Dompé

Study code NGF0216

An 8-week, Phase II, single-center, randomized, double-masked, vehicle-controlled, parallel-group study with 4 weeks of follow-up to evaluate safety and efficacy of recombinant human nerve growth factor (rhNGF) eye drops solution versus vehicle in patients with dry eye

Test Formulation:	Recombinant human nerve growth factor (rhNGF) 20 µg/ml ophthalmic sterile buffered aqueous solution, Dompé farmaceutici S.p.A., Italy
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Version and Date:	Final Version 2.0, 27Oct2016

PROTOCOL APPROVAL

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Flavio Mantelli, MD, PhD

Date	Signature

INVESTIGATORS

Principal Investigator

Investigator Agreement*: I have read the clinical study described herein, recognize its confidentiality, and agree to conduct the described trial in compliance with Good Clinical Practice (GCP), the ethical principles contained within the Declaration of Helsinki, this protocol, and all applicable regulatory requirements.*

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STUDY SYNOPSIS

Title of Study:
An 8-week, Phase II, single-center, randomized, double-masked, vehicle-controlled, parallel-group study with 4 weeks of follow-up to evaluate safety and efficacy of recombinant human nerve growth factor (rhNGF) eye drops solution versus vehicle in patients with dry eye

Study Number: NGF0216

Principal Investigators: Dr Giacomina Massaro Giordano

Study Center: Scheie Eye Institute, Philadelphia, PA, US

Phase of Development: II Safety, Efficacy

Investigational product(s):

Test Product: Recombinant human nerve growth factor (rhNGF) ophthalmic sterile buffered aqueous solution, Dompé farmaceutici S.p.A, Italy, 20 µg/mL vials (Group 1)

Background information:
NGF is a polypeptide discovered in the early 1950s by R. Levi Montalcini. NGF acts through specific receptors: high-affinity tropomyosin receptor kinase A (TrkA) and low-affinity p75 neurotrophin receptor (p75NTR), which are expressed not only on nerve fibers but also on anterior segment of the eye (iris, ciliary body, lens, cornea and conjunctiva) and by the lacrimal gland, providing the rationale for use of NGF in the treatment of diseases of the anterior segment of the eye. Murine NGF (mNGF), extracted and purified from the male mouse submaxillary gland, was administered to over 100 patients (45 published) with stage 2 and stage 3 neurotrophic keratitis (NK) in form of eye drops at a concentration of 200 µg/mL in balanced salt solution in two uncontrolled, unmasked, open-label studies. Compelling results were observed, with all affected eyes healed from persistent epithelial defects. Tolerability was good, with only mild, local, and transient side effects. As for dry eye disease, administration of NGF to dogs with surgically induced dry eye resulted in enhanced production and functional characteristics of tear film, with an associated improvement of ocular surface signs.

Based on these data, the Company has developed a recombinant human NGF (rhNGF) expressed in *E. coli* for the treatment of NK.

A phase I, double-masked, placebo-controlled clinical study in 74 healthy volunteers, using single and multiple doses of different concentrations of rhNGF eye drops, showed a safety profile rhNGF eye drops (Study NGF0112). A Phase I/II multicenter, double-masked, vehicle-controlled study evaluated the safety and efficacy of rhNGF at 10 and 20 µg/mL six times daily in 174 patients with stage 2 and 3 NK (study NGF0212) has been completed in 2015, demonstrating that rhNGF also was very well tolerated in patients with NK.

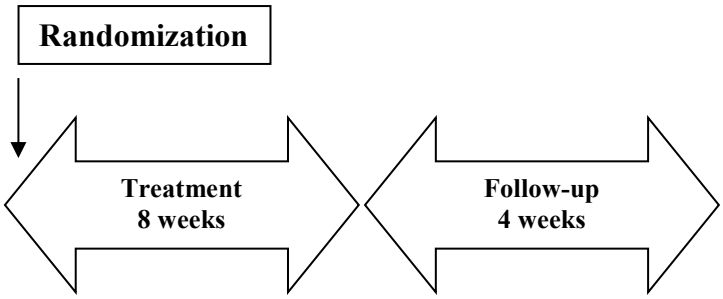
rhNGF, in the same formulation (containing L-methionine) proposed for the present study, has been evaluated in a Phase I/II study in retinitis pigmentosa (RP) patients at the doses of 60 and 180 µg/mL (Study NGF0113), and in a phase II study in NK patients at the same concentration of the present study, 20 µg/mL (6 times daily) (Study NGF0214).

An open-label, uncontrolled study showed that 4-week treatment with rhNGF eye drops (at 20 µg/mL and 4 µg/mL concentrations) was safe and effective in improving symptoms, corneal staining, and tear function in patients with dry eye as compared to baseline (NGF0213).

Safety and tolerability of rhNGF at 20 µg/mL six times daily has been demonstrated in both NGF0212 and NGF0214 studies.

Study Design and Methodology:
The proposed phase II study is a single-center, randomized, double-masked, parallel-arm, vehicle-controlled trial, designed to evaluate the safety and efficacy of rhNGF eye drops at 20 µg/ml concentration administered six times daily for 8 weeks in patients with dry eye.

After confirmation of inclusion and exclusion criteria all eligible patients will be randomized at 2:1 ratio to rhNGF or vehicle control treatment with 8 weeks of study treatments administration with 4 weeks Follow-up :



Treatment: either rhNGF eye drops 20 µg/ml or vehicle six times daily for 8 weeks.

Follow-up: 4 weeks with no further treatment except preservative-free artificial tears (use recorded in drops/day).

After baseline (day 0), enrolled patients will be evaluated for safety and efficacy at week 4 (day 28±2), week 8 (day 56±4) or early exit and at 4 weeks after the end of study treatment (28±4 days).

Treatment Groups:
Before randomization study eye will determined for primary efficacy and safety analysis as the "worse eye" (i.e. the eye with more severe dry eye – if both eyes are equally worse, the right eye will be the study eye). In all patients, both eyes will be treated as specified below:

Group 1: *rhNGF 20 µg/mL:* One drop (40 µL) corresponding to 0.80 µg of rhNGF will be instilled into each eye six times a day (every 2h), for a total daily dose of 9.6 µg (both eyes, if applicable), for 56 consecutive days.

Group 2: *Vehicle:* One drop (40 µL) will be instilled into each eye six times a day (every 2h).

In the 4week follow up no further treatment except preservative-free artificial tears, whose use will be recorded (drops/day).

Administration of rhNGF six times daily will maintain and improve lubrication of the ocular surface since the use of preservative-free artificial tears will be not allowed during treatment period.

Number of Subjects: In total 150 patients with dry eye will be enrolled in the study with 100 patients to be treated with rhNGF and 50 patients with vehicle.

Study Duration:
Treatment duration: 8 weeks

Follow-up period: 4 weeks

Maximum total study duration: 12 weeks

Main Criteria for Inclusion:

1. Patients (male or female) must be ≥ 18 years of age.
2. Patients must be diagnosed with any type of dry eye (e.g. Meibomian Gland Dysfunction, Blepharitis, Keratoconjunctivitis sicca etc) at least 3 months before enrollment.
3. Patients must present dry eye pathology characterized by the following clinical features:
   a) Corneal and/or conjunctival staining with fluorescein and lissamine green using National Eye Institute (NEI) grading system > 3
   b) Mean Symptom Assessment in Dry Eye (SANDE) questionnaire ≥30
   c) Schirmer test without anesthesia < 10 mm/5 minutes and/or tear film break-up time (TFBUT) < 10 seconds in the study eye
4. The same eye (study eye) must fulfill all the above criteria.
5. Patients must have best corrected distance visual acuity (BCDVA) score of ≥ 0.1 decimal units in both eyes at the time of study enrollment.
6. Female patients must have negative pregnancy test if at childbirth potential.
7. Only patients who satisfy all requirements for informed consent may be included in the study. Written Informed Consent must be obtained before the initiation of any study-specific procedures.
8. Patients must have the ability and willingness to comply with study procedures.

Main Exclusion Criteria:

1. Best corrected distance visual acuity (BCDVA) score of < 0.1 decimal units in either eye.
2. Evidence of an active ocular infection in either eye.
3. Presence or history of any ocular disorder or condition, including ocular surgery, trauma, or disease that could possibly interfere with the interpretation of study results in the opinion of the Investigator.
4. Intraocular inflammation defined as Tyndall score >0.
5. Active or recent diagnosis of malignancy (i.e., currently under chemo/radiotherapy).
6. Systemic disease not stabilized within 1 month before baseline visit (e.g., uncontrolled diabetes; 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.
7. Patients who have had a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds, or had a clinically significant allergy to drugs, foods, amide local anesthetics, or other materials, including commercial artificial tears containing carboxymethylcellulose (CMC) (in the opinion of the Investigator).
8. Use of topical cyclosporine, topical corticosteroids, or any other topical medication for the treatment of dry eye in either eye until the day of study enrollment.
9. Contact lenses or punctal plug use during the study (previous use not an exclusion criteria, but must be discontinued at the baseline visit.

10. An anticipated need of additional systemic treatments for dry eye during the study (all prior treatment must be continued for the entire duration of the study).
11. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:
    a) are currently pregnant or,
    b) have a positive result at the urine pregnancy test (Baseline/Day 0) or,
    c) intend to become pregnant during the study treatment period or,
    d) are breast-feeding or,
    e) are not willing to use highly effective birth control measures, such as: hormonal contraceptives - oral, implanted, transdermal, or injected - and/or mechanical barrier methods - spermicide in conjunction with a barrier such as a condom or diaphragm or IUD - during the entire course of and 30 days after the study treatment periods.
12. History of drug addiction or alcohol abuse.
13. Any prior ocular surgery (including refractive palpebral and cataract surgery) if within 90 days before the screening visit.
14. Participation in a clinical trial with a new active substance during the past 30 days.
15. Participation in another clinical trial study at the same time as the present study.

