Clinical Trial Manager, CRO Project Team and Investigator or their designated staff, to reach patients who are lost to follow-up. Lost to follow-up is defined as patients who miss one or more study visits and are not reachable despite every reasonable attempt made at contacting them (at least three documented attempts by phone and/or email).

7.4 TRIAL STOPPING RULES

The Sponsor has the right to discontinue the study at any time for reasonable medical and/or administrative reasons. Reasons for discontinuation have to be documented appropriately. In

this event, no further patients will receive doses of the IMPs, and patients already having received a dose of IMP will not receive any further doses of the IMP but will undergo all safety assessments scheduled after the last dose of IMP, up to and including the end of study examination.

8 TRIAL ASSESSMENTS AND PROCEDURES

8.1 SCREENING/BASELINE

Site staff will conduct the following assessments in the order outlined below during each visit.

Visit 1 - Day -12±2: SCREENING

1. Informed Consent/HIPAA (only for USA).
2. Demographics, Ocular and Medical History, Previous and Concomitant Ocular and Systemic Medications and Conditions.
3. Pregnancy Test.
4. SANDE questionnaire.
5. Ophthalmic Examinations for each eye:
   - **A.** Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS.
   - **B.** External Ocular Examination.
   - **C.** Schirmer-I test without anesthesia.
   - **D.** Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes).
   - **E.** Assess bulbar redness (VBR 10).
   - **F.** Instill fluorescein and wait 30 seconds.
   - **G.** Assess fTBUT.
   - **H.** After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15).
   - **I.** Apply lissamine green.
   - **J.** Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18).
6. Inclusion/Exclusion Criteria Screen the subject for protocol inclusion/exclusion criteria.
7. Dispense IMP (vehicle) eye drops to be instilled; the first drop will be instilled by the investigator or delegate and then the IMP (vehicle) will be instilled by the patient at home 3 times daily in both eyes for 12 days ±2.
8. Deliver instructions to patient.
9. Dispense the Patient Diary to record the administration of Study Product vehicle during the Run-In period (vehicle accountability), any new or changes in concomitant medications, any unusual medical condition.
10. Investigator will document the first administration of the Study Product (vehicle) in the patient's medical record following Visit 1 to confirm the first administration of the Study Product (vehicle).
11. AE monitoring and recording, as applicable.

RUN-IN (From Day -12±2 to Day 1):

Patients at home will proceed with the Run-In of 12 days ± 2 using the dispensed

Study Product vehicle eye drops (1 drop in both eyes 3 times a day). No other ophthalmic topical administrations are permitted.

Visit 2 - Day 1: BASELINE
Patients will be randomized if they continue to meet eligibility criteria at Visit 2/Baseline.

1. Concomitant Ocular and Systemic Medications and Conditions
2. AEs monitoring (recording and evaluation, as applicable)
3. Pregnancy Test
4. SANDE questionnaire
5. CCI
6. Ophthalmic Examinations in each eye:
   - **A.** Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS
   - **B.** External Ocular Examination
   - **C.** Assess NIKBUT (at selected centers)
   - **D.** Schirmer-I test without anesthesia.
   - **E.** Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes)
   - **F.** Assess bulbar redness (VBR 10 scale)
   - **G.** Instill fluorescein and wait 30 seconds.
   - **H.** Assess fTBUT
   - **I.** After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15).
   - **J.** Apply lissamine green.
   - **K.** Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18).
   - **L.** Perform CCI eyedrop CCI (only in patients with CCI
   - **M.** Perform specular microscopy to assess corneal endothelial cells density (at selected sites that have a specular microscope already available).
   - **N.** Instill tropicamide eye drop solution 1% after 15 minutes/once pupils are adequately dilated perform fundus examination.
7. Inclusion/Exclusion: Patients must continue to meet the Screening/Enrollment Criteria. At the end of the Baseline Visit (Day 1), it will be determined if the subject qualifies for continued participation and treatment in the study.
8. Collect used/unused Study Product (vehicle) of the Run-In phase (to be checked by independent personnel)
9. Retrieve the Patient Diary for the run-in period.
10. Randomization
11. Study Product dispensing: eligible patients will be supplied with a bi-weekly kit of Study product (IMP or vehicle) after randomization, sufficient for 2 weeks of treatment per visit.
12. Deliver instruction to Subjects.
13. Dispense Patient Diary to record adherence to the administration of IMPs during the treatment period, any new or changes in concomitant medications, any unusual medical conditions.

14. AEs monitoring and reporting, as applicable.

8.2 TRIAL VISITS AND FOLLOW-UP ASSESSMENTS

Site staff will conduct the following assessments in the order outlined below during each visit.

Visit 3 - Week 2 (Day 13±1) STUDY PRODUCT PICK-UP VISIT

1. Concomitant Ocular and Systemic Medications Update.
2. AEs monitoring (recording and evaluation, as applicable).
3. Check the Patient Diary.
4. Collect Used/Unused Study Products (IMP or vehicle; to be checked by independent personnel).
5. Study Product Dispensing: Patients will be supplied with a biweekly kit of Study product (IMP or vehicle) sufficient for 2 weeks of treatment.

Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT or EARLY EXIT VISIT

1. Concomitant Ocular and Systemic Medications Update.
2. AEs monitoring (recording evaluation, as applicable).
3. Check the Patient Diary of the treatment period.
4. SANDE questionnaire.
5. CCI [redacted]
6. Ophthalmic Examinations:
   - **A.** Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS.
   - **B.** External Ocular Examination.
   - **C.** Assess NIKBUT (at selected centers).
   - **D.** Schirmer-I test without anesthesia.
   - **E.** Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes).
   - **F.** Assess bulbar redness (VBR 10 scale).
   - **G.** Instill fluorescein and wait 30 seconds.
   - **H.** Assess fTBUT.
   - **I.** After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI, score range 0-15).
   - **J.** Apply lissamine green.
   - **K.** Assess total conjunctival lissamine green staining score ([NEI, score range 0-18).
   - **L.** Perform CCI [redacted] eyedrop CCI [redacted] (only in patients with CCI [redacted]
7. Collect used and unused Study Product (to be checked by independent personnel)
8. Retrieve Patient's Diary for the treatment period.
9. Dispense preservative free artificial tears (AT) eye drops to be administered 3 times daily.
10. Deliver instruction to Patient.
11. Dispense Patient Diary to record adherence to preservative free AT eye drops during the FU period, any new or changes in concomitant medications, any unusual medical conditions.

Visit 5 - Week 8 (day 56±2) FOLLOW-UP & END OF STUDY VISIT or EARLY EXIT VISIT*

1. Concomitant Ocular and Systemic Medications Update.
2. AEs monitoring (recording evaluation, as applicable).
3. Check the Patient's Diary of the Follow-Up period.
4. SANDE questionnaire.
5. CCI
6. Ophthalmic Examinations:
   - **A.** Best-Corrected Distance Visual Acuity (BCDVA) by ETDRS.
   - **B.** External Ocular Examination.
   - **C.** Assess NIKBUT (at selected centers).
   - **D.** Schirmer-I test without anesthesia.
   - **E.** Slit Lamp Examination (SLE) of the anterior segment of the eye (cornea, conjunctiva, anterior chamber, iris, lids and lashes).
   - **F.** Assess bulbar redness (VBR 10 scale).
   - **G.** Instill fluorescein and wait 30 seconds.
   - **H.** Assess fTBUT.
   - **I.** After 2-5 minutes from the time of fluorescein instillation, assess total corneal fluorescein staining score (NEI scale, score range 0-15).
   - **J.** Apply lissamine green.
   - **K.** Assess total conjunctival lissamine green staining score (NEI scale, score range 0-18).
   - **L.** Perform specular microscopy to assess corneal endothelial cells density (at selected sites that have a specular microscope already available).
   - **M.** Instill tropicamide eye drop solution 1%.
   - **N.** After 15 minutes/once pupils are adequately dilated perform fundus examination in both eyes.
7. Retrieve the Patient's Diary for the Follow-Up period.

End of Trial (EoT)/End of Study (EOS): as per **article 2 (26)** of the European Clinical Trial Regulation (CTR), EoT/End of Study (EOS) is the last visit of the last patient globally.

At the investigator's discretion, any patient could be seen for an Unscheduled Visit for safety evaluation.

Premature Discontinuation and Early Exit Visit: (*) In case of premature discontinuation of the study, the patient will be required to perform the Early Exit Visit that will have the same assessments of Visit 4 (in case of early treatment discontinuation before Visit 4) or the same assessments 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 Visit 5 is a per protocol visit or if it is done as Early Exit Visit.

8.3 ASSESSMENTS AND CLINICAL DEFINITIONS

Table 6: List of assessments

Assessment type	Parameters to be analyzed (units)
SANDE questionnaire	SANDE questionnaire includes two CCI-based questions that assess: (i) DED symptom frequency (from 0 to 100) (ii) 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. SANDE is copyrighted by Massachusetts Eye and Ear Infirmary (MEEI). A license will be purchased prior to use in the clinical trial.
CCI CCI	Scores for sub-scales within the dimensions of CCI (intensity 24h and past 2 weeks: 0-10), CCI (intensity: 0-10, time spent thinking about CCI 0-100%), CCI (interference in activities of daily life: 0-10, time spent thinking about CCI CCI CCI CCI . CCI is a validated questionnaire that is copyrighted by the CCI CCI A license will be purchased prior to use in the clinical trial. This form will be administered to all subjects but completed only by those that either have CCI or have filled this form before.
NIKBUT	Non-Invasive Keratograph tear Break-Up Time (measured in seconds) will be assessed at select centers that have the Oculus keratograph 5M (Oculus, Inc., Arlington, WA) Two types of NIKBUT will be collected: (i) NIKBUT-first is the time at which the first distortion is recorded, (ii) NIKBUT-average is the average time of first breakup incidents in different cornea locations during recording
Schirmer-I without anesthesia	After NIKBUT, but prior to slit-lamp examination and vital dye staining, Schirmer-I test will be performed without anesthesia to determine wetting of the strip within 5 minutes and the length of the moistened strip will be measured in millimeters (mm).
Slit lamp examination	A slit-lamp examination of ocular adnexa (lids, lashes, conjunctival fornix, lacrimal gland and drainage) and anterior segment (cornea, conjunctiva, aqueous humor, iris, lens, vitreous humor) will be performed using white light and prior to vital dye instillation.
VBR 10 scale	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)

Assessment type	Parameters to be analyzed (units)
Fluorescein Tear Break-Up Time (fTBUT)	fTBUT will be measured by determining the time to tear film break-up. The fTBUT will be performed after instillation of 1 drop of fluorescein solution into the inferior conjunctival cul-de-sac of each eye. To thoroughly mix the fluorescein with the tear film, the subject will be instructed to blink several times. In order to achieve maximum fluorescence, the examiner should wait approximately 30 seconds after instillation before evaluating fTBUT. With the aid of a slit lamp at 10X magnification using cobalt blue illumination, the examiner will monitor the integrity of the tear film, noting the time it takes to form lacunae (clear spaces in the tear film) from the time that the eye is opened after the last blink. 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 will be the average of the 2 or 3 measurements. The measured value will be expressed in seconds.
Corneal fluorescein staining	To avoid the phenomenon of quenching, which is increased in dry eye patients, staining will be assessed after 2-5 minutes from fluorescein instillation for fTBUT evaluation. Examination will be performed at the slit-lamp using blue light. Corneal fluorescein staining will be graded according to the NEI scale (5 zones, each zone score 0-3, total score range 0-15).
Lissamine green conjunctival staining	Lissamine green will be applied to the lower conjunctival fornix and bulbar conjunctival staining will be assessed at the slit-lamp using white light within 2 minutes of lissamine green application. Staining will be scored according to the NEI grading scale (6 zones, each zone score 0-3, total score range 0-18).
CCI CCI CCI (to perform only in patients with CCI CCI	1 drop of CCI (CCI will be instilled in the inferior fornix of the palpebral conjunctiva of each eye. After 30 seconds of instillation, CCI will be recorded and will be compared to a pre-test CCI
Specular microscopy	Corneal endothelial cell density will be determined using specular microscopy and expressed as the number of cells/mm^2 using the Cell Density readout provided by the software.
Dilated fundus exam	After specular microscopy, mydriatic drops will be instilled in both eyes and once adequate dilation is achieved, a fundal exam shall be performed to examine the 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:disc ratio.

8.4 PHARMACOKINETICS

No samples for pharmacokinetic evaluations will be collected during the study.

8.5 GENETICS

No sample for genetic analyses will be collected during the study.

8.6 BIOMARKERS

No sample for Biomarker assessment will be collected during the study.

8.7 IMMUNOGENICITY ASSESSMENT

No sample for immunogenicity assessment will be collected during the study.

9 EVALUATION OF ADVERSE EVENTS AND SAFETY INFORMATION

9.1 DEFINITIONS

9.1.1 Adverse Event

An **Adverse Event** (**AE**) is defined as any untoward medical occurrence in a patient or clinical investigation subject 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.

