

## **Study code NGF0116**

**A 8 weeks, Phase II, single-centre, randomized, double-masked, vehicle-controlled, parallel group study with 4 weeks of follow-up to evaluate preliminary efficacy and safety of recombinant human Nerve Growth Factor (rhNGF) eye drops solution versus vehicle in patients after cataract and refractive surgery.**

*Double-masked, single-centre, two-group, one dose, efficacy and safety study*

**EudraCT Number: 2016-002172-27**

Test formulation:	Recombinant human nerve growth factor (rhNGF), 20 µg/ml, ophthalmic sterile buffered aqueous solution, Dompé farmaceutici s.p.a., Italy
Sponsor:	Dompé farmaceutici S.p.A., Via Santa Lucia 6, 20122 Milan, Italy Phone: +39.02.583831 Fax: +39.02.58383324 Contact: Paolo Battigello Email: <a href="mailto:paolo.battigello@dompe.com">paolo.battigello@dompe.com</a>
Principal Investigator	Prof. Leonardo Mastropasqua, MD Clinica Oftalmologica - Centro regionale di Eccellenza in Oftalmologia Università G. D' Annunzio Via dei Vestini 66100 Chieti, Italy Phone: +39.0871.35713581 Fax: +39.0871.358794
Development phase:	Phase II
Version and date:	Final Version 3.0, 12 Apr 2017

*This study will be conducted in accordance with Good Clinical Practice (GCP), ICH topic E6*

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**PROTOCOL APPROVAL**

**SPONSOR**

Dompé farmaceutici S.p.A. , Italy

**Clinical Development Senior Specialist**

Paolo Battigello, M.Sc.

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

**Medical Expert**

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.*

Prof. Leonardo Mastropasqua, MD  
Clinica Oftalmologica - Centro regionale di Eccellenza in Oftalmologia  
Università G. D' Annunzio  
Chieti, Italy

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature

## CONTACTS

### **Dompé Drug Safety**

Email to: [farmacovigilanza@dompe.com](mailto:farmacovigilanza@dompe.com)  
or Fax: +39.02.36026913

### **Dompé Medical Expert**

Flavio Mantelli – Chief Medical Officer Ophthalmology,  
Email to: [flavio.mantelli@dompe.com](mailto:flavio.mantelli@dompe.com)  
or Fax: +39.02.58383324

### **Dompé Clinical Development**

Paolo Battigello – Clinical Development Senior Specialist  
Email to: [paolo.battigello@dompe.com](mailto:paolo.battigello@dompe.com)  
or Fax: +39.02.58383324

## **CRO: CROMSOURCE**

**Mauro D’Apolito** – Clinical Research Senior Director  
Email to: [mauro.dapolito@cromsource.com](mailto:mauro.dapolito@cromsource.com)  
Or Fax: +39.045.8222812

**Maria Conson** - Project Manager  
Email to: [maria.conson@cromsource.com](mailto:maria.conson@cromsource.com)

### **CromSource Pharmacovigilance**

Email to: [NGF0116-PV@cromsource.com](mailto:NGF0116-PV@cromsource.com)  
or Fax: +39 045 82 50 574

### **CromSource Medical Monitor**

Email to: [NGF0116-MM@cromsource.com](mailto:NGF0116-MM@cromsource.com)  
or Fax: +39 045 82 50 574

## Study Synopsis

<p><b>Title:</b> A 8 weeks, Phase II, single-centre, randomized, double-masked, vehicle-controlled, parallel group study with 4 weeks of follow-up to evaluate preliminary efficacy and safety of recombinant human Nerve Growth Factor (rhNGF) eye drops solution versus vehicle in patients after cataract and refractive surgery.</p> <p><b>Protocol number:</b> NGF0116</p> <p><b>EudraCT Code:</b> 2016-002172-27</p>	
<p><b>Clinical phase:</b> Phase II</p>	
<p><b>Study design:</b> Double-masked, single-centre, two-group, one dose, efficacy and safety study</p>	
<p><b>Study Centre:</b> Clinica Oftalmologica - Centro regionale di Eccellenza in Oftalmologia - Università G. D'Annunzio – Chieti, Italy</p>	
<p><b>Principal Investigator:</b> Prof. Leonardo Mastropasqua, MD - Clinica Oftalmologica - Centro regionale di Eccellenza in Oftalmologia - Università G. D' Annunzio - Chieti, Italy</p>	
<p><b>Investigational product(s):</b></p>	
<p><b>TEST PRODUCT:</b></p>	<p>Recombinant human nerve growth factor (rhNGF) ophthalmic sterile buffered aqueous solution, Dompé farmaceutici S.p.A, Italy, 20 µg/mL vials (Group 1)</p>
<p><b>Background information</b></p> <p>NGF is a polypeptide discovered in the early 1950s by R. Levi Montalcini. NGF acts through specific high affinity (i.e., TrkA) and low affinity (i.e., p75NTR) NGF receptors which are expressed not only on nerve fibres 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 a hundred 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 eye(s) 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 where dry eye had been surgically induced resulted in enhanced production and functional characteristics of tear film, with an associated improvement of ocular surface signs.</p> <p>Based on these data, the Company has developed a recombinant human NGF (rhNGF) expressed in E. coli for treatment of NK.</p>	