Primary objectives:
Clinical efficacy and safety parameters will be evaluated at each time point:

Primary endpoint:

- Change from baseline in SANDE scores for severity and frequency assessed at 8 weeks of treatment(with LOCF imputation at earlier end of trial assessment)

Secondary endpoints:

- Change from baseline in SANDE scores for severity and frequency assessed at 4 and 8 weeks of treatment (without imputation)
- Changes in Cornea and conjunctival vital staining with fluorescein, National Eye Institute (NEI) scales
- Changes in Tear film break-up time (TFBUT) and Schirmer test I

Exploratory endpoint:

- Changes in levels of the inflammatory biomarker matrix metallopeptidase 9 (MMP-9) in tears, measured by the RPS InflammaDry detector

Safety endpoints

- Incidence and frequency of Treatment-emergent adverse events (TEAEs), assessed throughout the study

Analyses of endpoints will be performed based on evaluations obtained on days 0, 28±2, 56±4 and 28±4 days after discontinuation of study treatment. Details of analyses will be presented in the Statistical Analysis Plan finalized before Database Lock.

Sample size calculation
Sample size was calculated by assuming both for severity and frequency a mean difference from baseline SANDE scores of -30±20 in the rhNGF groups versus -20±20 in vehicle group (80% power, alpha =0.05; 2-sided test), considering that the active compound has already proven safe

and effective in dry eye patients. The sample size has been calculated based on a 2:1 randomization scheme by the following formula:

$n1 = (0.5) \times n \times (1 + k)$

$n2 = (0.5) \times n \times (1 + (1/k))$

With n1 = active treatment, n2 = vehicle, n = 63, and $k = n1/n2 = 2/1=2$. Therefore, n1= 94.5 and n2=47.25. Assuming a 5% dropout rate, a total of 100 patients will be enrolled in the active arm, and 50 in the vehicle arm.

Study procedures				
Study procedures	**Baseline Visit Day 0**	**Week 4** [a]	**Week 8** [b]	**Follow-up Visit Week 12 or early termination** [b]
Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Urine Pregnancy Test	X		X	
Randomization	X			
Demographics	X			
Ocular and General Medical History	X			
Previous and Concomitant Ocular And Systemic Medications	X	X	X	X
Frequency of Artificial Tear Use	X			X
Record Adverse Events (AEs)	X	X	X	X
Verify Patient Study Medication Dosing Compliance		X	X	
SANDE[c] questionnaire	X	X	X	X
Best Corrected Distance Visual Acuity (BCDVA)	X	X	X	X
Schirmer test I (without anesthesia)	X		X	
External Ocular Examination	X	X	X	X
Slit-lamp Examination (SLE)	X	X	X	X
Fluorescein and lissamine green staining (NEI scale)	X	X	X	X
Tear Film Break-up Time (TFBUT)	X	X	X	X
InflammaDry (MMP-9)	X		X	
Study Drug Dispensation	X	X		

a.) Visit window of ± 2 days; b.) Visit window of ± 4 days; c.) SANDE - Symptom Assessment In Dry Eye

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
ANOVA	Analysis Of Variance
BCDVA	Best Corrected Distance Visual Acuity
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CMC	Carboxymethylcellulose
eCRF	electronic Case Report Form
CRO	Contract Research Organization
DSUR	Development Safety Update Report
ETDRS	Early Treatment Diabetic Retinopathy Study
ETV	Early Termination Visit
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPI	First Patient In
FSFV	First Subject (Patient), First Visit
FU	Follow-Up
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
IB	Investigator's Brochure
ICH	International Conference On Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IMP	Investigational Medicinal Product
IND	Investigational New Drug
LPO	Last Patient Out
LSLV	Last Subject (Patient), Last Visit
MedDRA	Medical Dictionary For Regulatory Activities
mL	milliLitres
mmHg	millimetres of mercury
MMP-9	Matrix Metallopeptidase 9
MVPVU	Materiovigilance And Pharmacovigilance Unit
mNGF	Murine Nerve Growth Factor
Mg	Micrograms
NA	Not Applicable
NEI	National Eye Institute
NGF	Nerve Growth Factor
NK	Neurotrophic Keratitis
NSAE	Non-Serious Adverse Event
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
NT	Neurotrophin
OTC	Over-The-Counter
PP	Per Protocol Set
PT	Preferred Term
rhNGF	Recombinant Human Nerve Growth Factor
RP	Retinitis Pigmentosa
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SANDE	Symptom Assessment In Dry Eye
SAP	Statistical Analysis Plan
SLE	Slit-Lamp Examination
SOC	System Organ Class
SOP	Standard Operating Procedure
SDTM	Study Data Tabulation Model
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event

TFBUT	Tear Film Break-Up Time
TrkA	Tropomyosin receptor kinase A (a.k.a. neurotrophic tyrosine kinase receptor type 1; high-affinity nerve growth factor receptor)
VA	Visual Acuity
VAS	Visual Analog Scale
VHP	Voluntary Harmonisation Procedure
WHODDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

1.1 Background Information

1.1.1 Nerve growth factor - overview

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. It binds with at least two classes of receptors: high-affinity tropomyosin receptor kinase A (TrkA), a transmembrane tyrosine kinase, and low-affinity NGF receptor (LNGFR), also known as p75 neurotrophin receptor (p75NTR).

NGF and TrkA are expressed in the anterior segment of the eye (iris, ciliary body, lens, cornea and conjunctiva), and NGF is released in the aqueous humor. Several pieces of experimental evidence suggest that NGF affects all tissues of the anterior ocular segments, playing a crucial role in the physiopathology of several anterior ocular segment diseases.

1.1.2 Chemical and formulation data

As recombinant human NGF (rhNGF) production in mammalian cells does not achieve adequate yields, a manufacturing process based on the use of recombinant *Escherichia coli* (*E. coli*) has been developed. However, because the biological activity of NGF relies on the formation of three disulfide bonds, and because disulfide bonds cannot occur in the reducing cytosol, the purification and renaturation of NGF produced in *E. coli* is problematic. Based on the knowledge that the prosequence increases the yield and rate of refolding of NGF, we have developed a manufacturing process starting from proNGF. After expression of proNGF in *E. coli*, the insoluble protein is isolated in the form of insoluble inactive aggregates (inclusion bodies), solubilized in a strong denaturing agent and subsequently converted into the natural conformation, which is determined by the disulfide bridges present in the natural NGF. Biologically active rhNGF is finally obtained by splitting off the prosequence by enzymatic cleavage. The DNA sequence of human proNGF has been optimized for *E coli* expression (codon adjustment) and two changes in the furin cleavage site, R101V and K103A, have been introduced. These two changes are important to ensure a homogeneous rhNGF preparation during the process with the mature protein starting with serine 105.

The investigational medicinal product (IMP) consists of a sterile isotonic solution for ocular administration (containing L-methionine as excipient), containing rhNGF 20 µg/mL (i.e. 0.020 mg/mL) as drug substance.

1.1.3 Rationale for rhNGF therapy in patients with dry eye

Dry eye is a chronic inflammatory condition of the ocular surface with severe symptoms and visual impairment, leading to worse efficiency to perform duties for an average of 184 work days and resulting in an average loss of productivity estimated in 5,000USD per year per patient (13).

Dry eye results from systemic diseases (Sjögren's syndrome, rheumatoid arthritis, systemic lupus erythematosus, Stevens-Johnson syndrome, thyroid disease, Bell's palsy), ocular conditions (Meibomian gland dysfunction, blepharitis, ocular rosacea, corneal dystrophies), elective surgeries (refractive surgery, blepharoplasty), eyelid conditions (lagophthalmos, entropion/ectropion), cranial surgeries, side effects of drugs (antihistamines, diuretics, beta-blockers), ocular injuries and burns, chemotherapy and radiation, aging, menopause, etc. (2).

Dry eye pathogenesis is multifactorial; however, a number of common mechanisms can be identified: (i) chronic inflammation of the conjunctiva; (ii) decrease of ocular surface sensitivity; (iii) impairment of quantity of tears and/or quality of the tear film, including tear film hyperosmolarity; (iv) changes of conjunctival epithelium with squamous metaplasia and decrease of goblet cells density; (v) corneal epithelium damage.

Until now, treatment has been limited to the use of artificial tears to temporarily improve lubrication of the ocular surface, or the use of steroids to decrease the inflammatory reaction. However, chronic use of steroids is associated with severe complications such as cataract and glaucoma (2). Cyclosporine eye drop therapy for dry eye patients has been approved in the United States but not in Europe. This drug seems to affect only inflammation and tear film production, without any effect on ocular surface sensitivity or the corneal epithelium. On the other hand, experimental and clinical evidence suggests that NGF may affect all the pathogenic mechanisms of dry eye, potentially restoring ocular surface homeostasis (11).

Indeed, several studies have shown that NGF is involved in the regulation of tear film production. In fact, NGF, TrkA and p75, as well as other neurotrophins (NTs) and related receptors, are expressed by the rat lacrimal gland tissue; moreover, NGF has been quantified in human tears, indicating that NGF is basally released by the lacrimal gland (12, 6, 17). These data suggest that NGF may play a role in the maintenance of the tear film and in its alterations in drying ocular surface diseases. Specifically, considering that NGF potentially affects all the components of the ocular surface (cornea, conjunctiva, lacrimal gland, and sensory innervation), it might play an important role during dry eye disease. In line with this hypothesis: 1) NGF eye drop administration in a dog experimental model of dry eye increases tear production, conjunctival goblet cell density and corneal transparency (4, 2) an increased tear concentration of NGF has been reported in patients affected by dry eye (9; 3) NGF stimulates glycoconjugate secretion by conjunctival goblet cells, without affecting cell proliferation (14).

1.1.4 Previous experience in humans with rhNGF

NGF is a polypeptide discovered in the early 1950s by R. Levi Montalcini. NGF acts through specific receptors: high-affinity tropomyosin receptor kinase A (TrkA) and low-affinity p75 neurotrophin receptor (p75NTR), which are expressed not only on nerve fibers but also on anterior segment of the eye (iris, ciliary body, lens, cornea and conjunctiva) and by the lacrimal gland, providing the rationale for use of NGF in the treatment of diseases of the anterior segment of the eye. Murine NGF (mNGF), extracted and purified from the male mouse submaxillary gland, was administered to over 100 patients (45 published) with stage 2 and stage 3 neurotrophic keratitis (NK) in form of eye drops at a concentration of 200 µg/mL in balanced salt solution in two uncontrolled, unmasked, open-label studies. Compelling results were observed, with all affected eyes healed from persistent epithelial defects. Tolerability was good, with only mild, local, and transient side effects. As for dry eye disease, administration of NGF

to dogs with surgically induced dry eye resulted in enhanced production and functional characteristics of tear film, with an associated improvement of ocular surface signs.