9.1.2 Adverse Drug Reaction

An **Adverse Drug Reaction (ADR)** is defined as any noxious and unintended response to a medicinal product related to any dose. Any responses to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

The definition also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. For the purposes of regulatory safety reporting, “reasonable possibility” means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event. Adverse events are to be considered unrelated if the relationship to the study drug, as described in the table in § 9.3.2, is none or unlikely; whereas any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

9.1.3 Serious Adverse Event

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.
  NOTE: In general, hospitalization means that the individual remained at the hospital or emergency ward for observation and/or treatment (usually involving an overnight stay) that would not have been appropriate in the physician's office or an outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred, the event should be considered serious.
- results in persistent or significant disability/incapacity.
  NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions, but do not constitute a substantial disruption.
- is a congenital anomaly/birth defect,
- is an important medical event.
  NOTE: An important medical event is an event that may not result in death, be life threatening, or require hospitalization, but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the patient's wellbeing and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization or the development of drug dependency or drug abuse.

Pre-planned hospitalization or hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition are not considered to be SAEs (see § 9.4.2). These events must be recorded in the AE page of the eCRF where a variable will be ticked to indicate that they are not SAEs.

Death shall always be reported as SAE and cause of death shall always be specified when known.

9.1.4 Unexpected Adverse Events

An AE or ADR is considered unexpected if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed and listed in the Investigator

Brochure. Events that are mentioned in the Investigator Brochure (section Reference Safety Information) as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation are considered unexpected. The determination of expectedness is made by the Sponsor on the basis of the IB Reference Safety Information (RSI) section.

9.1.5 Suspected Unexpected Serious Adverse Reaction

A **suspected unexpected serious adverse reaction** (**SUSAR**) is defined as an adverse reaction that is both unexpected (not consistent with the applicable reference safety information) and also meets the definition of a Serious ADR.

9.1.6 Adverse Events (AEs) of Special Interest (Sight-threatening Events)

The following adverse events are considered to be of Special Interest 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.

9.2 MONITORING AND REPORTING ADVERSE EVENTS

Following study informed consent form signature, at each visit, after the patient has had the opportunity to spontaneously mention any problems, the Investigator or appropriate designee should inquire about AEs by asking the standard questions:

- “Have you had any health problems since your last study visit?”
- “Have there been any changes in the medicines you take since your last study visit?”

AEs should be reported for any clinically relevant change in concomitant condition(s) that is the result of an untoward (unfavorable and unintended) change in patient's medical conditions. Changes in any protocol-specific ocular or systemic parameter evaluated during the study are to be reviewed by the Investigator. In addition, the patient’s responses to any questionnaire utilized during the study are to be reviewed by the Investigator. Any untoward (unfavorable and unintended) change in a protocol-specific parameter or questionnaire response that is clinically relevant is to be reported as an AE. These clinically relevant changes will be reported regardless of causality.

All AEs should be followed-up to determine the outcome of the reaction.

In order to collect as complete as possible information in the clinical study database, all ADRs and SAEs ongoing at the time the subject's study participation ends should be evaluated within 10 days after the final visit. After this period, all unresolved ADRs and SAEs will be reported as "ongoing" in the eCRF.

9.3 RECORDING ADVERSE EVENTS

AE data should be obtained through observation of the patient, from any information volunteered by the patient, and through patient questioning.

Adverse Events:

All AEs (non-serious and serious) that occur during the course of the study will be recorded in the eCRF. Any pre-existing medical conditions or signs/symptoms present in a patient prior to the start of the study (i.e., before informed consent is signed) should be specified in the dedicated eCRF sections. Subsequent to signing an informed consent form, all untoward medical occurrences that occur during the course of the study must be documented on eCRF. AEs will be collected till the last follow-up visit (week 8).

When possible, signs and symptoms indicating a common underlying pathology should be documented as one comprehensive event. For each recorded event, the AE documentation must include the onset date, outcome, resolution date (if event is resolved), intensity (i.e., severity), any action with study treatment taken as a result of the event, and an assessment of the adverse event relationship to the study treatment.

Serious Adverse Events:

The Investigator must record all SAEs, including sight-threatening events, occurring at any time during the study (after signature of the informed consent) regardless of presumed causal relationship, on the Serious Adverse Event form in the eCRF of the EDC system within 24 hours of learning of the event; information on the SAE must also be recorded on a specific Non-Carbon Repeat SAE form (included in the Investigator's Site File).

9.3.1 Follow-Up of Patients with Adverse Events

The Investigator is responsible for adequate and safe medical care of patients during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. All AEs should be followed-up to determine outcome of the reaction or until 10 days after the final visit (EOS or Early Exit Visit). The Investigator should follow-up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the patients experiencing AEs receive definite treatment for any AE, if required.

If a patient was hospitalized due to a SAE, a copy of the discharge summary is to be forwarded to Dompé as soon as it becomes available. In addition, a letter from the Investigator that

summarizes the events related to the case as well as results of any relevant laboratory tests may also be requested. Further, depending upon the nature of the SAE, Dompé may request copies of applicable segments of the patient's medical records.

For pharmacovigilance purposes, all SAEs should be followed-up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or the subject is lost to follow-up. Follow-up may therefore continue until after the patient has left the study up to 10 days after his/her discontinuation from the study for unrelated SAEs, and without timelines for related SAEs, unless the patient denies consent.

9.3.2 Relationship of AEs to the Investigational Medicinal Product

The Investigator will assess the relationship between the AE and the IMP, according to the criteria in Table below.

Table 7: Relationship of the Adverse Event to the IMP

Relationship	Description
None (Intercurrent Event)	An event that is not and cannot be related to the Investigational Product, e.g., patient is a passenger in a road traffic accident or surgical intervention performed during the study but planned before patient enrolment into the study.
Unlikely (remote)	Relationship is not likely e.g., a clinical event including laboratory test abnormality with temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide more plausible explanations
Possible	Relationship may exist, but could have been produced by the patient's condition or treatment or other cause
Probable	Relationship is likely, the AE abates upon discontinuation of Investigational Product and cannot be due to the patient's condition
Highly Probable	Strong relationship, the event abates upon discontinuation of Investigational Product and, if applicable, re-appears upon repeated exposure

An adverse drug reaction (ADR) is defined as an adverse experience which is reasonably likely to have been caused by the drug. Events considered "Possible", "Probable" and "Highly Probable" related to the IMP treatment and implying a reasonable possibility, if considered unexpected, will be reported to appropriate Regulatory Authorities by Dompé.

9.3.3 Severity of AEs

The Investigator will grade the severity of any AE using the definitions in the Table below. For each episode, the highest severity grade attained should be reported.

Table 8: Severity of the Adverse Event

Severity	Description
Mild	Grade 1 - Does not interfere with patient's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).
Moderate	Grade 2 - Interferes to some extent with patient's usual function (enough discomfort to interfere with usual activity [disturbing]).
Severe	Grade 3 - Interferes significantly with patient's usual function (incapacity to work or to do usual activities [unacceptable])

9.4 SERIOUS ADVERSE EVENT REPORTING

9.4.1 Reporting Procedure for Investigators

The Investigator must report all SAEs filling in and signing a SAE Report form, including sight threatening events, regardless of presumed causal relationship, to Dompé Global Pharmacovigilance, Safety and Surveillance Dept and CRO Pharmacovigilance, by e-mail (preferred) or fax immediately, anyway **within 24 hours** of learning of the event. Contact details for SAE reporting are provided below:

CRO Safety reporting
PPD Pharmacovigilance
4-Hour Safety Hotline: +44 1223 374240
Safety Hotline Fax: +44 1223 374102
Email: EMEAASIASafetyCentral.SM@ppd.com

Dompé Contact Information
Dompé Global Pharmacovigilance, Safety and Surveillance Department
PPD

Dompé Medical Expert
PPD

PPD

Dompé Clinical Operations

PPD

In addition to reporting the SAE to Dompé, the Investigator must also comply with the requirements related to the reporting of SAEs to the IRB/IEC which approved the study. The requirements of IRBs/IECs vary from one IRB/IEC to another; however, as a minimum requirement, the Investigators must promptly report all suspected unexpected serious adverse reaction (SUSAR) to their IRB/IEC.

If assistance is needed with the reporting of a SAE, CRO/Dompé may be contacted at the addresses provided above.

SAEs will be managed directly by the Dompé Pharmacovigilance, Safety and Surveillance department, with CRO support for follow-up requests.

The investigator should also report information on SAEs that continue after the patient has completed his/her participation in the study (whether study completion or withdrawal) unless the patient has withdrawn his/her consent.

Follow-up reports (as many as required) should be completed and faxed/emailed following the same procedure above, marking the SAE form as "follow-up Number XX".

Whenever more than one SAE is observed, the Investigator should identify which is the primary adverse event, i.e., the most relevant one. If other events are listed in the same report, the Investigator, along with their relatedness to the IMP, should identify which AEs are serious and which are non-serious. In any case, the Investigator is requested to record his/her opinion about the relatedness of the observed event(s) with the IMP.

An assessment of expectedness and causality of each SAE will be performed case by case by Dompé Pharmacovigilance. For SAE reported by the Investigator as not related that is subsequently assessed to be related by Dompé, the Investigator will receive a notification. Depending on the nature and seriousness of the AE, further information, including copies of appropriate medical records of the patient, as well as results of laboratory tests performed will need to be included in the patient's chart. If the patient was hospitalized, a copy of the discharge summary should be available, if possible.

In line with CT3 Detailed Guidance and ICH E2A provisions, although the Investigator does not usually need to actively monitor patients for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a patient after that patient has ended his/her

participation in the study (whether study completion or withdrawal), the SAE should be reported by the Investigator to the Dompé Pharmacovigilance. Such "post-study cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment and determination of expectedness are needed for a decision on whether or not expedited reporting is required.

9.4.2 Conditions that should not be reported as Serious Adverse Events

The conditions listed below, that may require hospitalization of a patient, are not considered to be SAE and shall not be reported as such, but only need to be recorded in the eCRF:

- Hospitalizations planned before entry into the clinical study which is part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition.
- Hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Hospitalization for treatments, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen.
- Hospitalization for general care not associated with any deterioration in condition.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of SAEs given above and not resulting in hospital admission.

In addition, the following situation shall not be considered SAE:

- Abnormal test results that do not induce clinical signs and/or symptoms and do not require intervention/therapy, i.e., are not clinically significant.

9.4.3 Adverse Events Exemption

There are no adverse event exemptions for this study.

9.4.4 Reporting Procedure to IRB/IEC and to Regulatory Authorities

During the course of the clinical trial, the Sponsor shall report any SUSAR to the concerned Regulatory Authority(ies) and IRBs/IEC(s), as soon as possible and in no event later than:

a) seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
b) fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Follow-up information shall be reported within the same timelines.

If the results of an investigation show that an ADR not initially determined to be reportable is reclassified as reportable, the Sponsor shall report such SUSAR in a written safety report as soon as possible, but in no event later than 7/15 calendar days after the determination is made.

Treatment will be unmasked by Sponsor Pharmacovigilance prior to regulatory submission of a SUSAR to Regulatory Authorities and IRBs/IECs and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law

requirements.

The Sponsor shall also inform worldwide Regulatory Authorities of SUSARs, as applicable (cross reporting).

Dompé shall also notify FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible after Dompé determines that the information qualifies for reporting, in particular shall notify of:

- any suspected adverse reaction that is both serious and unexpected. Dompé must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event.
- findings from other studies that suggest a significant risk in humans exposed to the drug. Such a finding would result in a safety-related change in the overall conduct of the clinical investigation.
- findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug.
- increased rate of occurrence of serious suspected adverse reactions.

In line with provisions set forth in 21CFR312, Dompé shall notify all participating US Investigators in an IND safety report of any SUSAR and of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than:

- Seven calendar days after becoming aware of the information if the event is fatal or life threatening; to be followed by any relevant information within eight days.
- Fifteen calendar days after becoming aware of the information if the event is serious but neither fatal nor life threatening.

The Investigators in turn shall notify their IRB/IEC.

Copies of all correspondence relating to reporting of any SAEs to the IRB/IEC should be maintained in the Investigator's Files.

According to US regulations, the Investigators will be informed in an unmasked manner: Dompé will open the masking and report only SUSAR referred to rhNGF ophthalmic solution.

EU Investigators shall be informed periodically, in a masked fashion, in line with applicable requirements.

As part of periodical information, the Sponsor shall notify the Investigator on a six-monthly basis of any serious ADRs (maintaining the masked condition) that occur during ongoing clinical trials.

9.4.5 Periodical Reporting to Regulatory Authorities

Dompé shall be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities in the countries concerned, as per applicable requirements.

9.5 EXPOSURE TO INVESTIGATIONAL PRODUCT DURING PREGNANCY

Women of childbearing potential are not excluded from the study as long as adequate birth control methods are being utilized. Women of childbearing potential are defined as all women physiologically capable of becoming pregnant. Adequate birth control methods are summarized in the protocol's exclusion criteria.