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

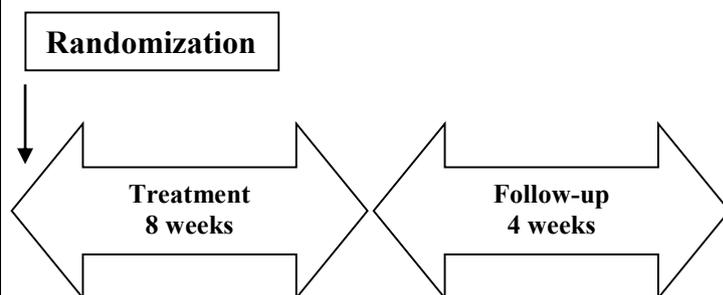
In addition to NK, rhNGF, in the same formulation (containing L-methionine) proposed for the present study, has been evaluated in a Phase I/II study in retinitis pigmentosa (RP) patients at the doses of 60 and 180 µg/mL, and in a phase II study in neurotrophic keratitis patients at the same concentration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

An open uncontrolled study showed that 4 weeks 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).

### **Study Design and Methodology**

The proposed phase II study is a single-centre, randomized, double masked, parallel arm, vehicle-controlled trial, designed to evaluate the preliminary efficacy and safety of rhNGF eye drops at 20 µg/ml concentration administered six times daily for 8 weeks in patients who underwent cataract and corneal refractive surgery, both known to damage the corneal sensory nerve plexus.

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 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±2) or early exit and at 4 weeks (day 84±2) after the end of study treatment.

### **Treatment Groups**

Eligible patients will be randomized to either rhNGF eye drops 20 µg/ml or vehicle:

**Group 1: *rhNGF 20 µg/mL*:** One drop (40 µL) corresponding to 0.80 µg of rhNGF will be instilled into each eligible 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 eligible eye six times a day (every 2h).

In all patients, both eyes will be treated, if meet the eligibility criteria. If only one eye is eligible, then only that eye will be treated.

In the 4 week follow up no further treatment is planned except artificial tears, per Investigator decision. Administration of such medications will be recorded in eCRF (number of drops per day).

### **Primary Objective**

The primary objective of this study is to assess efficacy and safety of rhNGF when administered as eye drops to patients after cataract and refractive surgery.

### **Study end-points**

Clinical efficacy and safety parameters will be evaluated at each time point (days 0, 28±2, 56±2 and 28±2 days after discontinuation of treatment) with the following endpoints:

#### **Primary efficacy end-point:**

- Change from baseline in SANDE scores for severity and frequency assessed at 8 weeks of treatment

#### **Co-primary Efficacy Endpoint**

- Changes in Cornea vital staining with fluorescein (National Eye Institute [NEI] scales) assessed at 8 weeks of treatment

#### **Secondary efficacy end-points:**

- Changes Conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales)
- Changes in Tear Film Break-Up Time (TFBUT)
- Changes in Cochet-Bonnet corneal aesthesiometry
- Changes in Nerve count and morphology at scanning laser in vivo corneal confocal microscopy (only patients who had LASIK surgery)
- Changes in SANDE scores (face values) for severity and frequency

**Safety end-point:**

- Incidence and frequency of Treatment-Emergent Adverse Events (TEAEs), assessed throughout the study

Details of efficacy and safety analyses will be presented in the Statistical Analysis Plan finalised before Database Lock.