Based on these data, the Company has developed a recombinant human NGF (rhNGF) expressed in E. coli for the treatment of NK.

A phase I, double-masked, placebo-controlled clinical study in 74 healthy volunteers, using single and multiple doses of different concentrations of rhNGF eye drops, showed a safety profile rhNGF eye drops (Study NGF0112). A Phase I/II multicenter, double-masked, vehicle-controlled study evaluated the safety and efficacy of rhNGF at 10 and 20 µg/mL six times daily in 174 patients with stage 2 and 3 NK (study NGF0212) ; this study was has been completed in 2015, demonstrating that rhNGF also was very well tolerated in patients with NK.

rhNGF, in the same formulation (containing L-methionine) proposed for the present study, has been evaluated in a Phase I/II study in retinitis pigmentosa (RP) patients at the doses of 60 and 180 µg/mL (Study NGF0113), and in a phase II study in NK patients at the same concentration of the present study, 20 µg/mL (6 times daily) (Study NGF0214).

An open-label, uncontrolled study showed that 4-week treatment with rhNGF eye drops (at 20 µg/mL and 4 µg/mL concentrations) was safe and effective in improving symptoms, corneal staining, and tear function in patients with dry eye as compared to baseline (NGF0213).

1.2 Study Rationale

The data reported above, together with the evidence of rhNGF eye drop effectiveness in the treatment of patients affected by corneal ulcers, make rhNGF a strong candidate for the treatment of dry eye disease (7, 8, 3).

As part of the development plan to evaluate the potential efficacy of the rhNGF solution, the present exploratory study was designed in order to preliminary evaluate the potential efficacy and safety of the IMP.

For additional information regarding the development of rhNGF, please consult the current IB (1)].

1.2.1 Dose and Schedule Rationale

The dose proposed for this trial is 20 µg/mL (1 drop to both eyes, six times daily for 8 weeks).

In previous studies with Dompé rhNGF in human volunteers, the compound has been administered in a wide range of doses after single and multiple administrations up to 180 µg/mL (three times daily for five days, one eye).

In the Phase I/II of the NK studies (NGF0212 and NGF0214), rhNGF administered at 20 µg/mL (six times daily for 8 weeks, one eye) demonstrated to be safe and well tolerated at this dose regime.

Dose regime at six times daily will also ensure the lubrication of the ocular surface in the present study where the use of artificial tears will be not allowed during the treatment period.

1.3 Risks and benefits

In the present study, potential risks of multiple rhNGF applications to patients with dry eye are not expected to surpass the frequency of adverse reactions and untoward effects previously reported in the Phase I/II studies.

The patients with dry eye participating in this study may potentially benefit from the application of rhNGF for 56 days.

2 STUDY OBJECTIVES

The primary objective of this study is to assess the efficacy and safety of rhNGF when administered as eye drops to patients with dry eye.

Evaluation of the clinical efficacy and safety during and at the end of treatment with rhNGF, on the basis of the following assessments at each time point:

2.1 Primary endpoint

- Change from baseline in SANDE scores for severity and frequency assessed at 8 weeks of treatment (with LOCF imputation at earlier end of trial assessment)

2.2 Secondary endpoints

- Change from baseline in SANDE scores for severity and frequency assessed at 4 and 8 weeks of treatment (without imputation)
- Changes in Cornea and conjunctival vital staining with fluorescein, National Eye Institute (NEI) scales
- Changes in Tear film break-up time (TFBUT) and Schirmer test I

2.3 Exploratory endpoint

- Changes in Levels of the inflammatory biomarker matrix metallopeptidase 9 (MMP-9) in tears, measured by the RPS InflammaDry detector

2.4 Safety endpoint

- Incidence and frequency of Treatment-emergent adverse events (TEAEs), assessed throughout the study

Evaluations will be performed on days 0, 28±2, 56±4 and 28±4 days after discontinuation of treatment.

3 CLINICAL SUPPLIES

3.1 Treatment

3.1.1 Description of products

The analytical certificates will be enclosed with the IMP.

Test product

TEST PRODUCT	
IMP	Recombinant human nerve growth factor (rhNGF), containing L-methionine as excipient - 20 µg/mL vials (Group 1), - Vehicle vials (Group 2)
Manufacturer active substance	Dompé Farmaceutici S.p.A., Italy
Manufacturer finished product	Bulk drug product is manufactured by Patheon Italia S.p.A-Italy. Packaging and labelling is performed by PCI Inc. USA
Pharmaceutical form	Sterile buffered aqueous solution
Dose	6 times daily for 8 weeks
Administration route	Ophthalmic

3.1.2 Dose regimen

The dosing scheme of the different study groups is summarized in the following table:

Table 3.1.2.1 Doses for dose Groups

Group	Doses to be tested	Dose (µg / day / eye)	Total daily dose (µg)/ both eyes	Total dose (µg) in 56 days
Group 1	20 µg/mL	4.80 µg	9.60 µg	537.6 µg
Group 2	vehicle	Not Applicable	Not Applicable	Not Applicable

3.1.3 Route and method of administration

Administration route and dose regimen for Groups 1 and 2 will be:

Group 1: *rhNGF 20 µg/mL:* One drop (40 µL) corresponding to 0.80 µg of rhNGF will be instilled into each eye six times daily, for a total daily dose of 9.60 µg (both eyes), for 56 consecutive days. Total dose will be 537.6 µg/56 days.

Group 2: *vehicle*: One drop of vehicle (40 µL) will be instilled into each eye six times daily, for 56 consecutive days.

For both groups 1 and 2, the patients will self-administer the drops at home. In all patients, both eyes will be treated as specified above, whereas only the "worse eye" (i.e. the eye with the more severe dry eye) will be the study eye. The Investigator will check that all patients take the IMP appropriately verifying the diary and the used medication returned.

3.2 Packaging, labeling, distribution and storage

3.2.1 Formulation and packaging

The Investigator will be provided with frozen IMP solutions (-20 ± 5°C) containing rhNGF at concentrations of 20 µg/mL (for the dosing Group 1) and vehicle (for group 2) in a monthly box containing 4 weekly boxes.

The monthly boxes containing the treatments for each day will be delivered to the patient at each study visit according to the following scheme:

Table 3.2.1.1 Investigational Medicinal Product (IMP) Delivery Plan

Box delivery	**Boxes to be provided**
Day 0	4 Weeks (Day 0 - Day 28) – 1 Box rhNGF/Vehicle
Day 28 ±2	4 Weeks (Day 28 - Day 56) – 1 Box rhNGF/Vehicle

Each monthly box will contain a total of 28 vials of rhNGF (Group 1) or Vehicle (Group 2).

Together with the IMP monthly box, the patients will be provided with a sufficient number of syringes and adaptors to be used for the administration of the IMP for the following 4 weeks. Syringes and adaptors will be provided separately in single sterile polyethylene packages and may be kept at room temperature.

The syringe is used with an adaptor consisting of a connecting device with dual connections: one end for the syringe and one end for the vial. Patients will need to:

1) Put the adaptor on the top of the vial (after removing the plastic seal) by piercing the septum
2) Put the syringe on adaptor inlet
3) Draw the solution contained in the vial with the syringe until this reaches its predetermined capacity
4) Remove the syringe and use it as a dropper to administer one drop of IMP into each eye

3.2.2 Labeling, Storage, and Handling

Labeling

The monthly boxes containing the vials of drug will be labeled in accordance with the relevant labeling standards (according to the Annex 13).

The medication labeling will report all the information requested according to the Annex 13 to the good manufacturing practices (published by the European Commission in "The rules governing medicinal products in the European Union," Volume 4) as follows:

a. Name, address and telephone number of the Sponsor, contract research organization (CRO) or Investigator (the main contact for information on the product, clinical study)

b. Pharmaceutical dosage form, route of administration, quantity of dosage units (and in the case of open studies, the name and strength)

c. The batch code number to identify the contents and packaging operation

d. A study reference code allowing identification of the study, site, Investigator, and Sponsor if not given elsewhere

e. The study patient identification study number and where relevant, the treatment period

f. Directions for use (reference may be made to a leaflet or other explanatory document intended for the study patient or person administering the product)

g. "For clinical study use only" or similar wording

h. The storage conditions

i. Period of use (use-by date, expiry date, or re-test date as applicable), in month/year format and in a manner that avoids any ambiguity

j. "Keep out of reach of children"

Labels will be in local language.

Storage and handling

The Pharmacist and/or Investigator will be responsible for receipt, proper storage, and usage of study drug, as well as for the IMP distribution, collection of used and unused vials and final disposal of the remaining IMP.

The investigational product must be stored at -20 ± 5 °C at the investigational sites, in an appropriate locked room accessible only to the pharmacist, the Investigator, or a duly designated person.

A temperature probe and data logger will accompany the drug on shipment. It is essential that the investigational sites will verify the temperature excursion during shipment vs. the acceptable storage conditions, 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. The IMP will be stored in a locked place, sheltered from light. The vials will be not shaken since agitation of vials may cause foaming and/or particle formation.

On Day 0 and at Week 4 visits, the study personnel will give the patient monthly boxes containing the study medications in a refrigerated bag. The refrigerated bag will be used to ensure that the medications will maintain refrigeration temperatures during transport to the patient's home.

Patient should bring the study medication, one box containing 4 weekly kits, at home as soon as possible and immediately store it in a freezer at -20 ± 5 °C.

The weekly kit must be kept at 2-8°C for 7 days, the daily vial can be kept at room temperature before the patient will use the single vial for each instillation (both eyes) as far as 12 hours are not exceeded.

Together with the IMP monthly box, the patient will also receive a separate kit of vial adapters (one per vial), pipettes (6 per vial) and disinfectant wipes (6 per vial).

IMP eye drops solution instruction will be provided to patients.

Patients will use one vial to instill one drop in both eyes six times daily between 8 AM and 6 PM for 8 weeks (six times daily every 2 hours).

The IMP contains L-methionine as an excipient. The contents of each vial are for the daily administration to both eyes only (1 drop of 40 µL per eye 6 times per day). After the last administration the used vial should be returned to the original medication box and is not to be reused.