Prior to enrollment in the clinical trial, female patients of childbearing potential and their partners must be advised of the importance of avoiding pregnancy during the entire course of the study treatment and for the 30 days after the study treatment period ends and of the potential risks associated with an unintentional pregnancy. During the trial (during the study treatment period and during the follow-up), female patients are to be instructed to contact the Investigator immediately if they suspect they might be pregnant; in the same way, male patients who become aware that the partner might be pregnant, are to be instructed to contact the Investigator immediately.

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy) to CRO/Dompé Pharmacovigilance, Safety and Surveillance contacts reported at § 9.4.1, even if no AE has occurred, and follow it to term.

The pregnancy form will be utilized to capture all pregnancy-related information until the birth of the child for both the patient and the partner.

If the pregnancy is associated with a SAE (e.g., if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed as described in § 9.4 with the appropriate serious criterion (e.g., hospitalization) indicated on the SAE report form. Miscarriage, stillbirth, and any malformation/disease must be reported as a SAE. Any pregnancy leads to the immediate cessation of the study treatment.

9.6 ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION

If a patient is withdrawn from the study as a consequence of an AE, this must be recorded and reasoned in the eCRF, and the patient must be followed up until the resolution of the AE or as instructed by the medical monitor.

9.7 OVERDOSE

Overdose (accidental or intentional) of Study Products (including vehicle) which may or may not result in serious adverse reactions, shall be reported to Sponsor Global Pharmacovigilance, Safety and Surveillance /CRO by email or fax, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to intake through different routes (e.g., ingestion) or with suicidal intentions and consequent drug overdose.

Since in the preclinical toxicology studies in animals and in the multiple ascending dose study

performed in healthy volunteers none of the dose has caused an overdose as documented by adverse reaction, for the purpose of this study we define that the administration of more than 3 times the total daily dose on any given treatment day will be reported as an overdose, even if not associated with adverse reactions. The Investigator shall provide in the SAE form information about symptoms, corrective treatment, and outcome of overdose.

The Medical Expert should be contacted to discuss corrective treatment, if necessary.

10 STATISTICS

10.1 SAMPLE SIZE

The sample size of the study is calculated based on results from the previous study NGF0118 using the subgroup of patients with baseline SANDE Global Score > 50. 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 (eye drop) formulation, a total sample size of 276 evaluable patients (92 per group) is needed. This will allow to achieve an overall power of 80% 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 of 0.025.

The sample size calculation takes into consideration a Bonferroni correction for two comparisons (2 concentration groups versus vehicle), i.e., the alpha level has been set to 0.0125 one-sided for each comparison.

Assuming a 5% rate 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). Enrollment will be competitive.

In Dompé previous studies, a screen failure rate of about 20% has been observed (NGF0121 - PROTEGO-1). This fact implies that approximately 350 subjects will need to be screened in order to have 291 patients enrolled and at least 276evaluable. In any case, the screen procedures should be interrupted when the expected sample size of 291 subjects has been reached.

10.2 OVERVIEW OF PLANNED STATISTICAL ANALYSIS

The study plans only a final analysis: this analysis will be conducted when all randomized subjects have completed the study [treatment at week 4 (V4) and follow-up at week 8 (V5)] and the study database has been locked and unmasked.

10.3 INTERIM ANALYSIS

No interim analyses are foreseen.

10.4 PATIENT POPULATION

10.4.1 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.

10.4.2 Enrolled Population (ENR)

A patient in the SCR population will be defined as enrolled if meets all the inclusion criteria and no one 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.

10.4.3 Randomized Population (RND)

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

10.4.4 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. Safety population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.

10.4.5 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 (ITT) 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.

10.4.6 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 population will be determined in the masked Data Review Meeting (DRM). A non-exhaustive list of possible reasons for exclusion from the PP can be the following:

- Lack of compliance with IMP and NIMP administration. (§1.1, §4.1, §9.7, §11.6)
- Missing of any efficacy data post randomization.
- Intake of prohibited medications. (§4.1, §6.6.1)

10.5 ESTIMANDS

The estimands for the primary and key secondary objectives are defined in §s 3.2.1 and 3.2.2, respectively.

10.6 STATISTICAL METHODOLOGY

Statistical analysis will be performed by the CRO appointed by Dompé.

10.6.1 Descriptive Statistics

Appropriate descriptive statistics will be produced by treatment arms according to the nature of the variable. For continuous data, the number of observations, mean, standard deviation, median (25th and 75th percentile) and range (minimum and maximum) will be presented. For qualitative data, frequency distributions and percentages per category will be presented. If appropriate, confidence intervals around the mean or the proportion will be presented. The number of subjects 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 will be summarized, only the non-missing values will be evaluated for computing summary statistics. Any exception will be declared.

For the purpose of efficacy analysis in this trial, the statistical analysis (both descriptive and inferential) will be done on the Study Eye. Details will be provided in the statistical analysis outputs as notes. In case analysis involves both eyes, it will be clearly declared.

The significance level used for primary analysis will be 0.0125 one-sided for each active arm versus vehicle and will be determined in accordance with the Hochberg approach (Hochberg Y. 1988) for the key secondary endpoints (in order to preserve an overall 0.025 one-sided). Unless otherwise specified, the significance level used for other statistical testing will be 0.05 and two-sided tests will be used. All patient data collected on the eCRF will be listed by patient and center.

Data transformation (e.g., 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 of log transformation, also a back transformation of the results will be provided in order to facilitate the clinical interpretation.

The Statistical Analysis Plan (SAP) will be issued before database lock with more technical and detailed elaboration of the principal features of statistical analyses. Additional post-hoc analysis may be produced to further allow comparison between treatment and control, according to the results obtained. Any deviation from the original statistical plan (including unplanned analyses) will be documented in the Clinical Study Report.

10.6.2 Primary Analysis of Efficacy Variables

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

H_0: CCI $\geq \mu_{VEHICLE}$ AND CCI $\geq \mu_{VEHICLE}$

H_1: CCI $< \mu_{VEHICLE}$ OR CCI $< \mu_{VEHICLE}$

where for SANDE Global Scale lower score is better.

Considering the multiple comparisons (each concentration group versus vehicle) to claim superiority, a Bonferroni multiplicity correction of type I error will be applied on primary endpoint comparison. Each concentration group will be consequently tested at one-sided alpha = 0.0125, in order to preserve the overall one-sided alpha of 0.025. The null hypothesis H0 will be rejected if the associated primary analysis one-sided p-value will be lower than 0.0125 for at least one of the two concentration groups versus vehicle.

The primary endpoint (i.e., the change from baseline in SANDE global score at week 8) will be analyzed by means of a mixed model for repeated measures (MMRM) in the FAS. The analyses will include the fixed effects of treatment (3 levels: rhNGF C mcg/mL, rhNGF C mcg/mL and vehicle), visit (2 levels: Weeks 4, 8), baseline value, and treatment by visit interaction. The covariance matrix used will be "unstructured". Each concentration group (IMP1 and IMP2) will be compared with the vehicle group at Week 8 using the least square mean differences (i.e., the mean difference between each concentration group and vehicle over time). 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 subjects. Further details and SAS code will be described in the SAP that will be generated before the DB lock and the unmasking.

For patients analyzed under the hypothetical strategy (see § 3.2.1), any data collected in the week after the ICE of interest (intake of prohibited medications) will be considered as missing for the purpose of the primary analysis and will be imputed, as well as the other missing data (for example for lost to follow-up, patients who withdrew their consent), by using the multiple imputation described below with a copy-reference approach.

Missing data that may be obtained notwithstanding the intent to apply the treatment policy strategy and after having applied the hypothetical strategy when needed, 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:

1) 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.
2) For each imputed dataset, the change from baseline at Week 4 and Week 8 is derived.
3) 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. Subject will be considered as cluster variable and an unstructured covariance matrix will be set to model the within-subject correlation.
4) Results obtained on each imputed dataset will be combined using Rubin's rule to draw inference.

The adjusted estimated treatment difference between each concentration of the rhNGF new formulation and vehicle will be displayed together with the corresponding two-sided 95% confidence interval (CI) and one-sided p-value.

In case both concentrations of rhNGF differ from placebo, the choice between the two concentrations will be based on the size of the effect (and corresponding CI), on the results of secondary endpoints and on safety data.

10.6.3 Sensitivity and Supportive 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. They will be done after having applied the hypothetical strategy for ICEs, when expected (i.e., after having set to missing the observed values collected in the week after the use of a prohibited medication):

- The comparison between treatment and control will be performed in the FAS population by means of MI under the missing at random (MAR) instead of the MNAR assumption.
- A tipping point strategy will be used as a sensitivity analysis for missing data for assessment of superiority (if shown) of rhNGF. 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 described in § 10.6.2, 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 $p \geq 0.0125$) for each arm. Tipping point analysis will be run only if the superiority has been proven for at least one of the two concentrations of the rhNGF. The tipping point will be firstly searched in the values defined by ±SD. If not found, the range will be enlarged to ±2SD.
- Analysis on complete cases only (i.e., without considering patients with missing primary endpoint for any reason and after having discarded any observed case after the ICEs under the hypothetical strategy).
- Analysis on all observed cases (i.e., without considering patients with missing primary

endpoint for any reason and including data discarded due to ICEs handled under the hypothetical strategy).
- Analysis on the PP set instead of FAS.

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 estimand:
  - 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 § 10.6.2 for further details).
  - Use of a prohibited medication listed in § 6.6.1 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 discarded. Missing data will be imputed by using a multiple imputation with copy-reference approach (see § 10.6.2 for further details).
- Modified strategy #2 to handle ICEs for primary estimand:
  - 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 § 10.6.2 for further details).
  - Use of a prohibited medication listed in § 6.6.1 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 at week 8.

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

For the modified estimand #2, the trimmed approach by Permutt and Li (Permutt T and Feng L. 2017) will be used. 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 best SANDE values at week 8 (i.e., with lowest values, since for SANDE questionnaire lower values are better). For this analysis k=50%, 70%, and 90% will be used. 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.

Further details on sensitivity and supportive analyses will be provided in the SAP.

10.6.4 Secondary and Exploratory Analysis

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.

This 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 subjects 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).

Key secondary endpoints will be analyzed with the same strategy described for the primary endpoint for the multiple imputation. Multiple imputation for Schirmer-I test will be done once for both continuous and dichotomous endpoints that will be derived (i.e., change from baseline and improvement $\geq$ 10 mm/5min will be derived after the imputation of the continuous values). An ANCOVA model with treatment and baseline value will be used instead of the MMRM for the two continuous endpoints since these are evaluated at Week 4. For the same reason, only baseline and week 4 data will be used in the multiple imputation described in § 10.6.2. The binary key secondary endpoint will be analyzed in analogy with the other key secondary endpoints, but a logistic model will be used instead of an ANCOVA model with baseline value and treatment as covariates.

In case of not rejection of the null hypothesis (or for the concentration for which the superiority is not shown), the above testing strategy will not be performed. Instead, independently of results on primary endpoint, descriptive in nature analyses will be performed on all key secondary and secondary endpoints at each available timepoint by means of descriptive statistics and by appropriate parametric or non-parametric tests depending on the distribution of each parameter. Change from baseline values and shift tables versus baseline may be summarized for all available post-baseline visits.

Further details will be provided in the SAP.

Details on the statistical methods that will be used for the additional objective (i.e., the validation of the Italian version of the CCI will be provided in the SAP.

10.6.5 Analysis of Safety Variables

Adverse events (AEs) will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).

Treatment-emergent AEs (TEAEs) are all events occurring or worsening after the first dose of the IMP. TEAEs will be presented by treatment arm in terms of number of AEs and their incidence by System Organ Class (SOC) and Preferred Terms (PT) using MedDRA. Analyses will be provided also by seriousness, relationship to treatment, severity, and study period

(treatment or follow-up).

Individual AEs will be listed in patient data listings.

10.6.6 Missing Data

All reasonable efforts will be made to reduce the rate of missing data by encouraging the patients to attend the scheduled visits, even if they have interrupted the study treatment. Investigators will be trained about the importance of patient retention and full data capture. Also, all reasonable attempts should be made by the Investigators to emphasize continued subject's participation for the full duration of the trial. However, in order to minimize missing data, if a patient cannot refer to the site for a planned visit, the Investigator will try to obtain any relevant information from the patients, including documents results available from local medical care.

10.6.7 Intermediate Analyses for the DMC

Not applicable.

10.6.8 Specification of Subgroups for Analysis

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

- Age class (≤Median, >Median)
- Race/ethnicity
- Gender
- Region (EU, US)
- Etiology of dry eye
- Medical history
- Concomitant medication

Statistical details and potential new subgroups definitions will be reported in the SAP.