**Number of Subjects**

In total 180 post cataract or refractive surgery patients will be enrolled in the study with 120 patients to be treated with rhNGF and 60 patients treated with vehicle.

**Sample size calculation**

Sample size was calculated by assuming at single eye level a mean difference from baseline mean SANDE scores of  $-30 \pm 20$  in the rhNGF groups versus  $-20 \pm 20$  in vehicle group (80% power,  $\alpha = 0.05$ ; 2-sided test), considering that the active comparator has already proven safe and effective in dry eye patients. The sample size has been calculated based on a 2:1 randomization 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 and  $n2$ = vehicle and  $n = 63$  and  $k = n1 / n2 = 2/1 = 2$ . Therefore  $n1 = 94.5$  and  $n2 = 47.25$ . Assuming a 5% drop out rate, a total of 100 patients will be enrolled in active arm and 50 in the vehicle arm. This calculation is based on one study eye per patient. Whereas also two study eyes per patient may occur, that will increase power to above 80%).

However, in order to provide the mean of detecting a significant difference also on a clinical sign of the effect of the rhNGF therapy (namely Co-primary Efficacy Endpoint cornea and conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales)), sample size calculation was also performed by assuming, at single eye level, a mean difference from baseline in Cornea NEI scale scores of  $-3.9 \pm 2.2$  in the rhNGF groups versus  $-2.9 \pm 2.2$  in vehicle group (80% power,  $\alpha = 0.05$ ; 2-sided test), considering that the active comparator has already proven safe and effective in dry eye patients. The sample size has been calculated based on a 2:1 randomization 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 and  $n2$ = vehicle and  $n = 76$  and  $k = n1 / n2 = 2/1 = 2$ . Therefore  $n1 = 114$  and  $n2 = 57$ . Assuming a 5% drop out rate, a total of 120 patients will be enrolled in active arm and 60 in the vehicle arm. This calculation is based on one study eye per patient. Whereas also two study eyes per patient may occur, that will increase power to above 80%).

Considering the two calculations performed to determine the sample size, in this study will be enrolled 180 patients (120 patients enrolled in active arm and 60 in the vehicle arm) in order to detect both changes at the same time in the variables.

No adjustment for multiplicity has been made considering that rhNGF efficacy is to be evaluated on the contemporaneous change of both variables (see ICH-E9)

**Main selection criteria**

**Inclusion Criteria:**

1. Male or female  $\geq 18$  years old
2. Patients who are characterized by the following clinical features:
  - a. History of cataract or refractive corneal surgery in the study eye(s) in the previous 6 months;
  - b. Mean Symptom Assessment in Dry Eye (SANDE) score for severity and frequency of at least 30 at baseline
3. The same eye (study eye) must fulfill all the above criteria
4. Best corrected distance visual acuity (BCDVA) score of  $\geq 0.1$  decimal units in both eyes at the time of study enrolment
5. Female patients must have negative pregnancy urine test if at childbirth potential.
6. 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.
7. Patients must have the ability and willingness to comply with study procedures.

**Exclusion Criteria:**

1. Any ocular disease other than Dry Eye requiring treatment with topical medications in either eye at the time of study enrolment.
2. Any active ocular infection or active inflammation in either eye unrelated to Dry Eye.
3. Presence or history of any systemic or ocular disorder, condition or disease (with particular attention to malignancies and neuro-oncological diseases) that could possibly interfere with the conduct of the required study procedures or the interpretation of the study results.
4. Use of therapeutic or Refractive Contact lenses in either eye at the time of study enrolment;
5. History of ocular surgery in the study eye(s), excluding corneal refractive or cataract procedures, within 90 days of study enrolment.
6. 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.
7. Participation in another clinical study at the same time as the present and within 30 days of study enrolment;
8. History of drug, medication or alcohol abuse or addiction.

**Data analysis**

Statistical analysis of demographic, efficacy and safety data will be defined in the statistical analysis plan and will be performed using SAS, Version 9.1.3 Service Pack 4 for Windows (or higher). The data documented in this study will be summarized using classic descriptive statistics for quantitative variables and frequencies for qualitative variables. Details on the final study analysis will be provided in the study Statistical Analysis Plan.