Any deviations from the recommended storage conditions should be immediately reported by the pharmacist to the Sponsor and Investigator, and the use of the drug should be suspended until they have given authorization for its continued use. The IMP supplies are to be used only in accordance with this protocol. The Investigator will not use any drug 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 drug samples to a third party.

3.3 Drug accountability

The Pharmacist and/or Investigator will confirm the receipt of the IMP supply in writing by signing and dating standard drug accountability forms.

At the week 4 and 8 visits the patients will return the used or unused study boxes to the Investigator.

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 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.

At each scheduled visit, the diary should be reviewed by the Investigator with the patient for completeness. Missing information should not be provided during the diary check but reported as missing.

Partially used or unused study drug boxes will be verified and returned by the Investigator/ Institution to the IMP manufacturer, at the end of the study.

At the conclusion of the study, and if appropriate during the course of the study, the Investigator will complete the drug accountability forms. Within one month after completion of the trial the unused study medication will be shipped to the Sponsor or will be destroyed after authorization by the Sponsor by an authorized company according to GCP regulations.

4 INVESTIGATIONAL PLAN

4.1 Overall study design

This is a Phase II, single-center, randomized, double-masked, parallel-arm, vehicle-controlled trial, designed to evaluate the safety and efficacy of rhNGF eye drops at 20 µg/ml concentration administered six times daily for 8 weeks in patients with dry eye.

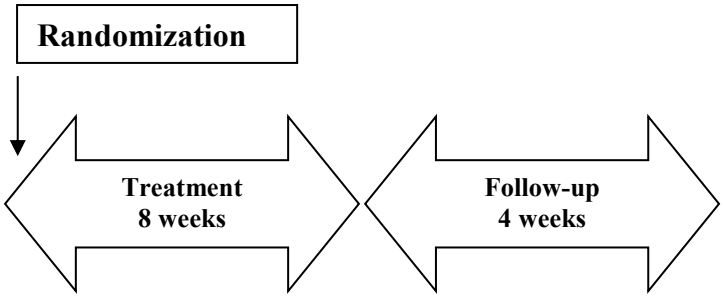
Patients will be evaluated at baseline (day 0), week 4 (28±2 days), week 8 (day 56±4) or early exit and 4 weeks after the end of study treatment (28±4 days).

Eligible patients will be randomized 2:1 to either rhNGF eye drops 20 µg/ml or vehicle six times daily and treated for 8 weeks.

In the 4 weeks following the end of the study treatment, no further treatment will be allowed, except preservative-free artificial tears (use recorded in drops/day).

The study flow chart is given below:

Figure 4.1.1 Study flow chart



4.2 Discussion of design

The study is designed in order to investigate the clinical efficacy and safety of rhNGF in patients with dry eye.

In the previous studies with Dompé rhNGF in human volunteers the compound has been administered in a wide range of doses after single and multiple administrations up to 180 µg/mL (three times daily for five days, one eye).

In the Phase I/II of the NK studies, rhNGF was administered up to 20 µg/mL (six times daily for 8 weeks, one eye).

In the Phase I/II of the NK studies (NGF0212 and NGF0214), rhNGF administered at 20 µg/mL (six times daily for 8 weeks, one eye) demonstrated to be safe and well tolerated at this dose regime.

Dose regime at six times daily will also ensure the lubrication of the ocular surface in the present study where the use of artificial tears will be not allowed during the treatment period.

In the present study, the dose planned (Group 1) is 20 µg/mL (1 drop, 40µL, six times daily, both eyes, for 56 days) corresponding to a daily dose of 9.60 µg and a total dose in 56 days of 537.6 µg.

5 STUDY POPULATION

5.1 Target population

Male and female patients ≥ 18 years old, affected by dry eye syndrome.

5.2 Inclusion criteria

To be enrolled in this study, patients must fulfill all these criteria:

1. Patients (male or female) must be ≥ 18 years of age.
2. Patients must be diagnosed with any type of dry eye (e.g. Meibomian Gland Dysfunction, Blepharitis, Keratoconjunctivitis sicca etc) at least 3 months before enrollment.
3. Patients must present dry eye pathology characterized by the following clinical features:
   a. Corneal and/or conjunctival staining with fluorescein and lissamine green using National Eye Institute (NEI) grading system > 3
   b. Mean Symptom Assessment in Dry Eye (SANDE) questionnaire ≥30
   c. Schirmer test without anesthesia < 10 mm/5 minutes and/or tear film break-up time TFBUT < 10 seconds in the study eye
4. The same eye (study eye) must fulfill all the above criteria.
5. Patients must have BCDVA score of ≥ 0.1 decimal units in both eyes at the time of study enrollment.
6. Female patients must have negative pregnancy test if at childbirth potential
7. Only patients who satisfy all requirements for informed consent may be included in the study. Written Informed Consent must be obtained before the initiation of any study-specific procedures.
8. Patients must have the ability and willingness to comply with study procedures.

5.3 Exclusion criteria

Patients meeting any of these criteria will not be enrolled in the study:

1. Best corrected distance visual acuity (BCDVA) score of < 0.1 decimal units in either eye.
2. Evidence of an active ocular infection in either eye.
3. Presence or history of any ocular disorder or condition, including ocular surgery, trauma, or disease that could possibly interfere with the interpretation of study results in the opinion of the Investigator.
4. Intraocular inflammation defined as Tyndall score >0.
5. Active or recent diagnosis of malignancy (i.e., currently under chemo/radiotherapy).
6. Systemic disease not stabilized within 1 month before baseline visit (e.g., uncontrolled diabetes; 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.
7. Patients who have had a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds, or had a clinically significant allergy to drugs, foods,

amide local anesthetics, or other materials, including commercial artificial tears containing carboxymethylcellulose (CMC) (in the opinion of the Investigator).

8. Use of topical cyclosporine, topical corticosteroids, or any other topical medication for the treatment of dry eye in either eye until the day of study enrollment.
9. Contact lenses or punctal plug use during the study (previous use not an exclusion criteria, but must be discontinued at the baseline visit.
10. An anticipated need of additional systemic treatments for dry eye during the study (all prior treatment must be continued for the entire duration of the study).
11. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:
    a. are currently pregnant or,
    b. have a positive result at the urine pregnancy test (Baseline/Day 0) or,
    c. intend to become pregnant during the study treatment period or,
    d. are breast-feeding or,
    e. are not willing to use highly effective birth control measures, such as: hormonal contraceptives - oral, implanted, transdermal, or injected - and/or mechanical barrier methods - spermicide in conjunction with a barrier such as a condom or diaphragm or IUD - during the entire course of and 30 days after the study treatment periods.
12. History of drug addiction or alcohol abuse.
13. Any prior ocular surgery (including refractive palpebral and cataract surgery) if within 90 days before the screening visit.
14. Participation in a clinical trial with a new active substance during the past 30 days.
15. Participation in another clinical trial study at the same time as the present study.

5.3.1 Concomitant Medication

All medications (including over-the-counter drugs, herbal products, vitamins, and antacids) taken within 4 weeks prior to the start of and throughout the study must be recorded on the case report form. Medication entries should be specific to product name (if a combination drug product) 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 STUDY SCHEDULE

The schedule of the study is summarized at the end of the synopsis (page 10).

6.1 Study visits and procedures

Each study patient will undergo 4 visits.

Maximum study duration will be 84 days. A written informed consent will be obtained before any study assessment or procedure.

The first patient first visit (FSFV) is defined as the 1st visit performed at the clinical center by the 1st screened patient. The "last patient, last visit" (LSLV) is defined as the last visit performed at the clinical center (or the telephone follow-up, if applicable) by the last patient (i.e., the last visit foreseen by the study protocol), independently of whether the patient completed or withdrew from the study.

The following phases, visits and procedures will be performed:

- **Interventional phase**
  - Baseline Visit - Day 0
  - Week 4 Visit - Day 28 ± 2
  - Week 8 Visit - Day 56 ± 4

- **Follow-up phase**
  - Week 12 Follow-up Visit - Day 84 ± 4 / Early Termination Visit (ETV): In case of early discontinuation, discontinued patients will undergo an early termination visit (ETV)

	Day	Procedures/Assessments
Baseline Visit	*Day 0*	The following procedures will be performed (the below order is not mandatory): ➢ Explanation to the patient of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number allocation (as S001, S002, etc.) ➢ Demographic data ➢ Medical and surgical history/current medical conditions ➢ Prior/concomitant medications ➢ AE collection ➢ Ocular examination of both eyes: 1. Assessment by SANDE questionnaire *2.* Assessment of best corrected distance visual acuity (BCDVA) 3. Slit-lamp examination (SLE) to assess the eyelid (Meibomian glands), eyelid (erythema), eyelid (edema), lashes, conjunctiva erythema, lens, iris, anterior chamber *4.* External Ocular Examination 5. TFBUT 6. Schirmer test I (without anesthesia) *7.* Ocular surface staining (NEI score - fluorescein and lissamine green) 8. InflammaDry (MMP-9) ➢ Frequency of patient's own artificial tear use (to be reported in eCRF) ➢ Pregnancy test for female patients if childbirth potential. ➢ Patient eligibility: Inclusion/exclusion criteria evaluation ➢ Assignment of the study eye ➢ Randomization ➢ Study Drug Dispensation The Investigator will dispense to the patients their monthly box containing the study drug for the following 4 weeks together with an adequate number of adapters and pipettes After completing baseline evaluation patients will start the study treatment as per instructions and will self-administer at home the subsequent doses till the evening of Day 28 (±2). Patients will return to the clinical site on Day28 (Week 4 Visit). The patients will be instructed to enter time of self-administration, AE occurrence, and concomitant medication intake into the diary, and to return the refrigerated box on the Week 4 visit.
At home	*Days 0-28* *±2*	➢ Self-administration at home of the IMP, six times daily every 2 h for both eyes (diary) ➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) Data will be recorded by the patient in the patient's diary.