11 GENERAL CONSIDERATIONS: REGULATORY, ETHICAL, AND TRIAL OVERSIGHT

The study will be performed in accordance with the Protocol, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (ICH-GCP E6-R2) and any local regulations.

11.1 REGULATORY AND ETHICAL CONSIDERATIONS

United States. Approval by the Institutional Review Board (IRB) before the start of the study will be the responsibility of the Principal Investigator (PI). A copy of the approval letter will be supplied to the Sponsor, along with a roster of IRB members or the US Department of Health and Human Services (DHHS) general assurance number. During the course of the

study, the PI will provide timely and accurate reports to the IRB on the progress of the study, at intervals not exceeding 1 year (or as appropriate) and will notify the IRB of serious adverse events (SAEs) or other significant safety findings. The study Protocol, informed consent form (ICF), information sheet advertisements, and amendments (if any) will be approved by the IRBs at the study centers in conformance with Code of Federal Regulations (CFR), Title 21, Part 56. For institutions/PIs that also utilize the study central IRB, the main study protocol and other main study related materials will be submitted to the study central IRB by the Sponsor/CRO on behalf of the PI. All documents that are site-specific will be submitted by the PI directly to the central IRB and local IRB, as applicable.

Europe. This study will be carried out in full compliance with the guidelines of the independent ethics committee (IEC) and government agencies of each respective country as well as Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use. Before the study begins, the Sponsor will require approval from an IEC and Regulatory agency. During the course of the study, the Sponsor or authorized contract research organization (CRO) representative will provide timely and accurate reports to the IEC on the progress of the study, at intervals not exceeding 1 year (or as appropriate) and will notify the IEC of SUSARs or other significant safety findings. The study Protocol, eCRF sample, ICF, information sheet advertisements, and amendments (if any) will be approved by the EC in conformance with the EU Regulation (EU) No 536/2014), and local regulations.

11.2 COMMITTEES

Data Monitoring Committee (DMC)/ Data Safety Monitoring Board (DSMB) and Data Safe Monitoring Board (DSMB) are not required for this trial considering the following points:

- The drug under investigation is well characterized and known for not harming patients.
- This clinical trial does not foresee an interim analysis.
- The study design is not complex and already performed in other clinical trial in DED.
- The study does not have a long duration.

11.3 INFORMED CONSENT PROCESS

Patients, after being given adequate information and explanation related to the study, will give voluntary and written informed consent before participating in any study-related procedures.

The informed consent statement contains all the elements of informed consent and contains all the core elements and mandatory statements as defined in the Code of Federal Regulation (CFR). Signed copy of the ICF and the Patient Information Sheet will be given to the patient, and both signed documents will be placed in the Investigator's Site File. A unique Patient identification (PID) number will be assigned according to § 6.4 of the Protocol at the time the patient signs the ICF.

Each patient will read or be read (if he/she cannot read or write), assent understanding of, and sign or thumbprint an instrument of informed consent, after having had an opportunity to discuss them with the Investigator before signing.

Patients have the right to choose to stop their participation in the clinical trial at any time during the study.

11.4 DATA PROTECTION AND CONFIDENTIALITY

All information obtained during the conduct of the study will be regarded as confidential. An agreement for disclosure will be obtained in writing by the patient and will be included in the ICF. Patient's data collected during the study will be handled in accordance with applicable data protection laws and regulations.

On the eCRFs, Questionnaires or Patient Diary, patients will be identified ONLY by the assigned patient number.

If patient names are included on copies of documents submitted to Dompé or CRO, the names will be obliterated or masked, and the assigned patient number added to the document.

The Investigator should keep a separate log (Patient Master List) of patient's codes, names and addresses.

11.5 EARLY SITE CLOSURE OR TRIAL TERMINATION

Dompé reserves the right to terminate the study in its entirety or at a specific study center at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory (poor/ no patient recruitment, inadequate research staffing, site quality issues, lack of adherence to protocol etc.), or for other valid administrative reasons**.**

11.6 QUALITY TOLERANCE LIMITS

Gross noncompliance (see § 6.5 for derivation of compliance by using the number of drops collected in the diary) will be defined as compliance lower than 80% or greater than 120% and in case of gross noncompliance the patient will be excluded from the Per Protocol dataset. Quality tolerance limits are indicated in the centralized monitoring plan.

11.7 DATA QUALITY ASSURANCE

The study site may be audited by CRO on behalf of the Dompé or inspected by a Regulatory Agency on one or more occasions. The Investigator may be informed in advance of such a visit.

11.8 DISSEMINATION OF CLINICAL TRIAL DATA AND PUBLICATION POLICY

All data generated in this study will be the property of Dompé.

The study results will be communicated in full to the related competent authorities for the countries involved by the submission of a complete Clinical Study Report. On an exceptional basis, Dompé may temporarily delay registration of certain data elements (e.g., compound,

name, outcome, measures etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

Publication of the results by the PI will be subject to mutual agreement between the PI and Dompé.

The Sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the Investigator publishing in peer reviewed journals; presenting results at scientific congresses; and posting information and results on internet-based public registers and databases.

As the Sponsor agrees that the study results can be published by the Investigator(s), the Investigator agrees to submit any manuscript (abstract, publication, paper etc.) to the Sponsor before any public disclosure.

This will be done in order to ensure that clinical trial results are reported in an objective, accurate and balanced manner. The Sponsor reviews proposed manuscripts prior to submission within a reasonable period of time (30-90 business days in relation with the complexity of the work).

The Investigator(s) will also be provided by the Sponsor with the clinical study report and the results of any additional analysis, tables, figures etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).

11.9 MONITORING ACTIVITIES

Before any patient enters the study, a representative of Dompé/CRO, will meet with the PI and his or her staff to review the procedures to be followed during the study and to train them on recording the data in the eCRFs using the electronic data capture (EDC) system. After the first patient is enrolled, the progress of the study will be periodically monitored by the Dompé representative, a monitor (CRA), conducting on-site visits. This CRA will also be able to review query statuses remotely, possibly warranting more frequent communication with the PI and his or her staff. The PI will make available to the CRA the eCRFs, the source documents, the signed consent forms, and all other study-related documents. The PI and his or her staff will be responsible for reviewing eCRFs, resolving data queries generated by the CRA/Data Management via the system, providing missing or corrected data, approving all changes performed on his or her data, and endorsing the patient data within the EDC system. This approval method will include applying an electronic signature, a uniquely assigned username and password that together will represent a traditional handwritten signature.

The CRA will visit the study site on a regular basis to perform Source Data Verification (SDV) and/or will perform remote checks to ensure that the study is conducted and documented in accordance with this protocol, ICH GCP guidelines, regulatory requirements, and any study specific documents such as eCRF completion guidelines.

Monitoring visits or remote monitoring will be conducted to confirm that e.g.:

- The investigational team is adhering to the study Protocol.
- Informed consent has been adequately obtained from all participants prior to study specific procedures being performed.
- AEs have been reported and followed up as required and any SAEs have been reported according to the requirements.
- Data are being accurately recorded in the eCRFs in a timely manner.
- The Investigator's Site File is being adequately maintained.
- Facilities and staffing resources are, and remain, acceptable and sufficient throughout the study.
- The Investigator and the site are receiving sufficient information and support throughout the study.

Moreover, during on-site monitoring visits the data recorded in the eCRFs, source documents and other study-related records will be compared against each other in order to ensure accurate data that reflect the actual existence of the subject in the study i.e. source data verification.

Monitoring procedures are described in the concerned Standard Procedural Documents of the CRO in charge and in the Monitoring Plan, which also encompasses situational changes, e.g., intermediate switch to remote monitoring only if site visits are not possible; details concerning the nature and extent of SDV are also provided in the Monitoring Plan.

11.10 COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

Before the trial formally starts, Dompé will take out a study-specific insurance contract according to national laws for patients/Investigators/Institutions participating in the clinical trial. In case of questions about medical care, cost for medical care or insurance, patients can talk to their Investigator. Contact details will be given in the Patient Informed Consent Document.

11.11 SERIOUS BREACHES MANAGEMENT

Process (as defined under art. 52 of European Regulation 536/2014):

Institutions, CRO, and investigators involved in the clinical trial management must notify the Sponsor about any Serious Breaches and suspected Serious Breach within 24 hours from the identification of such Serious Breach.
If not otherwise specified in relevant study plans, manuals and agreements with the contracted CRO, the Sponsor will manage the event according to the internal process for the management and notification of the relevant competent authority.

Definitions:
Serious Breach: a breach likely to affect to a significant degree the safety and rights of a subject, or the reliability and robustness of the data generated in the clinical trial.

Suspected Serious Breach: an incident, which at the time of communication from the

investigators or from the service providers to the Sponsor, has not yet been assessed by the Sponsor to be a Serious Breach.

12 DATA HANDLING AND RECORD KEEPING

The Investigator will allow designated Sponsor representatives, including staff from the CRO, and Regulatory/Ethics bodies to have direct access to the source documents to verify the data reported in the eCRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and all related information to substantiate the integrity of the data collected during the trial.

12.1 SOURCE DOCUMENTS AND CASE REPORT FORMS

Main Data Management activities and procedures will be accurately described in the Data management Plan (DMP), created by CRO and approved by Dompé.

All data relating to the study will be recorded on eCRFs to be provided by the CRO, through the EDC system. eCRF data are the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

Data collection will involve the use of an EDC system, to which only authorized personnel will have access. In addition to periodic monitoring occurring within the system by Dompé/CRO monitors, programmatic edit checks will be used to review the data for completeness, logic, and adherence to study protocol (following the Data Validation Plan). As a result of this monitoring and these checks, queries may be electronically issued to the study centers and electronically closed by those study centers. The identifying information (assigned username, date, and time) for both the originator of the query (if created during the monitoring process) and the originator of the data change (if applicable), as well as the PI's approval of all changes performed on his or her patients' data, will be collected. Reconciliation of study data and SAEs between Clinical and Drug Safety database will be performed by the CRO on an ongoing basis and before the database lock. Procedure will be detailed in the DMP.

Encoding of specific data will be carried out by the CRO. For this trial, Medical History, Adverse Events and Concomitant Medication will be coded; Medical Dictionary for Regulatory Activities (MedDRA) and World Health Organisation (WHO)-DRUG Enhanced dictionaries will be used, version number of each dictionary will be documented in the DMP. Dictionary version numbers will not be changed during the study.

eCRF is required and should be completed for each patient that signed the informed consent, including the screening failures. Source documents should be available to support all the data recorded in the eCRF; location of source documents, including those for which the eCRF might be accepted as being the sole source document, will be specified and listed at the Site Initiation Visit.

All data collected in the context of this study will be stored and evaluated per regulatory

requirements and applicable guidance for electronic records. Also, data will be stored and evaluated in such a way as to guarantee patient confidentiality in accordance with the legal stipulations applying to confidentiality of data. Study records (e.g., copies of eCRFs, regulatory documents) will be retained at the study site, along with adequate source documentation, according to ICH requirements. All study records must be available for audit by Dompé; its authorized representatives; and Regulatory Inspection by Regulatory Authority.

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, complete, consistent, enduring and available. Changes to source data should be traceable, should not obscure the original entry, and should be explained, if necessary, via an audit trail.

12.1.1 Systems Validation

Please refer to the Data Management Plan (DMP) created by the CRO and approved by the Sponsor.

12.2 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL

The following documents will be required from the Investigator prior to the site Initiation Visit:

- Current, signed, and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/Site Study Staff. Document updates should be provided at least every two years.
- A signed page of the final protocol and any amendments.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with the CRO, including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- A financial disclosure agreement completed and signed by the PI and all Sub-Investigators listed on Form FDA 1572 for US and on the Declaration of Interest (DoI) for EU. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.
- IRB/EC approval to conduct the study, including IRB/EC approval of protocol and any amendment, ICF, and any patient facing material.
- Documents of GCP training for PI and all Sub-Investigators listed on the Form FDA 1572 and according to ICH E6 (R2) for GCP.
- Other documents may be required and will be described in the essential document checklist outlined in the CRO Procedural Manual for site initiation and activation.

A copy of the IRB/IEC approval will be sent to Dompé along with relevant correspondence with the IRB/IEC, a roster of IRB/IEC members or the US Department of Health and Human Services general assurance number. The study will not be started until full written approval

has been obtained from the appropriate IRB/IEC. The letter of approval should be dated and should specify the study references (e.g., protocol number) and the date of the documents which were reviewed and approved.

The CRO appointed by Dompé, or the PI will submit any future amendment to the Protocol to the IRB/IEC which granted the original approval. Any amendment will be implemented only when full approval has been obtained from the appropriate IRB/IEC, except for those amendments which involve only logistical or administrative aspects of the study.

The current version of the Investigator brochure.is also required to be submitted to IRB/IEC prior to trial initiation. The CRO appointed by Dompé, or the PI will also send to the IRB/IEC any updated Investigator's Brochure.
The CRO appointed by Dompé, or the PI will also submit to the IRB/IEC which approved the protocol, at least annually, any required progress reports and study update, and will inform the IRB/IEC of the termination of the study. The CRO appointed by Dompé, or the PI will report to the IRB/EC any serious ADRs, life-threatening problems or deaths occurred at other sites participating to this clinical trial and/or in other clinical studies.