## Study Schedule

Study procedures	Baseline Visit (Day 0)	Week 4 (Day 28±2)	Week 8** (ETV) (Day 56±2)	Week 12 (Follow-up Visit) (Day 84±2)
Informed Consent	X			
Inclusion/Exclusion Criteria	X			
Urine Pregnancy Test	X	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	X	X
Record Adverse Events (AEs)	X	X	X	X
Verify patient study medication dosing compliance		X	X	
SANDE questionnaire	X	X	X	X
Best Corrected Distance Visual Acuity (BCDVA)	X		X	X
Cochet-Bonnet aesthesiometry	X		X	
External Ocular Examination	X	X	X	X
Slit Lamp Examination (SLE)	X	X	X	X
Fluorescein staining (NEI scale)	X	X	X	X
Tear Film Break-up Time (TFBUT)	X	X	X	X
Corneal confocal microscopy*	X		X	
Study drug dispensation	X	X		
<p>* Only for patients who underwent LASIK surgery            ** ETV – Early Termination Visit to be performed according to Visit Week 8 procedures, if premature treatment discontinuation occurs.            Note: Patient diary will be reviewed for potential AEs, and details as applicable            Abbreviations: SANDE - Symptom Assessment In Dry Eye</p>				

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## LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
BCDVA	Best Corrected Distance Visual Acuity
CDISC	Clinical Data Interchange Standards Consortium
eCRF	Electronic Case Report Form
CRO	Contract Research Organisation
ETDRS	Early Treatment Diabetic Retinopathy Study
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IMP	Investigational Medicinal Product
IUD	Intra-Uterine Device
iv	intravenous
LNGFR	Low-Affinity Nerve Growth Factor Receptor
MedDRA	Medical Dictionary for Regulatory Activities
mL	Millimetres
mNGF	Murine Nerve Growth Factor
µg	Micrograms
NA	Not Applicable
NEI	National Eye Institute
NGF	Nerve Growth Factor
NK	Neurotrophic Keratitis
OTC	Over The Counter
PT	Preferred Term
PTAE	Pre-Treatment Adverse Event
PR	Pulse rate
rhNGF	Recombinant human nerve growth factor
RP	Retinitis Pigmentosa
SAE	Serious Adverse Event
SANDE	Symptom Assessment In Dry Eye
SBP	Systolic Blood Pressure
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	neurotrophic tyrosine kinase receptor type 1
VA	Visual Acuity
VAS	Visual analogical scale
WHODDE	World Health Organisation 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: TrkA, a transmembrane tyrosine kinase and p75 LNGFR (for "low-affinity nerve growth factor receptor") often abbreviated as 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 humour. 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* has been developed. However, because the biological activity of NGF relies on the formation of three disulphide bonds and because disulphide 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 pro-NGF. After expression of pro-NGF in *E. coli*, the insoluble protein is isolated in the form of insoluble inactive aggregates (inclusion bodies), solubilised in a strong denaturing agent and subsequently converted into the natural conformation, which is determined by the disulphide 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 rh-NGF 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 at 20 µg/mL i.e. 0.020 mg/mL) as drug substance.

#### **1.1.3 Previous experience in humans with rhNGF**

NGF acts through specific high affinity (i.e., TrkA) and low affinity (i.e., p75NTR) NGF receptors which are expressed not only on nerve fibres 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 a hundred 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 where dry eye had been surgically induced resulted in enhanced production and functional characteristics of tear film, with an associated improvement of ocular surface signs.

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

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

In addition to 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 and in a phase II study in neurotrophic keratitis patients at the same concentration of the present study, 20 µg/mL (6 times a day).

An open uncontrolled study showed that 4 weeks 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 and dry eye disease make rhNGF a strong candidate for the treatment of patients who underwent cataract and corneal refractive surgery, both known to damage the corneal sensory nerve plexus.

As part of the development plan to evaluate the potential efficacy of the rhNGF solution, the present exploratory study was designed in order to further evaluate the potential efficacy and safety of rhNGF eye drops in this population of patients.

For additional information regarding the development of rhNGF, please consult the current Investigator Brochure.

### **1.2.1 Dose and Schedule Rationale**

The dose proposed for this trial is 20 µg/mL (1 drop/eye, 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.

### **1.3 Risks and benefits**

In the present study, potential risks of multiple rhNGF applications to post cataract and refractive surgery patients are expected not to surpass in frequency the adverse reactions and the untoward effects reported in the Phase I study (NGF0112).