	Day	Procedures/Assessments
Week 4 Visit	*Day 28±2*	The following procedures will be performed (order below is not mandatory): ➢ Assessment of compliance to treatment (from patient diary and IMP reconciliation from returned weekly boxes) ➢ Current medical conditions ➢ Concomitant medications ➢ AE monitoring ➢ Ocular examination of both eyes: 1. Assessment by SANDE questionnaire 2. Assessment of best corrected distance visual acuity (BCDVA) 3. Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber 4. External Ocular Examination 5. Tear Film Break-up Time (TFBUT) 6. Ocular surface staining (NEI score - Fluorescein and Lissamine green) ➢ Study Drug Dispensation The Investigator will deliver to the patients their monthly box containing the study drug for the following 4 weeks together with an adequate number of adapter and pipettes. After discharge the patients will continue the therapy as per instructions and will self-administer at home the subsequent doses from the evening of Day 28±2 to the evening of Day 56±4. Patients will return to the clinical site on Day56 ± 4 (Week 8 Visit). The patients will be instructed to enter time of self-administration, AE occurrence, and concomitant medication intake into the diary, and to return the refrigerated box on the Week 8 visit.
At home	*Days 28±2 – 56±4*	➢ Self-administration at home of the IMP, six times daily for every 2 h (diary) ➢ Recording any new or changes inconcomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments
Week 8 Visit	*Day 56*±4	The following procedures will be performed (the below order is not mandatory): ➢ Compliance to treatment (from patient diary and control of returned weekly box) ➢ current medical conditions ➢ Concomitant medications ➢ AE monitoring ➢ Ocular examination in both eyes: 1. Assessment by SANDE questionnaire 2. Assessment of best corrected distance visual acuity (BCDVA) 3. Slit-lamp examination (SLE) to assess the eyelid (Meibomian glands), eyelid (erythema), eyelid (edema), lashes, conjunctiva erythema, lens, iris, anterior chamber 4. External Ocular Examination 5. TFBUT 6. Schirmer test I (without anesthesia) 7. Ocular surface staining (NEI score - Fluorescein and Lissamine green) 8. InflammaDry (MMP-9) ➢ Pregnancy test for female patients (urine). At completion of the test the patient will be discharged and will be asked to return for the follow-up visit on day 84±4 (Week 12 Visit). The patients will be instructed to enter time of self-administration, AE occurrence, and concomitant medication intake into the diary, and to return the refrigerated box.
At home	*Days 56* ±4– *84 ± 4*	➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Artificial tears use during 4 weeks of FU Data will be recorded by the patient on the patient's diary.
Week 12 Visit **Final visit or early termination visit (ETV)**	*Day 84 ± 4*	The final visit is defined as the visit performed 28 ± 2 days after the last IMP administration (i.e., at day 84 ± 4). In case of premature study discontinuation, patients will undergo an early termination visit (ETV). The following procedures will be performed (the below order is not mandatory): ➢ Medical and surgical history/current medical conditions ➢ Prior/concomitant medications ➢ AE monitoring ➢ Ocular examination of both eyes: 1. Assessment by SANDE questionnaire 2. Assessment of best corrected distance visual acuity (BCDVA) 3. Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber 4. External Ocular Examination 5. Tear Film Break-up Time (TFBUT) 6. Ocular surface staining (NEI score - Fluorescein and Lissamine green) ➢ Frequency of patient's own artificial tear use during 4 weeks of FU (to be reported in eCRF)

7 DESCRIPTION OF SPECIFIC PROCEDURES

7.1 Ophthalmological Evaluations

Ocular evaluations will be performed on both eyes. The assessment will be performed at Day 0 Baseline Visit, Week 4 Visit, Week 8 Visit and Week 12 Visit. The study eye will be the worse eye determined at Day 0 Baseline Visit (the right eye if both eyes are equally worse). The ophthalmological assessment will include:

7.1.1 External Ocular Examination

External Ocular Examination assesses the motility of the extraocular muscles and the appearance and function of the eyelids before the instillation of any dilating or anesthetic eye drops.

7.1.2 Symptom Assessment in Dry Eye (SANDE):

The Symptom Assessment in Dry Eye (SANDE) questionnaire is a short questionnaire to evaluate both dry eye intensity and frequency by using a 100 mm VAS. The patient symptoms of ocular dryness and/or irritation will be quantified on the scale based on two questions that assess both severity and frequency of symptoms. A VAS is a measurement instrument that tries to measure a characteristic or attitude that is believed to range across a continuum of values and cannot easily be directly measured. For example, the amount of irritation that a patient feels ranges across a continuum from none to an extreme amount of irritation. From the patient's perspective this spectrum appears continuous (i.e. their irritation does not take discrete jumps, as a categorization of none, mild, moderate and severe would suggest). It was to capture this idea of an underlying continuum that the VAS was devised.
For the assessment, the patients mark on the 100 mm VAS line the point that they feel represents their perception of their current state. The VAS score is determined by measuring in millimeters from the left hand end of the line to the point that the patient marks. The SANDE scores will be then evaluated for the 2 questions severity (0-100) and frequency (0-100).

7.1.3 Best corrected distance visual acuity (BCDVA):

Refraction and visual acuity measurements will be performed for all patients by trained vision examiners only. The name and certification number of the vision examiner should be documented in the patient’s visual acuity (VA) worksheet (provided by the Sponsor) at each visit. Refraction should be conducted prior to visual acuity testing to obtain best-corrected vision as described below. Best-corrected visual acuity is measured at all trial visits using standard charts, lighting, and procedures. Best correction is determined by careful refraction at that visit according to the standard protocol for refraction as described below.

Equipment

Refraction equipment required includes:

- Retroilluminated Light box and ETDRS 4 meter distance acuity chart set
- Trial lens frames
- Trial lens set with plus or minus cylinder lenses
- Jackson cross-cylinders of 0.25, 0.50, and 1.00 diopters
- Pinhole occluder
- Tissues or eye pads and tape
- A 1 meter rigid measuring stick

Visual acuity charts: Chart 1 is used for testing the visual acuity of the RIGHT eye; Chart 2 for testing the LEFT eye; and Chart R (or 3) for refraction only. Patients should not be allowed to see any of the charts before the examination**.**

A distance of 4 meters is required between the patient's eyes and the visual acuity chart. With the box light off, not more than 15 foot-candles of light (161.4 Lux) should fall on the center of the chart. To measure the amount of light, the room is set up for visual acuity testing, but with the box light off. The light meter is placed at the fourth line from the top of the chart, with its back against the chart and the reading is taken. If more than one line available for testing visual acuity, the visual acuity of an individual patient should be measured in the same line at each visit, if possible. If different lines are used to test visual acuity, they must each meet the same standards.

Retroilluminated ETDRS charts are used in this trial. The illuminator box will be either wall-mounted or mounted on a stand. The light box should be mounted at a height such that the top of the third row letter is 49 + 2 inches from the floor.

The visual acuity light box is equipped with two General Electric 20-watt fluorescent tubes and ballast. Each tube is partly covered by a 14-inch fenestrated sleeve, which is centered on the tube and open in the back**.** This serves as a "baffle" to produce even illumination over the testing chart. Because the illumination of fluorescent tubes diminishes by 5 percent during the first 100 hours and by another 5 percent during the next 2000 hours, new tubes should be kept on for 4 days (96 hours) continuously, and should be replaced once a year.

A sticker should be placed on the back of the light box, indicating the date when the present tubes were installed. A spare set of burned in bulbs should be available on site.

Detailed instructions for VA assessment

As a reminder, Charts 1, 2 and R (or 3) are used for testing the right eye, left eye, and refraction, respectively. Patients should not see the charts until the test begins. The lens correction from the patient's refraction should be in the trial frame worn by the patient.

All eyes must be tested at 4 meters first, even if the refraction was performed at 1 meter.

The patient should be seated comfortably directly in front of the chart so that the eyes remain at the 4 meter distance. Testing always begins with the right eye. The fellow eye should be occluded with a folded tissue or eye pad lightly taped over the eye behind the trial frame serves as an effective occluder that allows eccentric fixation without inadvertent use of the covered eye. After testing the right eye, occlusion of the right eye should be done BEFORE Chart 2 is put up for testing the left eye.

The patient is asked to read the letters slowly, approximately one letter per second. The patient should be told that only one chance is given to read each letter, but may change their mind before moving to the next letter. If the patient is unsure about the identity of the letter, then the patient should be encouraged to guess.

The patient should begin by reading the top line of the chart and continue reading every letter on each smaller line, from left to right on each line. The patient should be encouraged to continue reading even if making mistakes. Each letter read is counted. The examiner circles every correct letter read and totals each line and the whole column (0 if no letters are correct) on the provided VA worksheet. An X is put through letters read incorrectly. Letters, for which no guess was attempted, are not marked. When a patient reaches a level where he/she cannot guess, the examiner may stop the test, provided that the patient has made errors on previous guesses, which is a clear indication that the best visual acuity has been obtained.

When a patient cannot read at least 20 letters on the chart at 4 meters, the patient is tested at 1 meter. The distance from the patient to the chart should be measured again using the rigid one meter stick. The distance is measured from the outer canthus to the center of the fourth letter (right eye) or the second letter (left eye) of the third line of the chart. The spherical correction in the trial frame should be changed by adding +0.75 to correct for the closer test distance. The patient may fixate eccentrically or turn or shake his/her head to improve visual acuity. Particular care should be taken to make sure the patient does not move forward when testing at 1 meter. The patient should be reminded to blink.

The examiner should not tell the patient if a letter was identified correctly. The patient may be encouraged by neutral comments, such as “good,” “next,” and “OK.”

The examiner should not stand close to the chart during testing. Attention should be focused on the patient and the VA worksheet. If the patient has difficulty locating the next line to read, the examiner may go up to the chart and point briefly to the next line to be read, but then must move away from the chart.

When 20 or more letters are read at 4 meters the visual acuity score for that eye is recorded as the number of letters correct at 4 meters plus 30 (refer to the VA worksheet). The patient gets credit for the 30 letters at 1 meter even though they did not have to read them. Otherwise, the visual acuity score is the number of letters read correctly at 1 meter plus the number, if any, read at 4 meters. If no letters are read correctly at either 4.0 meters or 1 meter, then the visual acuity score is recorded as “0.”

7.1.4 Slit-lamp examination (SLE)

The Slit-lamp examination (SLE) must be performed before the instillation of any dilating or anesthetic eye drops or the fluorescein agent.
The patient will be seated at the slit-lamp while being examined. Grading of the eyelids, lashes, conjunctiva, cornea, lens, iris and anterior chamber will be done according to the following scales:

Eyelid - Meibomian glands

Evaluation of the central ten Meibomian gland openings in the mid-portion of the upper eyelid:
0 = None (none are plugged).
1 = Mild (1 to 2 glands are plugged).
2 = Moderate (3 to 4 glands are plugged).
3 = Severe (All glands are plugged).