12.3 ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP E6 R2) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period however if required by the applicable regulatory requirements (e.g., in Europe 25 years). The Investigator should take measures to prevent accidental or premature destruction of these documents.

The essential documents include at least: the signed protocol, copies of the completed eCRFs, signed ICFs from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator's Site File and Pharmacy/Dispensing File.

The Investigator will inform Dompé of the storage location of these essential documents and must contact Dompé before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, he/she should consult with Dompé about this change.

No records may be transferred to another location or party without written notification to the Sponsor.

Dompé will inform the Investigator in writing when these documents no longer need to be retained.

12.4 LONG-TERM SAMPLE RETENTION

We will not acquire any biological samples as part of this study.

13 PRIOR PROTOCOL AMENDMENTS

AMENDMENT No. 1.0

To the CLINICAL STUDY PROTOCOL version No. 2.0 - Final November 6, 2023

in the "AMENDMENT version No. 1.0 – Final, November 16, 2023" to the "CLINICAL STUDY PROTOCOL version No. 2.0 – Final, November 16, 2023"
(Summary of Changes from Protocol version 1.1 to Protocol version 2.0)

SUMMARY OF CHANGES

Note:
The deletions from the original text are denoted in ~~red strikethrough text~~.
The additions to the original text are denoted in the green text.

Section	Original Text	Revised Text	Rationale for Changes
Section 1 PROTOCOL SUMMARY SYNOPSIS Number of Patients	**Number of Patients:** **Two hundred and ~~forty~~ (~~240, 80~~** per group) male or female patients need to be randomized in a 1:1:1 ratio to have at least ~~228~~ evaluable patients (~~76~~ per group). Patients randomized to the vehicle arm will be randomly assigned 1:1 to two different vehicle filling volumes, namely 1 ml or 2 ml of diluent, to preserve masking. The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrollment. Considering a screen failure rate of 20%, approximately ~~290~~ subjects need to be screened in order to have ~~240~~ patients enrolled.	**Number of Patients:** **Two hundred and ninety one** (**291, 97** per group) male or female patients need to be randomized in a 1:1:1 ratio to have at least **276** evaluable patients (**92** per group). Patients randomized to the vehicle arm will be randomly assigned 1:1 to two different vehicle filling volumes, namely 1 ml or 2 ml of diluent, to preserve masking. The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrollment. Considering a screen failure rate of 20%, approximately **350** subjects need to be screened in order to have **291** patients enrolled.	Increased the sample size as result of the statistical analysis revision requested by FDA
Section 1 PROTOCOL SUMMARY SYNOPSIS Criteria for Inclusion/Exclusion	**Criteria for Inclusion/Exclusion** **Inclusion criteria:** 3. Dry eye disease characterized by the following clinical features: a. Symptoms Assessment in Dry Eye (SANDE) questionnaire **(-)** ≥50,	**Criteria for Inclusion/Exclusion** **Inclusion criteria:** 3. Dry eye disease characterized by the following clinical features: a. Symptoms Assessment in Dry Eye (SANDE) questionnaire **Global Score** ≥50,	Added following Investigators' FAQ.
Section 1 PROTOCOL SUMMARY SYNOPSIS Criteria for Inclusion/Exclusion	**Criteria for Inclusion/Exclusion** **Exclusion criteria:** 13. ~~**Females of childbearing potential**~~ (ie, not surgically sterilized, or postmenopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions: (…)	**Criteria for Inclusion/Exclusion** **Exclusion criteria:** 13. **Fertile patients** (ie, not surgically sterilized, or postmenopausal **women** for at least 1 year) are excluded from participation in the study if they **do not practice abstinence from heterosexual intercourse as per**	Inserting update for compliance to US and EU guidelines on contraception rules in course of a clinical trial

Section	Original Text	Revised Text	Rationale for Changes
		usual and customary lifestyle, or are unwilling to use an acceptable form of contraception such as condom with spermicidal cream or jelly for males, or for females if they meet any one of the following conditions: (…)	
Section 1 PROTOCOL SUMMARY SYNOPSIS Other Endpoint	**Other Endpoint**	**Other Endpoint** [1st bullet point in revised text] *The key secondary efficacy endpoints are:* **● Proportion of patients improving to Schirmer-I test without anesthesia ≥10mm/5min in the Study Eye [Time Frame: at week 4 (V4)]**	Added as a first bullet point as requested by FDA
Section 1 PROTOCOL SUMMARY SYNOPSIS Other Endpoint	**Other Endpoint** [4th bullet point in Original Text] *The key secondary efficacy endpoints are:* ~~**● Mean change from baseline in CCI assessed by the CCI CCI scale [Time Frame: at week 4 (V4)]**~~	**Other Endpoint**	Moved to the secondary endpoints due to the change from hierarchical to Hochberg strategy, to handle multiplicity for key secondary endpoints.
Section 1 PROTOCOL SUMMARY SYNOPSIS Other Endpoint	**Other Endpoint** *The secondary efficacy endpoints are:* [2nd bullet point] ● Proportion of patients improving to Schirmer-I test without anesthesia ≥10mm/5min in the Study Eye [Time Frame: at week~~s 4 (V4) and~~ 8 (V5)]	**Other Endpoint** *The secondary efficacy endpoints are:* [Second 2nd bullet point] ● Proportion of patients improving to Schirmer-I test without anesthesia ≥10mm/5min in the Study Eye [Time Frame: at week 8 (V5)]	Deleted [at week 4 (V4)] since moved at 1st bullet point in *The key secondary efficacy endpoints* as per request of FDA.
Section 1 PROTOCOL SUMMARY SYNOPSIS Other Endpoint	**Other Endpoint** *The secondary efficacy endpoints are:* [7th bullet point]: ● Mean change from baseline in CCI assessed by the CCI CCI [Time Frame: at week 8 (V5)]	**Other Endpoint** *The secondary efficacy endpoints are:* [7th bullet point]: ● Mean change from baseline in CCI assessed by the CCI CCI Time Frame: at weeks **4 (V4) and** 8 (V5)]	Added [at week 4 (V4) and] since this is no longer evaluated in the Key Secondary Endpoints.
Section 1 PROTOCOL SUMMARY SYNOPSIS Methods, procedure and assessment	**Methods, procedure and assessment** **Visit 2 (Day 1): BASELINE** **6. Ophthalmic examinations in each eye:** L. ~~**Assess**~~ CCI CCI CCI	**Methods, procedure and assessment** **Visit 2 (Day 1): BASELINE** **6. Ophthalmic examinations in each eye:** L. **Perform** CCI **eye drop** CCI **response test**	Updated in order to better explain this procedure at Visit 2 for the use of

Section	Original Text	Revised Text	Rationale for Changes
		(only in patients with CCI CCI)	anesthetic eye drops
Section 1 PROTOCOL SUMMARY SYNOPSIS Methods, procedure and assessment	**Methods, procedure and assessment** **Visit 2 (Day 1): BASELINE** **6. Ophthalmic examinations in each eye:** **M. ~~Instill~~** CCI **~~eyedrop~~** **N. ~~Assess response to~~** CCI CCI **~~eyedrop using~~** CCI CCI	**Methods, procedure and assessment** **Visit 2 (Day 1): BASELINE** **6. Ophthalmic examinations in each eye:**	Removed
Section 1. PROTOCOL SUMMARY SYNOPSIS Methods, procedure and assessment	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT (OR EARLY EXIT VISIT)** **L. ~~Assess~~** CCI CCI	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT (OR EARLY EXIT VISIT)** L. Perform CCI eye drop CCI response test (only in patients with CCI CCI)	Updated in order to better explain this procedure at Visit 4 for the use of anesthetic eye drops
Section 1. PROTOCOL SUMMARY SYNOPSIS Methods, procedure and assessment	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT (OR EARLY EXIT VISIT)** **~~L. Instill~~** CCI **~~eye drop~~** **~~M. Assess response to~~** CCI CCI CCI CCI	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT (OR EARLY EXIT VISIT)**	Removed
Section 1. PROTOCOL SUMMARY SYNOPSIS Statistical considerations	**Statistical considerations** **Sample size and randomization (First paragraph, 2nd sentence):** >, a total sample size of **~~228~~** evaluable patients (**~~76~~** per group) allows to achieve an overall power of 80% to show superiority of at least one rhNGF ophthalmic solution dose over Vehicle on the improvement from baseline of SANDE Global Score at week 8, considering an overall one-sided alpha of **~~0.05.~~** The sample size calculation takes into consideration a Bonferroni correction for two comparisons (each active vs. vehicle). Assuming a 5% rate of patients not evaluable after enrollment, the total number of patients to be enrolled in the study will be about **~~240.~~** Considering a screen failure rate of 20%, approximately **~~290~~** subjects need to be screened in order to have **~~240~~** subjects enrolled. >	**Statistical considerations** **Sample size and randomization (First paragraph, 2nd sentence):** >, a total sample size of **276** evaluable patients (**92** per group) allows to achieve an overall power of 80% to show superiority of at least one rhNGF ophthalmic solution dose over Vehicle on the improvement from baseline of SANDE Global Score at week 8, considering an overall one-sided alpha of **0.025**. The sample size calculation takes into consideration a Bonferroni correction for two comparisons (each active vs. vehicle). Assuming a 5% rate of patients not evaluable after enrollment, the total number of patients to be enrolled in the study will be about **291**. Considering a screen failure rate of 20%, approximately **350** subjects need to be screened in order to have **291** subjects enrolled. >	Increasing the sample size as result of the statistical analysis revision requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 1. PROTOCOL SUMMARY SYNOPSIS Statistical considerations	**Statistical considerations** **Statistical methods** **(Third paragraph):** ~~**If the primary analysis of primary endpoint leads to rejection of the null hypothesis for at least one of the two concentrations vs. vehicle, key secondary endpoints will be tested in a conditional sequential manner to show superiority of that concentration vs. vehicle according to the pre-defined ranking sequence to control the type I error 0.025 one-sided for each concentration vs vehicle.**~~ Each key secondary endpoint will be analyzed in the same manner described for the primary endpoint, with the only difference that ANCOVA will replace the MMRM.	**Statistical considerations** **Statistical methods** **(Third paragraph):** **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.** Each key secondary endpoint will be analyzed in the same manner described for the primary endpoint, with the only difference that ANCOVA will replace the MMRM **for continuous endpoints and logistic model will replace MMRM for the binary endpoint.**	Changed the multiple testing strategy as requested by FDA.
Section 1. PROTOCOL SUMMARY SCHEDULE OF ACTIVITIES	**Table 1. Schedule of activities planned during the different visits (trial procedures section)** ~~**Instill** CCI [redacted] **eyedrop**~~	**Table 1. Schedule of activities planned during the different visits (trial procedures section)**	Updated table to optimize trial procedures
Section 1. PROTOCOL SUMMARY SCHEDULE OF ACTIVITIES	**Table 1. Schedule of activities planned during the different visits (trial procedures section)** CCI [redacted] eyedrop response test	**Table 1. Schedule of activities planned during the different visits (trial procedures section)** Topical anesthetic eyedrop response test (CCI [redacted] CCI **before and after** CCI [redacted] CCI [redacted] **eyedrop) ***** ***** Only in patients with** CCI [redacted] CCI [redacted]	Updated trial procedure and added Table footnote explanation (***)
Section 1. PROTOCOL SUMMARY SCHEDULE OF ACTIVITIES	**Table 1. Schedule of activities planned during the different visits (trial procedures section)** CCI [redacted]	**Table 1. Schedule of activities planned during the different visits (trial procedures section)**	Updated table to optimize trial procedures

Section	Original Text	Revised Text	Rationale for Changes
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives [1st bullet point]:** ● To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in ~~**increasing the number of patients with**~~ improving reflex tear production as compared to vehicle.	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives [1st bullet point]:** ● To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in **improving** reflex tear production as compared to vehicle	Revised text
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints [1st bullet point]:** ~~**● Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye [Time frame: at week 4 (V4)]**~~	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints [1st bullet point]:** **● Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye [Time Frame: at week 4 (V4)]**	Revised text as for FDA request
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** ● To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving ~~**mean**~~ reflex tear production as compared to vehicle.	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** ● To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving reflex tear production as compared to vehicle.	Revised text
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS AND ENDPOINTS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints** -	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints** **● Mean change from baseline in Schirmer-I score without anesthesia in the Study Eye [Time frame: at week 4 (V4)]**	Added the Endpoint
3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** ~~**● To evaluate the efficacy of the new formulation of rhNGF ophthalmic solution in improving associated symptoms in DED as compared to vehicle.**~~	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives-**	Removed the Objective from key secondary
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints** ~~**● Mean change from baseline of the**~~ CCI [redacted] CCI [redacted] ~~**Time Frame: at week 4 (V4)]**~~	**Table 3. Trial objectives and endpoints;** **Key Secondary Objectives** **Efficacy endpoints**	Moved the Objective to secondary