The post cataract and refractive surgery patients participating in this study may potentially benefit from the application of rhNGF for 56 days and restoration of the damages to the corneal sensory nerve plexus associated with the surgery.

## **2 STUDY OBJECTIVES**

The primary objective of this study is to assess the preliminary efficacy and safety of rhNGF when administered as eye drops to patients who underwent cataract and corneal refractive surgery.

### **2.1 Primary efficacy end-point**

Following refractive surgery, due to the iatrogenic surgical damage to corneal sensory nerves, a variable percentage of patients complain of dry eye symptoms that often do not correlate with specific ocular surface signs.

Therefore, a validated symptoms' questionnaire has been chosen to assess the preliminary efficacy of rhNGF eye drops in this clinical trial.

The two-item SANDE (Symptom Assessment in Dry Eye) is a validated questionnaire that has been widely reported to be quick, easy to perform for patients, and equally efficacious in scoring dry eye symptoms compared with the Ocular Surface Disease Index (OSDI), another validated questionnaire based on 12 questions that is commonly used in clinical trials on dry eye patients. However, while OSDI is designed for ocular surface disease in general, the SANDE questionnaire is designed specifically for dry eye symptoms, which are those commonly reported after corneal and refractive surgery procedures, including cataract surgery.

Preliminary evaluation of the clinical efficacy throughout the study on the basis of the following symptoms assessment:

- Change from baseline in SANDE scores for severity and frequency assessed at 8 weeks of treatment
- Assessments of SANDE score will be performed on days 0, 28±2, 56±2 and 28±2 days after discontinuation of treatment.

#### **2.1.1 Co-primary efficacy end-point**

Preliminary evaluation of the clinical efficacy throughout the study on the basis of the following sign assessment:

- Changes in Cornea vital staining with fluorescein (National Eye Institute [NEI] scales) assessed at 8 weeks of treatment

### **2.2 Secondary efficacy end-points**

Additional evaluation of the clinical efficacy throughout the study will be performed on the basis of the following assessments:

- Changes in Cornea and conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales)
- Changes in Tear Film Break-Up Time (TFBUT)

- Changes in Cochet-Bonnet corneal aesthesiometry
- Changes in Nerve count and morphology at scanning laser in vivo corneal confocal microscopy (only patients who had LASIK surgery)
- Changes in SANDE scores (face values) for severity and frequency

### **2.3 Safety end-point**

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

### 3 CLINICAL SUPPLIES

#### 3.1 Treatment

##### 3.1.1 Description of products

The analytical certificates will be enclosed with the investigational medicinal product (IMP).

##### Test product

TEST PRODUCT	
IMP	Recombinant human Nerve Growth Factor (rhNGF), containing L-methionine as excipient <ul style="list-style-type: none"> <li>- 20 µg/mL vials (Group 1),</li> <li>- Vehicle vials (Group 2)</li> </ul>
Manufacturer active substance	Dompé farmaceutici S.p.A., Italy
Manufacturer finished product	Bulk drug product manufactured by Patheon Italia S.p.A-Italy; Packaging and labelling performed by MontereSearch s.r.l. Italy
Pharmaceutical form	sterile buffered aqueous solution
Dose	6 times per day for 8 weeks
Administration route	Ophthalmic

##### 3.1.2 Dose regimen

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

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

(a) 1 drop/eye=40 µL

##### 3.1.3 Route and method of administration

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

**Group 1:** *rhNGF 20 µg/mL*: One drop (40 µL) corresponding to 0.80 µg of rhNGF will be instilled into each eligible eye six (both eyes, if applicable) times a day (every 2h), for a total daily dose of 9.6 µg (both eyes, if applicable), for 56 consecutive days. Total dose will be 537.6 µg/56 days if both eyes are treated.

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

Enrolled patients will self-administer the eye drops at home into both eyes unless only one eye meets the eligibility criteria (in both group 1 and 2).

The Investigator will check that all patients take the IMP appropriately verifying the diary and the used and/or unused medication returned.

### 3.2 Packaging, labelling, distribution and storage

#### 3.2.1 Formulation and packaging

The Investigator will be provided with frozen IMP solutions ( $-20 \pm 5^{\circ}\text{C}$ ) containing rhNGF at 20 µg/mL (for the dosing Group 1) and vehicle (for group 2) in one monthly box containing 4 weekly boxes.