Eyelid - Erythema

0 = None (normal).
1 = Mild (redness localized to a small region of the lid(s) margin OR skin).
2 = Moderate (redness of most or all lid margin OR skin).
3 = Severe (redness of most or all lid margin AND skin).
4 = Very severe (marked diffuse redness of both lid margin AND skin).

Eyelid - Edema

0 = None (normal).
1 = Mild (localized to a small region of the lid).
2 = Moderate (diffuse, most or all lid but not prominent/protruding).
3 = Severe (diffuse, most or all lid AND prominent/protruding).
4 = Very severe (diffuse AND prominent/protruding AND reversion of the lid).

Lashes

0 = Normal
1 = Abnormal (specify)

Conjunctiva – Erythema

0 = None (normal).
1 = Mild (a flush reddish color predominantly confined to the palpebral or bulbar conjunctiva).
2 = Moderate (more prominent red color of the palpebral or bulbar conjunctiva).
3 = Severe (definite redness of palpebral or bulbar conjunctiva).

Conjunctiva - Edema

0 = None (normal).
1 = Mild (slight localized swelling).
2 = Moderate (moderate/medium localized swelling or mild diffuse swelling).
3 = Severe (severe diffuse swelling).
4 = Very severe (very prominent/protruding diffuse swelling).

Lens

0 = No opacification (normal lens).
1 = Mild lens opacification.
2 = Moderate lens opacification.
3 = Severe lens opacification.
N/A = Patient with artificial lens

Iris

0 = Normal
1 = Abnormal.

Anterior Chamber Inflammation (Slit beam = 0.3 mm wide, 1.0 mm long)

0 = None (no Tyndall effect).
1 = Mild (Tyndall effect barely discernible).
2 = Moderate (Tyndall beam in the anterior chamber is moderately intense).
3 = Severe (Tyndall beam in the anterior chamber is severely intense).
Corneal findings of interest on the SLE include the horizontal diameter of the cornea (measured in mm at the time of the SLE using a ruler).

Relevant findings of the SLE will be entered in the eCRF.

7.1.5 Tear Film Break-up Time (TFBUT)

Patients with a Tear Film Break-up Time TFBUT test ≤ 10 in the study eye (study eye) at the screening visit are eligible for enrollment.

TFBUT will be measured by determining the time to tear break-up. The TFBUT will be performed after instillation of 5 µl of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. The patient will be instructed to blink several times to thoroughly mix the fluorescein with the tear film. In order to achieve maximum fluorescence, the examiner should wait approximately 30 seconds after instillation before evaluating TFBUT. 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. This measurement will be performed within 10 seconds maximum. The TFBUT will be measured twice during the first minute after the instillation of the fluorescein. If the 2 readings differ by more than 2 seconds a third reading is taken.

The TFBUT value will be the average of the 2 or 3 measurements.

Relevant TFBUT findings will be entered in the eCRF.

7.1.6 Schirmer test I (without anesthesia)

Patients with a Schirmer test without anesthesia ≤ 10 mm/5 minutes in the worse eye (study eye) at the screening visit are eligible for enrollment.
This test will be performed to measure aqueous tear secretion prior to the instillation of any dilating or eye drops. Both eyes may be tested at the same time.
This test will be conducted in a dimly lit room. While the patient looks upwards, the lower lid will be drawn gently downwards and temporally. The rounded bent end of a sterile strip will be inserted into the lower conjunctival sac over the temporal one-third of the lower eyelid margin. The test should be done without touching directly the Schirmer test strip with the fingers to avoid contamination of skin oils. The patients will be instructed to close their eyes gently.
After 5 minutes have elapsed, the Schirmer test strip will be removed and the length of the tear absorption on the strip will be measured (millimeters/5 minutes).
The wetting distance at 5 minutes for each eye will be recorded in the eCRF.

7.1.7 Ocular surface staining (NEI score - Lissamine green and fluorescein)

As grading scale of the corneal and conjunctiva damage, the NEI/Industry Workshop guidelines will be used (10). The cornea is divided into five sectors (central, superior, inferior, nasal and temporal), each of which is scored on a scale of 0–3, with a maximal score of 15. Both nasally and temporally, the conjunctiva is divided into a superior paralimbal area, an inferior paralimbal area and a peripheral area with a grading scale of 0–3 and with a maximal score of 9 for the nasal and temporal conjunctiva.
For a better reading it is also essential not to use an intense illumination beam, which may reduce the contrast and lead to an underestimation of grading (5).

7.1.8 InflammaDry:

InflammaDry is a rapid, in-office test that detects MMP-9, an inflammatory marker that is consistently elevated in the tears of patients with dry eye disease. Using direct sampling microfiltration technology, InflammaDry accurately identifies elevated levels of MMP-9 protein in tear fluid samples taken from the inside lining of the lower eyelid, the palpebral conjunctiva.

8 ASSIGNMENT OF STUDY TREATMENT

After obtaining informed consent a consecutive screening number will be assigned to each patient according to the sequence of study entry, from 001 to 150.

8.1 Randomization

Eligible patients will be randomized 2:1 to either rhNGF eye drops 20 µg/ml (100 patients) or vehicle solution (50 patients).

Each randomized patient will be allocated with randomization number to a consecutive list of starting with 1001 onwards.

Drop outs after randomization will not be replaced.

8.2 Treatment allocation

Each randomized patient will receive masked study treatment (either rhNGF eye drops 20 µg/ml or vehicle solution) according to the randomization code and determined by the allocated randomization number.

8.3 Masking

The identity of the treatments will remain unknown to the patient, Investigator, site staff and Sponsor's clinical research personnel until the study is unmasked for the final statistical analysis (after data base lock) except in case of specific events that will require unmasking of the patient.

The vials containing rhNGF (20 µg/ml) or vehicle will be identical in appearance, and the contents of the vials will be indistinguishable. All staff directly involved in the analysis of study results will remain masked to treatment assignments while the study is in progress.

A list of sequential kit numbers will be generated by a member of the CRO SAS programming group not involved in the conduct of the study. Each kit number will be randomly associated with a treatment group. Patients will be assigned to treatment in numerical order. A tear-off label from the kit box, with the kit number, will be attached to the investigational product dispensing log.

If the Investigator becomes unmasked for any reason, this information will be recorded on source data and in the eCRF of the study, specifying the date and the reason.

In the event of a medical emergency where the knowledge of patient treatment is required to provide the patient with appropriate care, Investigators will have the possibility to unmask the treatment assignment for a specific patient. . The Investigators are encouraged to contact the CRO staff before becoming unmasked if there is sufficient time.

9 EVALUATION PARAMETERS

9.1 Study variables

Clinical efficacy and safety parameters will be evaluated at each time point:

9.1.1 Primary endpoint

- Change from baseline in Symptom Assessment in Dry Eye (SANDE) scores for severity and frequency assessed at 8 weeks of treatment (with LOCF imputation at earlier end of trial assessment)

9.1.2 Secondary endpoints

- Change from baseline in SANDE scores for severity and frequency assessed at 4 and 8 weeks of treatment (without imputation)
- Changes in Cornea and conjunctival vital staining with fluorescein, National Eye Institute (NEI) scales
- Changes in Tear film break-up time (TFBUT) and Schirmer test I

9.1.3 Exploratory endpoint

- Changes in levels of the inflammatory biomarker matrix metallopeptidase 9 (MMP-9) in tears, measured by the RPS InflammaDry detector

9.1.4 Safety endpoints

- Incidence and frequency of Treatment-emergent adverse events (TEAEs), assessed throughout the study

Analyses of endpoints will be performed based on evaluations obtained on days 0, 28±2, 56±4 and 28±4 days after discontinuation of study treatment. Details of analyses will be presented in the Statistical Analysis Plan finalized before Database Lock.

Details on the assessments are given in § 7.1.

10 STATISTICAL METHODS

The data documented in this study will be summarized using descriptive summary statistics, i.e., arithmetic mean, standard deviation (SD), (%), minimum, median, and maximum values for quantitative variables, and frequencies for qualitative variables.

A statistical analysis plan (SAP) will be developed and finalized before database lock and de-masking. Final statistical analysis on the study variables will be presented in detail in the SAP.

10.1 Analysis Sets

10.1.1 Definitions

A patient will be defined as screened after the signature of the informed consent, regardless of the completion of all the screening procedures.

A patient will be defined as eligible if he/she respects all the inclusion/exclusion criteria. Otherwise he/she will be defined as a screen failure.

A patient will be defined as enrolled in the study if he/she is randomized.

- Enrolled Set: all enrolled patients. This analysis set will be used for demographic, baseline and background characteristics
- Safety Set (SAF): all enrolled patients who receive at least one dose of the investigational medicinal product at the study eye. This analysis set will be used for the safety analysis
- Full Analysis Set (FAS): all patients in the FAS who have at least one post-baseline efficacy measurement. This analysis set will be used for the primary efficacy analysis
- Per Protocol Set (PP): all patients in the FAS who fulfil the study protocol requirements in terms of investigational medicinal product intake and collection of primary efficacy data and with no major deviations that may affect study results. This analysis set will be used for supportive efficacy analysis

Each patient will be coded by the CRO Biometry Unit as valid or not valid for the Enrolled Set, FAS, FAS and PP. Patients will be evaluated according to the treatment dose they will actually receive.

10.1.2 Reasons for exclusion from the Full Analysis Set

Reasons for the exclusion from the Full Analysis Set are the following:

- failure to take at least one dose of the IMP at the study eye
- lack of any efficacy data post enrollment

10.1.3 Reasons for exclusion from the Per Protocol set

Reasons for the exclusion from the Per Protocol set will be determined in the Blind Data Review Meeting and can be the following:

- lack of compliance with IMP administration (see § 10.3)
- exposure to an IMP dose different from the one assigned to the patient
- missing primary efficacy data
- failure to satisfy any inclusion/exclusion criteria (eligibility violations)
- intake of prohibited medications

10.2 Sample size and power considerations

Sample size was calculated by assuming both for severity and frequency a mean difference from baseline SANDE score of -30±20 in the rhNGF groups versus -20±20 in vehicle group (80% power, alpha =0.05; 2-sided t-test), considering that the active comparator has already proven safe and effective in dry eye patients. Therefore, the sample size has been calculated based on a 2:1 randomization scheme by the following formula:

$n1 = (0.5) \times n \times (1 + k)$

$n2 = (0.5) \times n \times (1 + (1/k))$

With n1 = active treatment, n2 = vehicle, n = 63, and k = n1/n2 = 2/1=2. Therefore, n1= 94.5 and n2=47.25. Assuming a 5% dropout rate, a total of 100 patients will be enrolled in the active arm, and 50 in the vehicle arm.