Section	Original Text	Revised Text	Rationale for Changes
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Secondary and Exploratory (*)** **Efficacy Endpoints** Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye [Time Frame: at week~~s 4 (V4) and~~ 8 (V5)]	**Table 3. Trial objectives and endpoints;** **Secondary and Exploratory (*)** **Efficacy Endpoints** Proportion of patients improving to Schirmer-I test without anesthesia ≥ 10 mm/5min in the Study Eye [Time Frame: at week 8 (V5)]	Revised the Endpoint as V4 moved to key secondary endpoint
Section 3.1 TRIAL OBJECTIVES, ENDPOINTS AND ESTIMANDS	**Table 3. Trial objectives and endpoints;** **Secondary and Exploratory (*)** **Efficacy Endpoints** Mean change from baseline of the CCI CCI [Time Frame: at week 8 (V5)]	**Table 3. Trial objectives and endpoints;** **Secondary and Exploratory (*)** **Efficacy Endpoints** Mean change from baseline of the CCI CCI [Time Frame: at weeks 4 (V4) and 8 (V5)]	Revised the Endpoint

Section	Original Text	Revised Text	Rationale for Changes
Section 3.2. PRIMARY AND KEY SECONDARY ESTIMANDS	~~**3.1. PRIMARY/ SECONDARY/ EXPLORATORY**~~ ESTIMAND	**3.2. PRIMARY AND KEY SECONDARY** ESTIMANDS	Estimands for primary and key secondary endpoints updated as requested by FDA
Section 3.2.1. Primary estimand	~~**3.1.1.**~~ -	**3.2.1. Primary estimand**	Revised the list of "Intercurrent events and strategies to handle them" as requested by FDA
Section 3.2.1. Primary estimand	~~**3.1.1.**~~ [4th bullet point] • Intercurrent event	**3.2.1.** [4th bullet point] • Intercurrent events **(ICEs) and strategies** **(ICEs) and strategies to handle them:** **o 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 10.6.2 for further details).** **o Use of a prohibited medication, as listed in Section 6.6.1, in the week before any assessment of SANDE Global Score: a hypothetical strategy**	Revised the list of "Intercurrent events and strategies to handle them" as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
		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 10.6.2 for further details).	
Section 3.2.1. Primary estimand	~~3.1.1.~~ **[5th bullet point]** • Population-level summary: difference in mean change from baseline to week 8 in ~~SANDE Global Score~~ between each concentration group of IMP (groups IMP1 and IMP2) and the vehicle group.	**3.2.1. [5th bullet point]** • 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 IMP (groups IMP1 and IMP2) and the vehicle groups **as derived by the Mixed Model for repeated measures defined in Section 10.6.2.** **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 subjects in the week before any study evaluation.**	Updated as result of the revised estimand for primary endpoint as requested by FDA
Section 3.1.2 Key secondary estimands		**Key secondary estimands** ***Key secondary objective #1*** **The estimand for the key secondary objective #1 is defined as follows: •** **Treatment: 4 weeks of TID administration of rhNGF new formulation (two concentrations 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).** **• Intercurrent events (ICEs) and strategies to handle them:** **o 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**	Estimands for key secondary endpoints added as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
		copy-reference approach (see Section 10.6.3 for further details). **o Use of a prohibited medication, as listed in Section 6.6.1, 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 10.6.3 for further details), then the proportion of subjects 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 10.6.3.** **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 subjects 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 TID administration of rhNGF new formulation (two concentrations 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).** **• Intercurrent events (ICEs) and strategies to handle them:**	

Section	Original Text	Revised Text	Rationale for Changes
		o 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 10.6.3 for further details). o Use of a prohibited medication, as listed in Section 6.6.1, 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 10.6.3 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 10.6.3. 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 subjects 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 TID administration of rhNGF new formulation (two concentrations and vehicle). • Population: Male and female patients aged ≥ 18 years with dry eye. Full Analysis Set. • Variable: mean change from baseline in total corneal fluorescein staining (NEI scale) in the Study Eye as	

Section	Original Text	Revised Text	Rationale for Changes
		assessed by the investigator at week 4 (V4). **• Intercurrent events (ICEs) and strategies to handle them:** **o 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 10.6.3 for further details).** **o Use of a prohibited medication listed in Section 6.6.1 in the week before any assessment of corneal fluorescein staining: 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 10.6.3 for further details).** **• Population-level summary: difference in mean change from baseline to week 4 in total corneal fluorescein staining (NEI scale) 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 10.6.3.** **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 corneal fluorescein staining (NEI scale) 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 subjects in the week before any study evaluation.**	

Section	Original Text	Revised Text	Rationale for Changes
Section 4.1 DESCRIPTION OF TRIAL DESIGN	~~3.1.~~ DESCRIPTION OF TRIAL DESIGN **[Text under 2nd bullet point]** The total number of patients to be enrolled in the study will be about ~~240, 80~~ per group to target ~~228~~ evaluable patients (~~76~~ per group). The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrolment. Considering a screen failure rate of 20%, approximately ~~290~~ subjects need to be screened in order to have ~~240~~ patients enrolled and at least ~~228~~ patients ~~treated.~~	4.1 DESCRIPTION OF TRIAL DESIGN **[Text under 2nd bullet point]** The total number of patients to be enrolled in the study will be about 291, 97 per group to target 276 evaluable patients (92 per group). The total number has been obtained considering a 5% rate of patients not evaluable for primary analysis after enrolment. Considering a screen failure rate of 20%, approximately 350 subjects need to be screened in order to have 291 patients enrolled and at least 276 patients evaluable.	Increasing the sample size as result of the statistical analysis revision requested by FDA
Section 5.1 INCLUSION CRITERIA	**Inclusion Criteria** 3) Moderate-to-severe dry eye characterized by the following clinical features: a) Symptoms Assessment in Dry Eye (SANDE) questionnaire ≥50, and	**Inclusion Criteria** 3) Moderate-to-severe dry eye characterized by the following clinical features: a) Symptoms Assessment in Dry Eye (SANDE) questionnaire **global score** ≥50, and	Updated inclusion criteria following Investigators' FAQ.
Section 5.2 EXCLUSION CRITERIA	**Exclusion Criteria** **13) ~~Females of childbearing potential~~** (ie, not surgically sterilized or postmenopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:	**Exclusion Criteria** **13) Fertile patients** (ie, not surgically sterilized, or postmenopausal **women** for at least 1 year) are excluded from participation in the study if they **do not practice abstinence from heterosexual intercourse as per usual and customary lifestyle, or are unwilling to use an acceptable form of contraception such as condom with spermicidal cream or jelly for males, or for females if they** meet any one of the following conditions:	Updated exclusion criteria for a better compliance to US and EU guidelines on contraception rules in course of a clinical trial
Section 5.4 SCREEN FAILURES	**Screen Failures** We expect to screen approximately ~~290~~ subjects in order to have ~~240~~ subjects enrolled. The sample size remains at ~~240~~ subjects independent of screen failures.	**Screen Failures** We expect to screen approximately 350 subjects in order to have 291 subjects enrolled. The sample size remains at 291 subjects independent of screen failures.	Increasing the sample size as result of the statistical analysis revision requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 6.3 PREPARATION, HANDLING, STORAGE AND ACCOUNTABILITY	**A text in paragraph starting with "Upon receipt of the Study Products …..":** ~~Accountability of AT will be done through patient diary.~~	**A text in paragraph starting with "Upon receipt of the Study Products …..":**	Operational changes
6.3 PREPARATION, HANDLING, STORAGE AND ACCOUNTABILITY Page 56	**A text in paragraph starting with "During the course of the study …..":** Partially used or unused study drug boxes will be verified by the CRO delegated Monitor ~~within one month after completion of the trial.~~	**A text in paragraph starting with "During the course of the study …..":** Partially used or unused study drug boxes will be verified by the CRO delegated Monitor.	Operational changes
6.3 PREPARATION, HANDLING, STORAGE AND ACCOUNTABILITY Page 56 (57)	**A text in paragraph starting with "During the Run-in period …..":** Agitation of the reconstituted product may cause foaming ~~and/or particle formation~~, hence should be avoided at any time.	**A text in paragraph starting with "During the Run-in period …..":** Agitation of the reconstituted product may cause foaming, hence should be avoided at any time.	Updated due to a mistake
Section 6.4.2 Randomization	**Randomization:** **[2nd paragraph first sentence]:** Eligible patients will be randomized in a 1:1:1 ratio to rhNGF C mcg/mL TID (about ~~80~~ patients), rhNGF C mcg/mL TID (about ~~80~~ patients) or vehicle ophthalmic solution TID (about ~~80~~ patients).	**Randomization:** **2nd paragraph:** Eligible patients will be randomized in a 1:1:1 ratio to rhNGF C mcg/mL TID (about 97 patients), rhNGF C mcg/mL TID (about 97 patients) or vehicle ophthalmic solution TID (about 97 patients).	Updated statistical analysis as requested by FDA
Section 6.4.2 Randomization	**Randomization:** **Last paragraph:** The enrollment of patients will be scheduled in order to assure an inclusion of approximately ~~240~~ patients to ensure at least ~~228~~ evaluable patients assuming up to 5% dropout rate.	**Randomization:** **Last paragraph:** The enrollment of patients will be scheduled in order to assure an inclusion of approximately 291 patients to ensure at least 276 evaluable patients assuming up to 5% dropout rate.	Updated statistical analysis as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 6.5 TRIAL INTERVENTION COMPLIANCE	**Trial Intervention Compliance [2nd paragraph]:** -	**Trial Intervention Compliance [2nd paragraph]:** **Starting from the first IMP administration done at the baseline visit until the last expected administration (considering 1 drop TID, i.e. 3 drops per day), compliance for the study eye will be derived as the proportion (%) between the actual number of drops administered in the study eye and the expected number of drops in the study eye as if the subjects completed the study (up to 84 drops depending on the date of treatment completion status collected in the eCRF; for patients who prematurely discontinue the treatment, the expected number is set to 84). Further details will be inserted in the SAP.** **Assessment of the IMP accountability will be made by determining the number of Study Medication vials (CCI product) dispensed to the patient at Baseline/V2 and at Week 2/V3 and the number of unused Study Medication vials returned at Week 4/V4 (EOT). The number of used and unused vials of diluent will also be collected.**	Updated. The derivation of the compliance has been aligned through the entire document by considering the number of drops instilled collected in the patient diary.
Section 6.6.1 Prohibited Concomitant Therapy	**Prohibited Concomitant Therapy Last sentence:** -	**Prohibited Concomitant Therapy Last sentence:** **Before the study unmasking, a masked review of the concomitant medication taken by the patients during the study will be done in order to verify the presence of prohibited medication, their frequency and dosage in order to define the severity of the deviation.**	Updated to improve procedural explanation
Section 6.6.2 Permitted Concomitant Therapy	**Permitted Concomitant Therapy [4th paragraph]:** -	**Permitted Concomitant Therapy [4th paragraph]:** **During the entire duration of the study, any use of AT not provided by the sponsor will be recorded as concomitant medication and considered as protocol deviation.**	Updated

Section	Original Text	Revised Text	Rationale for Changes
Section 7.1. CRITERIA FOR DISCONTINUATION OF TRIAL INTERVENTION	~~5.1~~. CRITERIA FOR ~~PERMANENT~~ DISCONTINUATION OF TRIAL INTERVENTION	7.1. CRITERIA FOR DISCONTINUATION OF TRIAL INTERVENTION	Updated of the list of possible reasons for treatment and/or study discontinuation as request by FDA
Section 7.1.1. Criteria for Permanent Discontinuation of Trial Intervention	~~5.1.1.~~ **Criteria for Permanent Discontinuation of Trial Intervention** Patients with ~~insufficient therapeutic response,~~ tolerability issues, or worsening of symptoms and/or signs may be discontinued at any time during the study:	**7.1.1. Criteria for Permanent Discontinuation of Trial Intervention** **Treatment discontinuation** Patients with tolerability issues, or worsening of symptoms and/or signs may be discontinued at any time during the study **for the following reasons:** **1) AEs that at Investigator discretion require treatment discontinuation.** **2) Worsening of the clinical condition that requires treatment discontinuation per physician decision.** **3) Violation of study procedures that represent a protocol deviation deemed by the Investigator to require treatment discontinuation.** **4) Withdrawal of patient's consent for any reason.** **5) Patient is lost-to-follow-up notwithstanding all efforts to contact him/her (at least three documented attempts by phone and/or email).** **6) Other (e.g. death, pregnancy, planned operation).** **Except for items from #4 to #6 of the list reported above that imply also a study discontinuation, in case one of the events from #1 to #3 occurs, patients will be asked to complete the assessments planned by the protocol regardless the discontinuation of the study treatment. In case of more than one reason, the main one will be reported in eCRF.**	Updated of the list of possible reasons for treatment and/or study discontinuation as request by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 7.1.1. Criteria for Permanent Discontinuation of Trial Intervention	Patients should be discontinued from the study for one of the following reasons: ~~**Withdrawal of patient's consent for whatever reason.**~~ ~~**Any condition for which study procedures may compromise the safety of the patient.**~~	**Study discontinuation.** Patients should be discontinued from the study for one of the following reasons: **1) Withdrawal of patient's consent for any reason.** **2) AEs, protocol deviations and any other condition for which study procedures**	Updated of the list of possible reasons for treatment and/or study discontinuation as request by FDA