One monthly box including four weekly 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 delivery plan**

<b>Box delivery</b>	<b><u>Boxes to be provided</u></b>
Day 0	Week 1, Week 2, Week 3, Week 4 (Day 0 - Day $28 \pm 2$ ) - 1 Box rhNGF/Vehicle
Day $28 \pm 2$	Week 5, Week 6, Week 7, Week 8 (Day 29 - Day $56 \pm 2$ ) - 1 Box rhNGF/Vehicle

Each weekly box will contain a total of 7 vials of rhNGF (Group 1) or Vehicle (Group 2).

#### 3.2.2 Labelling, Storage and handling

##### Labelling

The medication labelling will report all the information requested according to the Annex 13 to the Good Manufacturing Practice (published by the Commission in The rules governing medicinal products in the European Community, Volume 4) as follows:

- a. Name, address and telephone number of the Sponsor, contract research organisation 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.

### **3.2.3 Storage and handling**

The Pharmacist and/or Investigator will be responsible for receipt, proper storage and usage of study drug.

The investigational product must be stored at  $-20 \pm 5$  °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 patients will receive from the study personnel 4 weekly 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 monthly box containing 4 weekly kits, at home as soon as possible and immediately store it in a freezer at  $-20 \pm 5$  °C.

The weekly kit in use 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 eye) as far as 12 hours are not exceeded.

Together with the IMP weekly 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 PE 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
- 5) the syringe must be discharged after each administration to both eyes, if applicable

### **3.3 Drug accountability**

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

Every four weeks from the start of the treatment the patients will return the used or unused study boxes to the Investigator.

The Pharmacist and/or Investigator, who will keep a cumulative inventory and dispensing records, will maintain all supplies under adequate security.

The Investigator will maintain an accurate record about the shipment and dispensing of the test drug, using a drug accountability ledger. An accurate drug disposition record will be kept specifying the date and amount dispensed to each patients.

Adequate record of receipt, use, return 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 at the conclusion of the study.

At each scheduled visit the diary should be reviewed 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 returned to the Sponsor, 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 authorisation by the Sponsor by an authorised company according to GCP regulations.

## **4 INVESTIGATIONAL PLAN**

### **4.1 Overall study design**

This is a Phase II study, single-centre, randomized, double masked, parallel arm, vehicle-controlled trial.

Randomization will be 2:1 of 180 post cataract or refractive surgery patients to rhNGF eye drops solution at 20 µg/ml (120 patients) or vehicle eye drops solution (60 patients) 6 times per day for 8 weeks.

The patients will be involved in the study for a maximum of 12 weeks from Day 0 visit to Week 12 follow-up visit.

### **4.2 Discussion of design**

The proposed phase II study is a single-centre, randomized, double masked, parallel arm, vehicle-controlled trial, designed to evaluate the preliminary efficacy and safety of rhNGF eye drops at 20 µg/ml concentration administered six times daily for 8 weeks in patients' eyes who underwent cataract and corneal refractive surgery, both known to damage the corneal sensory nerve plexus.

Patients will be evaluated at baseline (day 0), week 4 (28±2 days), week 8 (day 56±2) or early exit and 4 weeks (day 84±2) after the end of study treatment.

## **5 STUDY POPULATION**

### **5.1 Target population**

Male and female patients  $\geq 18$  years old, who had undergone post cataract and refractive surgery.

### **5.2 Inclusion criteria**

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

1. Male or female  $\geq 18$  years old
2. Patients who are characterized by the following clinical features:
  - a. History of cataract or refractive corneal surgery in the study eye(s) in the previous 6 months;
  - b. Mean Symptom Assessment in Dry Eye (SANDE) score for severity and frequency of at least 30 at baseline
3. The same eye (study eye) must fulfill all the above criteria
4. Best corrected distance visual acuity (BCDVA) score of  $\geq 0.1$  decimal units in both eyes at the time of study enrolment
5. Female patients must have negative pregnancy urine test if at childbirth potential.
6. 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.
7. 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. Any ocular disease other than Dry Eye requiring treatment with topical medications in either eye at the time of study enrolment.
2. Any active ocular infection or active inflammation in either eye unrelated to Dry Eye.
3. Presence or history of any systemic or ocular disorder, condition or disease (with particular attention to malignancies and neuro-oncological diseases) that could possibly interfere with the conduct of the required study procedures or the interpretation of the study results.
4. Use of therapeutic or Refractive Contact lenses in either eye at the time of study enrolment;
5. History of ocular surgery in the study eye(s), excluding corneal refractive or cataract procedures, within 90 days of study enrolment.
6. 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.
7. Participation in another clinical study at the same time as the present and within 30 days of study enrolment;
8. History of drug, medication or alcohol abuse or addiction.