Since this is an exploratory study, no adjustment has been made for multiplicity of SANDE parameters, neither has it been taken into account that the actual analysis will be descriptive only.

10.3 Compliance with IMP administration

The assessment of patients' compliance to the IMP will be made by determining the number of study medication vials dispensed to the patient at Day 0 baseline Visit, Week 4 Visit and the number of unused study medication vials returned at Week 4 Visit and Week 8 Visit, respectively. Compliance will be evaluated according to the following formula:

$$\text{Compliance} = \frac{\text{Number of vials dispensed} - \text{Number of unused vials returned}}{\text{Number of expected days on treatment}}$$

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 Set (see § 10.1.3). Since this definition does not warrant that the study eye treatment is compliant, if indicated the SAP will contain further definitions based upon the diary card information.

10.4 Demographic, baseline and background characteristics

Demographic and baseline characteristics will be examined per treatment group according to qualitative or quantitative data. Qualitative data will be listed and summarized in contingency tables. Quantitative data will be listed and summarized using descriptive statistics.

10.5 Analysis of ophthalmological evaluations

All efficacy variables will be summarized for their study eye, using descriptive statistics (i.e., arithmetic mean, SD, (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables) by dose and evaluation time point. Changes from baseline (Day 0 Baseline Visit assessment) will be presented as well, if applicable.

Additional details on the analyses will be provided in the statistical analysis plan.

10.6 Safety and tolerability evaluation

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

AEs are all events occurring or worsening after the first dose of the IMP.

Individual AEs will be listed in patient data listings. AEs will be summarized by treatment group. The number and percentage of patients with any AE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

11 DEFINITION AND HANDLING OF AES AND SAES

11.1 Applicable SOPs

AE definition, classification and management will follow the Sponsor and CRO SOPs, based upon applicable local and international regulations. A brief summary of AE definition, classification and management is reported below.

11.2 Definitions

11.2.1 Adverse Events

Adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. (CFR - Code of Federal Regulations Title 21 Sec. 312.32). 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 (investigational) product, whether or not related to the medicinal (investigational) product.

11.2.2 Adverse Drug Reaction

Any noxious and unintended response to a medicinal product related to any dose should be considered an Adverse Drug Reaction (ADR). 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 covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. For the purposes of IND safety reporting, "reasonable possibility" means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event.

11.2.3 Serious Adverse Event (SAE)

A serious adverse event (SAE) is defined in line with (CFR - Code of Federal Regulations Title 21 Sec. 312.32) 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

  (NOTE: Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a 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).*
- Results in 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 Par. 11.7.2).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.

If a SAE results in death, cause of death shall always be specified when known. In case cause of death is not initially available, adequate follow up to seek information shall be made by the Investigator.

11.2.4 Unexpected Adverse Event/Reaction

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 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 (21 CFR312.32(a)).

11.2.5 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 product information) and meets the definition of a Serious Adverse Reaction.

The determination of expectedness should be made on the basis of the IB.

11.2.6 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, in the opinion of the Investigator, may require medical intervention to prevent permanent loss of sight.

11.3 Adverse Event (AE) Monitoring

At each post-baseline 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.

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.

11.4 Recording

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. 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 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).

If the Investigator becomes aware of a related serious adverse event occurring to a subject after the treatment of that subject has ended, this event should be reported by the Investigator to Dompé. Such "post-study cases" should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment (by the Investigator and by Dompé) and determination of expectedness (by Dompé) are needed for a decision on whether or not expedited reporting is required.

11.5 Relationship of AEs to the Investigational Product

The Investigator will assess the relationship between the AE and the investigational medication, according to the criteria in Table below:

Table 11.5.1 Relationship of the Adverse Event to the IMP

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 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 repeat exposure

An 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.

11.6 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.

Specifically, the intensity of events should be classified as mild, moderate, or severe.

Table 11.6.1 Intensity (Severity) of the Adverse Event

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])

11.7 Serious Adverse Event Reporting Procedure (from Investigator to Dompé/CRO)

11.7.1 Reporting Procedure from Investigator to Dompé and CRO

As per Paragraph 11.4, the Investigator must record all SAEs, including sight-threatening events, occurring at any time during the study 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 and simultaneously the Investigator shall forward the filled in, signed and dated SAE form to Dompé Drug Safety and to Cromsource (MVPVU) to the following addresses:

Dompé Contact information
Dompé Drug Safety
Laura Boga, Senior Safety Manager
Email: farmacovigilanza@dompe.com
or Fax: +39.02.36026913

Dompé Medical Expert
Flavio Mantelli – Chief Medical Officer Ophthalmology,
Email: flavio.mantelli@dompe.com

or Fax: +39.02.58383324

Dompé Clinical Development
Email:valentina.vaja@dompe.com
or Fax: +39.02.58383324

Cromsource Contact Information:

- For Pharmacovigilance: DOMPE-NGF0216-PV@cromsource.com
- For Medical Monitor and Pharmacovigilance: DOMPE-NGF0216-MM@cromsource.com

or by Fax to:

- **Study specific eFax +1.2013267710** (linked to Dompé and Cromsource addresses).

Respective IRB must also be informed of all SAEs according to local specific requirements.

If assistance is needed with the reporting of a SAE, the CRO/Sponsor may be contacted at the addressed provided above

Serious adverse events will be managed directly by the Dompé Drug Safety department, with Cromsource MVPVU support for follow-up requests.

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 Investigational Product, should identify which adverse events are serious and which are non-serious. In any case, the Investigator is requested to record his/her opinion about the relationship of the observed event(s) with the investigational medication.

An assessment of expectedness and causality of each serious adverse event will be performed case by case by Dompé/CRO. 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 patients chart. If the patient was hospitalized, a copy of the discharge summary should be available, if possible.

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

11.7.2 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:

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

11.7.3 Reporting Procedure to IEC and to Regulatory Authorities

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 which approved the study. The requirements of IRBs vary from one IRB to another; however, as a minimum requirement, the Investigators must promptly report all suspected unexpected serious adverse reaction (SUSAR) to their IRB.

In line with provisions set forth in 21CFR312, Dompé shall notify all participating Investigators in an IND safety report of any suspected adverse reaction that is both serious and unexpected 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.

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 reaction 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 unblinded by Dompé Drug Safety Pharmacovigilance prior to submission of a SUSAR to Regulatory Authorities and only cases referred to active treatment will be considered expeditable for regulatory reporting, in line with law requirements.

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

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.

11.7.4 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.

11.8 Unmasking of the Study Treatment

Masked information on the identity of the assigned investigational product will be provided for each patient. If the treatment code needs to be broken in the interest of patient safety, the Dompé must be informed in all cases in which the code was broken and of the circumstances involved.

Additionally, Dompé Drug Safety may be need to unmask the patient's treatment if the a reported SAE meets criteria of a Suspected Unexpected Serious Adverse Reaction (SUSAR) in order to fulfil expedited regulatory reporting requirements. Unmasked information shall not be disclosed to Investigators.

The identity of the treatments will remain unknown to the patient, Investigator, site staff and Dompé's clinical research personnel and CRO staff (apart from pharmacovigilance).

11.9 Follow-up of patients with adverse events (AEs)

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. 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 patient was hospitalized due to a SAE, a copy of the discharge summary is to be forwarded to CRO/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 also may 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 subject has left the study up to 10 days after his/her discontinuation from the study for unrelated SAEs, and without timelines for related SAEs.

11.10 Pregnancy in the clinical trial

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 the CRO/Dompé Drug Safety contacts reported at Paragraph 11.7.1, 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 an SAE (eg, 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 Section 11.7 with the appropriate serious criterion (eg, 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.

11.11 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.

11.12 Overdose

Cases of overdose (accidental or intentional) which result in serious adverse reactions are to be handled following emergency procedures, and reported within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion), or drug intake 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, and shall be reported to Dompé Drug Safety by e-mail or fax within 24 hours, in order to have information about symptoms, corrective treatment and outcome of overdose.

12 DATA MANAGEMENT PROCEDURES

12.1 Data collection – case report forms (eCRFs)

The Investigator must ensure that the clinical data required by the study protocol are carefully reported in the eCRFs. He must also check that the data reported in the eCRFs correspond to those in the patients' source documents.

An electronic case report form (eCRF) will be provided for each randomized patient.

All protocol-required information collected during the study must be entered by the Investigator, or designated representative, in the eCRF. Details of eCRF completion and correction will be explained to the Investigator. If the Investigator authorizes other persons to make entries in the eCRF, the names, positions, signatures, and initials of these persons must be supplied to the Sponsor.

The Investigator, or designated representative, should complete the eCRF pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

12.2 Unique subject identifier

All the patients who sign the informed consent form for the present study will be coded with "unique subject identifiers" when data are extracted from the study database into the domains of the CDISC SDTM model. The unique subject identifier consists of the 4-digit screening number (e.g. S001, S002, etc.) and, if applicable, the 4-digit subject study number (e.g. 1001, 1002, etc.).

Screening number and subject study number are separated by slashes (e.g. "S001/1001").

12.3 Database management

Data management of all data captured within the eCRF will be performed by the CRO. CRO will update and verify the database and create the final SAS data sets. The tabulation datasets and analysis datasets created according to the standard CDISC (STDM and ADaM) will be provided to the Sponsor with all the other study documentation.

12.3.1 Coding dictionaries

Medical/surgical history and underlying diseases, physical examination abnormalities and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA™).

Prior and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHODDE). Version of coding dictionaries will be stated in the SAP and the study report.Study Monitoring, Quality Control And Quality Assurance.

12.4 Monitoring

The monitoring visits will be conducted by a local monitor.

Monitoring will comply with ICH-GCP chapter 5.18 requirements for what concerns monitoring purpose, selection and qualifications of monitors, extent and nature of monitoring, monitoring procedures, monitoring reports.