	~~Patient is lost-to-follow-up notwithstanding all efforts to contact him/her (at least three documented attempts by phone and/or email).~~	**may compromise the safety and well-being of the patient, as per physician decision.** **3) Patient is lost-to-follow-up notwithstanding all efforts to contact him/her (at least three documented attempts by phone and/or email).** **4) Other (e.g. death, pregnancy, planned operation).**	
Section 7.1.1. Criteria for Permanent Discontinuation of Trial Intervention	In all other situations, including cases where patients should be discontinued from study treatment, ~~for example because of the occurrence of an AE or for disease progression/worsening.~~ all efforts will be made to complete study visits and procedures as per protocol.	In all other situations, including cases where patients should be discontinued from study treatment, all efforts will be made to complete study visits and procedures as per protocol.	Updated
Section 8.1 SCREENING/ BASELINE	**Visit 2 - Day 1: BASELINE** **6. Ophthalmic Examinations in each eye:** ~~**L. Instill topical local anesthetic eyedrop**~~ **M.** ~~**Assess response to**~~ topical ~~**local**~~ anesthetic eyedrop.	**Visit 2 - Day 1: BASELINE** **6. Ophthalmic Examinations in each eye:** **L. Perform** CCI eyedrop CCI **esponse test (only in patients with** CCI	Updated
Section 8.2 TRIAL VISITS AND FOLLOW-UP ASSESSMENTS	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT or EARLY EXIT VISIT** **6. Ophthalmic Examinations:** ~~**L. Instill topical local anesthetic eyedrop**~~ ~~**M. Assess response to topical local anesthetic eyedrop.**~~	**Visit 4 - Week 4 (Day 28±1): END OF TREATMENT VISIT or EARLY EXIT VISIT** **L. Perform** CCI eyedrop **response test (only in patients with** CCI **.**	Updated due to a mistake
Section 8.3 ASSESSMENTS AND CLINICAL DEFINITIONS	**Table 6. List of assessments - Assessment type:** CCI	**Table 6. List of assessments - Assessment type:** CCI **(to perform only in patients with** CCI	Updated

Section	Original Text	Revised Text	Rationale for Changes
Section 10.1 SAMPLE SIZE	**SAMPLE SIZE Second sentence onwards:** 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 (eye drop) formulation, a total sample size of ~~228~~ evaluable patients (~~76~~ per group) is needed. This will allow to achieve an overall power of 80% 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 of ~~0.05.~~ The sample size calculation takes into consideration a Bonferroni correction for two comparisons (2 concentration groups versus vehicle), i.e. the alpha level has been set to ~~0.025~~ one-sided for each comparison. ~~**An overall one-sided alpha level of 0.05 is considerate appropriate for a phase II study**~~ Assuming a 5% rate of patients not evaluable for primary analysis after enrollment, the total number of patients to be enrolled in the study will be about ~~240~~. Enrollment will be competitive. In Dompé previous studies, a screen failure rate of about 20% has been observed (NGF0121 - PROTEGO-1). This fact implies that approximately ~~290~~ subjects will need to be screened in order to have ~~240~~ patients enrolled and at least ~~228~~ ~~**treated**~~. In any case, the screen procedures should be interrupted when the expected sample size of ~~240~~ subjects has been reached.	**SAMPLE SIZE Second sentence onwards:** 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 (eye drop) formulation, a total sample size of **276** evaluable patients (**92** per group) is needed. This will allow to achieve an overall power of 80% 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 of **0.025**. The sample size calculation takes into consideration a Bonferroni correction for two comparisons (2 concentration groups versus vehicle), i.e. the alpha level has been set to **0.0125** one-sided for each comparison. Assuming a 5% rate 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)**. Enrollment will be competitive. In Dompé previous studies, a screen failure rate of about 20% has been observed (NGF0121 - PROTEGO-1). This fact implies that approximately **350** subjects 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** subjects has been reached.	Updated Statistics as requested by FDA
Section 10.5 ESTIMANDS	~~**7.10.**~~ **ESTIMANDS** The estimand for the primary ~~**objective is defined as follows:**~~	**10.5 ESTIMANDS** The estimand**s** for the primary **and key secondary objectives are defined in Sections 3.2.1 and 3.2.2, respectively.**	Estimand for primary endpoint has been updated while

Section	Original Text	Revised Text	Rationale for Changes
	~~Treatment: 4 weeks follow up after 4 weeks of TID administration of rhNGF new formulation (two concentrations 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 event: a treatment policy strategy will be applied where all observed values will be used regardless of the occurrence of the intercurrent events. For patients with missing observations (such as those who have revoked their informed consent to participate in the study), the retrieve drop-out or the copy-reference imputation method will be used (see § 10.6.2).~~ ~~● Population-level summary: difference in least squared change from baseline to week 8 (V5) in SANDE Global Score between each concentration group of IMP (groups IMP1 and IMP2) and the vehicle group, as derived by the linear mixed model defined in §10.6.2.~~		estimands for the key secondary endpoints has been added as requested by FDA. To avoid duplicate sections, the description of the estimands have been moved to Section 3.2.1 and 3.2.2.
Section 10.6 STATISTICAL METHOD-OLOGY	~~7.11.~~ STATISTICAL METHODOLOGY	10.6 STATISTICAL METHODOLOGY	Updated the entire section as consequence of the changes requested by FDA.
Section 10.6.1 Descriptive Statistics	**Descriptive Statistics [3rd paragraph]:** ~~The significance level used for primary and key secondary analysis will be 0.025 one-sided for each active arm versus vehicle (in order to preserve an overall 0.05 one-sided).~~ **Unless otherwise specified, the significance level used for other statistical testing will be 0.05 and two-sided tests will be used. All patient data collected**	**Descriptive Statistics [3rd paragraph]:** **The significance level used for primary analysis will be 0.0125 one-sided for each active arm versus vehicle and will be determined in accordance with the Hochberg approach (Hochberg Y. 1988) for the key secondary endpoints (in order to preserve an overall 0.025 one-sided). Unless otherwise specified, the significance level used for other statistical testing will be 0.05 and**	Updated. Type-I error for primary and key secondary endpoints has been revised after accepting the comments of FDA. Multiple testing

Section	Original Text	Revised Text	Rationale for Changes
	on the eCRF will be listed by patient and center.	**two-sided tests will be used. All patient data collected on the eCRF will be listed by patient and center.**	procedure for key secondary endpoints has been modified as requested by FDA.
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables** **Second paragraph, second sentence onwards:** Each concentration group will be consequently tested at one-sided alpha = ~~**0.025**~~, in order to preserve the overall one-sided alpha of ~~**0.05**~~. The null hypothesis H0 will be rejected if the associated primary analysis one-sided p-value will be lower than ~~**0.025**~~ for at least one of the two concentration groups versus vehicle.	**Primary Analysis of efficacy variables** **Second paragraph, second sentence onwards:** Each concentration group will be consequently tested at one-sided alpha = **0.0125**, in order to preserve the overall one-sided alpha of **0.025**. The null hypothesis H0 will be rejected if the associated primary analysis one-sided p-value will be lower than **0.0125** for at least one of the two concentration groups versus vehicle.	Updated. Type-I error for primary and key secondary endpoints has been revised after accepting the comments of FDA.
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables** **[End of third paragraph]:** -	**Primary Analysis of efficacy variables** **[End of third paragraph]:** **The model can be summarized as follows:** **change from baseline=β_0+ β_1 treatment+ β_2 base+ β_3 visit+ β_4 treatment*visit+ ε** **where ε~N(0,R) and R is the unstructured covariance matrix with blocks defined by the subjects. Further details and SAS code will be described in the SAP that will be generated before the DB lock and the unmasking.** **For patients analyzed under the hypothetical strategy (see Section 3.2.1), any data collected in the week after the ICE of interest (intake of prohibited medications) will be considered as missing for the purpose of the primary analysis and will be imputed, as well as the other missing data (for example for lost to follow-up, patients who withdrew their consent), by using the multiple imputation described below with a copy-reference approach.**	Updated statistical analysis as requested by FDA. Model has been added as requested by FDA. Statistical methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.

Section	Original Text	Revised Text	Rationale for Changes
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables- [5th paragraph]:** ~~**Since patients who discontinue the treatment with the IMP will not be withdrawn from the study but will be asked to complete safety and efficacy assessments as per the protocol, the primary analysis will consider these evaluations as if the intercurrent events (such as AEs or disease progression leading to treatment discontinuation) did not occur. Missing data under treatment policy strategy (such as for patients who were lost to follow-up or who revoked their informed consent) will be addressed by using multiple imputation based on retrieve drop-outs (MI-RD) information. This approach is based on the assumption that missing data would have been similar to the assessments of the retrieved drop-outs if they were assessed. Retrieved drop-outs are defined as patients who discontinue study treatment and decide to remain in the study by following the schedule of assessments and continuing to adhere to protocol requirements.**~~ ~~**In order to apply the MI-RD approach, enough retrieved drop-out data must be available to allow convergence of a regression model. MI-RD will be implemented into different steps:**~~ ~~**Intermittent missing data up to primary available time point will be imputed using a MCMC method assuming missing at random (MAR) data.**~~ ~~**The remaining missing values (i.e. after withdrawal) will be imputed based on observed data of retrieve drop-outs, assuming a monotone missing data pattern. In this way, the imputation method will be missing not at random (MNAR). Specifically, MI-RD will be**~~	**Primary Analysis of efficacy variables -[5th paragraph]:** **Missing data that may be obtained notwithstanding the intent to apply the treatment policy strategy and after having applied the hypothetical strategy when needed, will be addressed by using multiple imputation (MI) based on copy-reference approach.**	Statistical methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.

Section	Original Text	Revised Text	Rationale for Changes
	~~performed based on subjects' allocated treatment arm and baseline and intermediate values (observed and imputed at Step 1) as covariates in a regression model using data from subjects that discontinued the treatment but have the primary endpoint measurement. Gender and age will also be added as covariates.~~ ~~The fully imputed datasets will be analyzed and results will be combined using Rubin's rule to draw inference.~~ ~~If not enough data are retrieved after study treatment discontinuation (in order words, in case of non-convergence of the MI-RD regression model), the same model described above will be fit using data from subjects of the control group (copy-reference method).~~		

Section	Original Text	Revised Text	Rationale for Changes
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables-End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **1)** Intermittent ~~missing data up-to primary available time point will be imputed using a MCMC method assuming MAR data.~~	**Primary Analysis of efficacy variables-End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **1)** 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.**	Statistical methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables-End of section:** **Specifically, the copy-reference approach will be**	**Primary Analysis of efficacy variables-End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **2) For each imputed dataset, the change**	Statistical methods updated as consequence of the revised estimand and strategy to

Section	Original Text	Revised Text	Rationale for Changes
	implemented in this way: ~~**2) The remaining missing values in the vehicle group with a monotone missing data pattern will be imputed based on observed data of the vehicle group with available endpoint, while the remaining missing values in the active treatment groups with a monotone missing data pattern will be imputed based on observed and imputed data of the vehicle patients.**~~	**from baseline at Week 4 and Week 8 is derived.**	handle intercurrent events as requested by FDA.
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables- End of section:** **Specifically, the copy-reference approach will be implemented in this way:** -	**Primary Analysis of efficacy variables- End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **3) 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. Subject will be considered as cluster variable and an unstructured covariance matrix will be set to model the within-subject correlation.**	Statistical methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.
Section 10.6.2 Primary Analysis of efficacy variables	**Primary Analysis of efficacy variables- End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **4)** ~~**The fully imputed datasets will be analyzed and**~~ results will be combined using Rubin's rule to draw inference.	**Primary Analysis of efficacy variables- End of section:** **Specifically, the copy-reference approach will be implemented in this way:** **4)** Results **obtained on each imputed dataset** will be combined using Rubin's rule to draw inference.	Statistical methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.
Section 10.6.2 Primary	**Primary Analysis of**	**Primary Analysis of efficacy variables**	Statistical

Section	Original Text	Revised Text	Rationale for Changes
Analysis of efficacy variables	**efficacy variables [second to last paragraph]:** ~~**The choice between MI-RD or the copy-reference approach will be done at the time of the analysis and reported in the CSR. Independently on the approach used for missing data,**~~ the adjusted estimated treatment difference between each concentration of the rhNGF new formulation and vehicle will be displayed together with the corresponding two-sided 95% confidence interval (CI) and one-sided p-value.	**[second to last paragraph]:** The adjusted estimated treatment difference between each concentration of the rhNGF new formulation and vehicle will be displayed together with the corresponding two-sided 95% confidence interval (CI) and one-sided p-value.	methods updated as consequence of the revised estimand and strategy to handle intercurrent events as requested by FDA.
Section 10.6.3 Sensitivity and Supportive Analyses	~~**5.1.2.** Sensitivity **Analysis**~~	**10.6.3** Sensitivity **and Supportive Analyses**	Updated Sensitivity and Supportive analyses as requested by FDA
Section 10.6.3 Sensitivity and Supportive Analyses	~~**5.1.2.** Sensitivity **Analysis**~~ **[2nd paragraph]:** 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.	**10.6.3** Sensitivity **and Supportive Analyses** **[2nd paragraph]:** 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. **They will be done after having applied the hypothetical strategy for ICEs, when expected (i.e. after having set to missing the observed values collected in the week after the use of a prohibited medication):**	Updated Sensitivity and Supportive analyses as requested by FDA
Section 10.6.3 Sensitivity and Supportive Analyses	~~**5.1.2.** Sensitivity **Analysis**~~ **[2nd paragraph, 1st bullet point]:** • The comparison between treatment and control will be performed in the FAS	**10.6.3** Sensitivity **and Supportive Analyses** **[2nd paragraph, 1st bullet point]:** • The comparison between treatment and control will be performed in the FAS population by means of MI under **the missing at random** (MAR) instead of the MNAR assumption.	Updated Sensitivity and Supportive analyses as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
	population by means of MI under the MAR instead of the MNAR assumption.		
Section 10.6.3 Sensitivity and Supportive Analyses	~~5.1.2.~~ Sensitivity ~~Analysis~~ **[2nd paragraph, Text under 2nd bullet point]:** Tipping point will be based on iterative application of ~~MI-RD (or MI under wash-out approach)~~, 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≥~~0.025~~) for each arm. Tipping point analysis will be run only if the superiority has been proven for at least one of the two concentrations of the rhNGF.	**10.6.3.** Sensitivity **and Supportive Analyses** **[2nd paragraph, Text under 2nd bullet point]:** Tipping point will be based on iterative application of **MI described in Section 10.6.2**, 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 two concentrations of the rhNGF. **The tipping point will be firstly searched in the values defined by ±SD. If not found, the range will be enlarged to ±2SD.**	Updated Sensitivity and Supportive analyses as requested by FDA
Section 10.6.3 Sensitivity and Supportive Analyses	~~5.1.2.~~ Sensitivity ~~Analysis~~ **[2nd paragraph, 3th bullet point]:** • Analysis on complete cases only (i.e. without considering patients with missing primary endpoint).	**10.6.3** Sensitivity **and Supportive Analyses** **[2nd paragraph, 3th bullet point]:** • Analysis on complete cases only (i.e. without considering patients with missing primary endpoint **for any reason and after having discarded any observed case after the ICEs under the hypothetical strategy**).	Updated Sensitivity and Supportive analyses as requested by FDA
Section 10.6.3 Sensitivity and Supportive Analyses	~~5.1.2.~~ Sensitivity ~~Analysis~~ **[2nd paragraph, 4th and 5th bullet point]:**	**10.6.3** Sensitivity **and Supportive Analyses** **[2nd paragraph, 4th and 5th bullet point]:** **• Analysis on all observed cases (i.e. without considering patients with missing primary endpoint for any reason and including data discarded due to ICEs handled under the hypothetical strategy).** **• Analysis on the PP set instead of FAS.**	Updated Sensitivity and Supportive analyses as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 10.6.3 Sensitivity and Supportive Analyses	~~5.1.2.~~ Sensitivity ~~Analysis~~ **[3rd paragraph onwards]:**	10.6.3 Sensitivity and Supportive Analyses **[3rd paragraph onwards]:** **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 estimand:** **o 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 10.6.2 for further details).** **o Use of a prohibited medication listed in Section 6.6.1 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 discarded. Missing data will be imputed by using a multiple imputation with copy-reference approach (see Section 10.6.2 for further details).** **• Modified strategy #2 to handle ICEs for primary estimand:** **o 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 10.6.2 for further details).** **o Use of a prohibited medication listed in Section 6.6.1 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 at week 8.**	Updated Sensitivity and Supportive analyses as requested by FDA
Section 10.6.3 Sensitivity and Supportive Analyses	~~5.1.2.~~ Sensitivity ~~Analysis~~ **[Last 3 paragraphs, end of section]** ~~**Further details on sensitivity analysis will be provided in the SAP.**~~	10.6.3 Sensitivity and Supportive Analyses **[Last 3 paragraphs, end of section]** **The modified estimand #1 will be analyzed as described for the primary estimand.** **For the modified estimand #2, the trimmed approach by Permutt and Li (Permutt T and Feng L. 2017) will be used. This method provides an exact test that does not require any modeling**	Updated Sensitivity and Supportive analyses as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
		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 best SANDE values at week 8 (i.e. with lowest values, since for SANDE questionnaire lower values are better). For this analysis k=50%, 70%, and 90% will be used. 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.** **Further details on sensitivity and supportive analyses will be provided in the SAP.**	

Section	Original Text	Revised Text	Rationale for Changes
Section 10.6.4 Secondary and Exploratory Analysis	~~5.1.3.~~ **Secondary and Exploratory Analysis [first three paragraphs]** If the primary analysis of primary endpoint leads to rejection of the null hypotheses, key secondary endpoints will be tested ~~in a conditional sequential manner~~ to show superiority of each concentration for which superiority vs. vehicle has been proven in the primary analysis ~~according to the pre-defined hierarchical sequence (see order in Section 3).~~ This ~~hierarchical~~ test strategy protects the family-wise false positive error rate at the overall one-sided ~~0.025~~ level for each concentration vs. vehicle.	**10.6.4 Secondary and Exploratory Analysis [first three paragraphs]** 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. This 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 subjects evaluable per treatment group, the study would have approximately a power of 67% to**	Updated the strategy to handle multiple testing as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
	Key secondary endpoints will be analyzed with the same strategy described for the primary endpoint for the multiple imputation. but an ANCOVA model with treatment and baseline value will be used instead of the MMRM.	**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).** Key secondary endpoints will be analyzed with the same strategy described for the primary endpoint for the multiple imputation. **Multiple imputation for Schirmer-I test will be done once for both continuous and dichotomous endpoints that will be derived (i.e. change from baseline and improvement ≥ 10 mm/5min will be derived after the imputation of the continuous values).** An ANCOVA model with treatment and baseline value will be used instead of the MMRM **for the two continuous endpoints since these are evaluated at Week 4. For the same reason, only baseline and week 4 data will be used in the multiple imputation described in Section 10.6.2. The binary key secondary endpoint will be analyzed in analogy with the other key secondary endpoints, but a logistic model will be used instead of an ANCOVA model with baseline value and treatment as covariates.**	
Section 10.6.8 Specification of Subgroups for Analysis	~~**5.1.7.**~~ Specification of Subgroups for Analysis **[4th bullet point]:** ~~**• Statistical tests for interaction (between subgroup variable and treatment arm) will be performed before investigating further the subgroups. Analyses will be performed if interaction tests between treatments and variables are statistically significant at 15% alpha nominal level. Variables that may be evaluated after test for interaction are:**~~	**10.6.8** Specification of Subgroups for Analysis: -	Removed test for interaction for subgroup analysis as requested by FDA

Section	Original Text	Revised Text	Rationale for Changes
Section 11.6 QUALITY TOLERANCE LIMITS	~~10.11.~~ QUALITY TOLERANCE LIMITS ~~The assessment of patient compliance for both the IMP and NIMP will be made by determining the number of study medication vials dispensed to the patient at Day1/Baseline/V2 and at Week 2/V3 and at Week 4/V4 (AT NIMP) and the number of unused study medication vials returned at Week 2/V3, Week 4/V4 (EOT) Week 8/FU (EOS)/V5. Compliance will be evaluated according to the following formula:~~ ~~Compliance = 100 x ((Number of vials dispensed) – (Number unused vials returned))/((Number of expected days of treatment))~~	11.6 QUALITY TOLERANCE LIMITS	Updated. The derivation of the compliance has been aligned through the entire document by considering the number of drops instilled collected in the patient diary. The accountability has been moved and described in par 6.5.
Section 11.6 QUALITY TOLERANCE LIMITS	~~10.11.~~ QUALITY TOLERANCE LIMITS Gross non compliance will be defined as compliance lower than 80% or greater than 120% and in case of gross non compliance the patient will be excluded from the Per Protocol dataset. ~~Since this definition does not warrant that the Study Eye treatment is compliant, if indicated the SAP will contain further definitions.~~	11.6 QUALITY TOLERANCE LIMITS Gross non compliance **(see Section 6.5 for derivation of compliance by using the number of drops collected in the diary)** will be defined as compliance lower than 80% or greater than 120% and in case of gross non compliance the patient will be excluded from the Per Protocol dataset. **Quality tolerance limits are indicated in the centralized monitoring plan.**	Updated

Section	Original Text	Revised Text	Rationale for Changes
Section 12.2 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL	~~11.7.~~ DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL **[5th bullet point]:** • A financial disclosure agreement completed and signed by the PI and all Sub-Investigators listed on Form FDA 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.	12.2 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL **[5th bullet point]:** • A financial disclosure agreement completed and signed by the PI and all Sub-Investigators listed on Form FDA 1572 **for US and on the Declaration of Interest (DoI) for EU**. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.	Updated for compliance to EU legislation
Section 12.2 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL	~~11.7.~~ DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL **[7th bullet point]:** • Documents of GCP training for PI and all Sub-Investigators listed on the Form FDA 1572.	12.2 DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE TRIAL **[7th bullet point]:** • Documents of GCP training for PI and all Sub-Investigators listed on the Form FDA 1572 **and according to ICH E6 (R2) for GCP.**	Updated for compliance to EU legislation

14 REFERENCES

1) Levi-Montalcini R. The nerve growth factor 35 years later. *Science (New York, N.*Y.*)*. 1987; 237: 1154-62.
2) Lambiase A, Mantelli F, Sacchetti M, Rossi S, Aloe L, Bonini S. Clinical applications of NGF in ocular diseases. Arch Ital Biol. 2011;149:283-92.
3) Craig JP, *et al.* TFOS DEWS II Definition and Classification Report. Ocul. Surf. 2017;15: 276–83.
4) Lambiase A, Sacchetti M, Bonini S. Nerve growth factor therapy for corneal disease. Curr Opin Ophthalmol. 2012;23:296-302.
5) Coassin M, Lambiase A, Costa N, De Gregorio A, Sgrulletta R, Sacchetti M, Aloe L, Bonini S. Efficacy of topical nerve growth factor treatment in dogs affected by dry eye. Graefe's Arch Clin Exp Ophthalmol 2005;243:151-5.
6) Lee HK, Ryu IH, Seo KY, Hong S, Kim HC, Kim EK. Topical 0.1% prednisolone lowers nerve growth factor expression in keratoconjunctivitis sicca patients. Ophthalmology. 2006;113:198-205.
7) Bonini S, Lambiase A, Rama P, Caprioglio G, Aloe L. Topical treatment with nerve growth factor for neurotrophic keratitis. Ophthalmology 2000;107:1347-51.
8) Ríos JD, Ghinelli E, Gu J, Hodges RR, Dartt DA Role of neurotrophins and neurotrophin receptors in rat conjunctival goblet cell secretion and proliferation. Invest. Ophthalmol. Vis. Sci. 2007; 48:1543–51.
9) OXERVATE ™ US package insert. Boston, MA: Dompe' U.S. Inc; 2018
10) Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. Biometrika 1988;75:800-2.
11) Permutt T and Feng L. Trimmed means for symptom trials with dropouts. Pharmaceuticals Statistics. 2017;16:20-28.

15 APPENDICES

APPENDIX 1-SPONSOR APPROVAL PAGE

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

PPD

Signature Date (dd/mmm/yyyy)

PPD

Signature Date (dd/mmm/yyyy)

PPD

Signature Date (dd/mmm/yyyy)

PPD

Signature Date (dd/mmm/yyyy)

APPENDIX 2-INVESTIGATOR'S SIGNATURE PAGE

Investigator's Statement

I have read trial protocol (*NGF0123: 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 Drops Solution at two different Concentrations in patients with Dry Eye Disease)* and agree to conduct the trial as outlined in the Protocol, and in accordance with the Declaration of Helsinki, ICH-GCP and any local regulations, being responsible for personally supervise the trial conduct and ensure trial staff complies with Protocol requirement.

Name of Principal Investigator: _____
(Block letters)

Signature: _____ **Date:** _____
dd/mmm/yyyy