Adequate time and availability for monitoring activities should be ensured by the Investigator and key study personnel.

Data verification is required and will be done by direct comparison with source documents, always giving due consideration to data protection and medical confidentiality. In this respect the Investigator will assure support to the monitor at all times.

The Investigator agrees, by written consent to this protocol, to fully co-operate with compliance checks by allowing authorized individuals to have access to all the study documentation. In addition to the monitoring activities performed by the study monitor, the Sponsor could perform some quality control activities to verify the compliance with the study procedures and the ICH-GCP guidelines.

12.5 Quality Control and Quality Assurance

The CRO has implemented and maintains a Quality System that includes quality controls and audits at different study steps with written SOPs to ensure that the study is conducted in compliance with the protocol and all effective amendments, ICH-GCP, and the applicable regulatory requirement(s) and that data have been reliably and correctly generated, recorded, processed and reported, in agreement with the ALCOA principles (Attributable-Legible-Contemporaneous-Original-Accurate).

12.6 Applicable SOPs

The Sponsor, the clinical center and the CRO will follow their respective SOPs in the conduct of the respective activities, unless otherwise stated in written agreements. SOPs will be made available for review, if required.

12.7 Data access

The Investigator and the CRO will ensure that all raw data records, medical records, eCRFs and all other documentation that is relevant to this study will be made accessible to monitoring activities, audits, IEC review, and regulatory inspection.

12.8 Audits and inspections

The Sponsors, independent bodies acting on behalf of the Sponsor and the CRO have the right to perform audits according to ICH-GCP responsibilities.

The study may also be inspected by regulatory authorities.

The Investigators agrees, by written consent to this protocol, to fully co-operate and support audits and inspections compliance checks by allowing authorized individuals to have access to all the study documentation.

13 ETHICAL CONSIDERATIONS

13.1 Ethics and Good Clinical Practice (GCP)

The study will be performed in accordance with the relevant guidelines of the Declaration of Helsinki.

The approval of the study protocol by the relevant Local (AKH) Research Ethics Committees Authorities will be obtained before the start of the study.

Study notification to the Competent Authorities (BASG, Austria) will be performed according to the Austrian current regulations.

The present clinical study will be carried out according to the general principles of "ICH Topic E6, CPMP/ICH/135/95," July 1996 including post Step 4 errata, status September 1997 and post Step errata (linguistic corrections), July 2002.

13.2 Informed consent

Before being enrolled into the clinical study, the patients must have expressed their consent to participate, after the Investigator has explained to them, clearly and in details, the scope, the procedures and the possible consequences of the clinical study. Information will be given in both oral and written form. The information sheet and informed consent form will be prepared in the local language by the CRO and must be approved by the EC and regulatory authorities. It will include all the elements required by law according to the ICH-GCP recommendations.

In addition to the standard requirements that physicians are currently obliged to observe when providing information, the following points must also be covered:

- a description of the aims of the study and how it will be organised
- the type of treatment
- any potential negative effects attributable to the study treatment
- the freedom to ask for further information at any time
- the patients' right to withdraw from the clinical study at any time without giving reasons and without jeopardising their further course of medical treatment
- the existence of patient insurance cover and obligations following from this cover

Adequate time and opportunity to satisfy questions will be given to the patients and the time will be recorded.

The Investigator will be supplied with an adequate number of blank informed consent forms to be used. The forms will be signed and dated by both the Investigator and the patients.

A copy of the signed form will be given to the patient.

To ensure medical confidentiality and data protection, the signed informed consent forms will be stored in the Investigator's study file according to the regulatory requirements (see § 14.2). The Investigator will allow inspection of the forms by authorized representatives of the Sponsor, EC members and regulatory authorities. He will confirm, by signing and dating the forms, that informed consent has been obtained.

13.3 Insurance policy

An insurance cover has been issued in favor of the patients participating in this clinical study. The insurance is in compliance with the local regulation and with the requirements of the Health Authorities.

13.4 Withdrawal of patients

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.

13.4.1 Primary reason for discontinuation

- **Adverse event (AE)**: Any significant AE that, in the opinion of the Investigator or concerned patient, is not compatible with study continuation. For the definition of AE, please refer to § 11.2.
- **Death**: the absence of life or state of being dead
- **Lack of efficacy**: the lack of expected or desired effect related to a therapy
- **Lost to follow-up**: the loss or lack of continuation of a patient to follow-up
- **Non-compliance with study drug**: an indication that a patient has not agreed with or followed the instructions related to the study medication
- **Physician decision**: a position, opinion or judgment reached after consideration by a physician with reference to the patient
- **Pregnancy**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth (please see § 11.10)
- **Protocol violation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **Study terminated by the Sponsor**: an indication that a clinical study was stopped by its Sponsor
- **withdrawal by patient**: study discontinuation requested by a patient for whatever reason
- **other**: different than the ones previously specified.

13.4.2 Discontinuation procedures

For any patient discontinuing the study, the Investigator will:

- ask the patient to undergo, as far as possible, a final medical visit (ETV) 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 of study discontinuation
- record in the eCRF any follow-up, if the patient is withdrawn for an AE.

13.4.3 Replacement

Discontinued patients will not be replaced.

13.5 Study termination

The study will be considered terminated at the date of the last visit of the last patient or upon completion of any follow-up procedure described in the protocol. The Investigator and the Sponsor have the right to discontinue the study at any time for reasonable medical and/or administrative reasons. As far as possible, this should occur after mutual consultation. Reasons for discontinuation have to be documented appropriately. In this event, no further patients will receive doses of the study drugs, and patients already having received a dose of study drug will not receive any further doses of the study IMP but will undergo all safety assessments scheduled after the last dose of study drug, up to an including the end of study examination.

14 ADMINISTRATIVE PROCEDURES

14.1 Protocol amendments

In order to obtain interpretable results, neither the Investigator nor the Sponsor will alter the study conditions agreed upon and set out in this protocol. Amendments should be made by mutual agreement between the Investigator and the Sponsor. Any amendment must be set out in writing, giving the reasons, and being signed by all concerned parties. The amendment becomes then part of the protocol.

All amendments will be sent to the EC and concerned Competent Authorities.

14.2 Study documentation and record keeping

The Investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported in the eCRFs and in all required reports.

The Investigator must keep source documents for each patient in the study. All information on the eCRFs must be traceable to these source documents, which are generally stored in the patient's medical file. The source documents should contain all demographic and medical information, including ophthalmic assessments, etc., and the original signed informed consent forms.

Data reported on the eCRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

The Investigator and the Sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by the applicable regulatory requirement(s).

These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study commences, during the clinical study, and after termination of the study and include but are not limited to, study protocol, amendments, submission and approval of EC, raw data of patients including ophthalmic assessments, insurance contracts, certificate of analysis of the IMP(s), drug accountability records, signed informed consent forms, confidential subjects identification code, eCRFs, curricula vitae of the Investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.

The Investigator and the Sponsor should take measures to prevent accidental or premature destruction of these documents.

Study documents must be retained by the Investigator and the Sponsor as long as needed to comply with ICH-GCP, national and international regulations. By signing the protocol, the Investigator and the Sponsor agree to adhere to these requirements.

14.3 Study patients' recruitment

The clinical site have detailed SOPs on recruitment process.

14.4 Confidentiality and data protection

By signing this protocol, the Investigator and the CRO agree to keep all the information provided by the Sponsor in strict confidentiality and to request similar confidentiality from his/her staff. Study documents provided by the Sponsor (protocols, IB, eCRFs and other materials) will be stored appropriately to ensure confidentiality. The information provided by the Sponsor to the Investigator and to the CRO cannot be disclosed to others without direct written authorisation from the Sponsor, except for the extent necessary to obtain the informed consent from the patients wishing to participate in the study.

Data on patients collected on the e CRFs during the study will be documented in an anonymous way (see § 12.2). If, as an exception, it becomes necessary to identify a patient for safety or regulatory reasons, the monitor, the Sponsor and the Investigator will be bound to keep this information confidential.

14.5 Publication policy

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.

In any case, study results will be communicated in full to the competent Health Authorities by the submission of a complete Clinical Study Report.

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).

On an exceptional basis, the Sponsor 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 a data could, in some circumstances, prevent or negatively impact patentability.

14.6 Liability Statement

On behalf of the Sponsor, the investigational sites will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the Investigators, the persons instructed by them and the hospital, practice or institute in which they are employed and the liability of the Sponsor in respect of financial loss due to personal injury

and other damage which may arise as a result of the carrying out of this study are governed by the applicable local laws.

As a precautionary measure, the Investigators, the persons instructed by them and the hospital, practice or institute are included in such cover in respect of work done by them in carrying out this study to the extent that the claims are not covered by their own professional indemnity insurance.

14.7 Financing of the Study

The financial aspects of this study are described in detail in the contract between Sponsor and CRO and between CRO and clinical site involved in this study.

14.8 Responsibilities of the Investigator

The Investigator is aware of his/her responsibility towards the Sponsor for all the actions delegated by him/her to other members of his/her staff assigned to the conduct of the study. Except where specifically required, the wording "Investigator" used in this protocol and in the eCRF, refers to the Investigator or the qualified person designated by him/her, who may carry out activities relevant to the clinical trial and sign the study documents on his/her behalf.

The Investigator is obliged to conduct the study in compliance with the study protocol and in adherence to GCP (ICH E6) and with the principles of the Declaration of Helsinki (1964) and subsequent revisions as well as in respect of applicable legislation.

14.9 Confidentiality and data protection

By signing this protocol, the Investigator(s) and the CRO agree to keep all the information provided by the Sponsor in strict confidentiality and to request similar confidentiality from his/her staff. Trial documents provided by the Sponsor (protocols, IBs, eCRFs and other materials) will be stored appropriately to ensure their confidentiality. The information provided by the Sponsor to the Investigator and to the CRO cannot be disclosed to others without direct written authorisation from the Sponsor, except for the extent necessary to obtain the informed consent from the patients wishing to participate in the trial.

14.10 Final study report

The final Clinical Study Report will be written by the CRO and then approved by Dompé Farmaceutici S.p.A., and the Principal Investigator. It will be written in compliance with the ICH E3 guideline for both content and format.

15 STUDY RESPONSIBLE PERSONS

15.1 Sponsor

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15.2 Institutes performing the study

15.2.1 Clinical centre

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