### **5.3.1 History of drug, medication or alcohol abuse or addiction. Allowed/Disallowed treatments**

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".

Any systemic or topical ocular treatment is allowed, as prescribed by the treating physician both during treatment and follow-up periods.

## **6 STUDY SCHEDULE**

The schedule of the study is summarized at the end of the synopsis (page 9).

### **6.1 Study visits and procedures**

Each enrolled patient will undergo 4 visits.

Maximum study duration will be 12 weeks, Baseline/Day 0 visit included. A written informed consent will be obtained before any study assessment or procedure.

The first patient first visit (FSFV) is defined as the 1<sup>st</sup> visit performed at the clinical centre by the 1<sup>st</sup> screened patient. The last patient last visit (LSLV) is defined as the last visit performed at the clinical centre (or the telephonic follow-up, if applicable) by the last patient, i.e. the last visit foreseen by the study protocol, independently of the fact that the patient is a completer or a withdrawn patient.

The following phases, visits and procedures will be performed:

#### **➤ Interventional phase**

- Baseline/Day 0 Visit - Day 0
- Week 4 Visit - Day 28 ± 2
- Week 8 Visit - Day 56 ± 2/Early Termination Visit (ETV). In case of study discontinuation patients will undergo the assessments foreseen at Week 8 visit.

#### **➤ Follow-up phase**

- Week 12 Visit Follow-Up - Day 84 ± 2

	Day	Procedures/Assessments
<b>Baseline Visit</b>	<i>Day 0</i>	<ul style="list-style-type: none"> <li>➤ Explanation to the patient of study aims, procedures and possible risks</li> <li>➤ Informed consent signature</li> <li>➤ Screening number allocation (as S001, S002, etc.)</li> <li>➤ Demographic data</li> <li>➤ Medical and surgical history/current medical conditions</li> <li>➤ Prior/concomitant medications</li> <li>➤ AE monitoring</li> <li>➤ Ocular examination of both eyes:               <ol style="list-style-type: none"> <li>1. Assessment by Symptom Assessment iN Dry Eye (SANDE)</li> <li>2. Assessment of best corrected distance visual acuity (BCDVA)</li> <li>3. Slit lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Oedema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber</li> <li>4. External Ocular Examination</li> <li>5. Corneal Confocal Microscopy (Only for patients who underwent LASIK surgery)</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>6. Tear Film Break Up Time (TFBUT)               <p style="margin-left: 20px;"><b>Five minutes break</b></p> <li>7. Corneal sensitivity (Cochet-Bonnet)               <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>8. Ocular surface staining (NEI score)               <p style="margin-left: 20px;"><b>Ten minutes break</b></p> </li> <li>➤ Frequency of patient's own artificial tear use (to be reported in eCRF)</li> <li>➤ Pregnancy test for female patients.</li> <li>➤ Patient eligibility: Inclusion/exclusion criteria evaluation</li> <li>➤ Assignment of study eye(s) (Note: if both eyes are eligible, study treatment will be administered to both eyes, and each eye will be considered as "study eye")</li> <li>➤ Randomization</li> <li>➤ Study Drug Dispensation</li> </li></li></li></ul> <p><i>Note: At the study visit the Investigator will provide each patient with diary and one monthly box of the study drug for the following 4 weeks together with an adequate number of adaptors and syringes.</i></p> <p><i>After visit completion patients will start topical ocular treatment as per instructions and will self-administer at home the IMP. Patients will return to the clinical site on Day 28 ± 2 (Week 4 Visit).</i></p> <p><i>The patients will be instructed to fill in the diary with time of self-administration, potential AE occurrence, concomitant medication intake and the quantity of artificial tears used, and to return the refrigerated box on the Week 4 visit.</i></p>
<b>At home</b>	<i>Days 0-28±2</i>	<ul style="list-style-type: none"> <li>➤ Self-administration at home of the IMP, six times daily every 2 h in every study eye (diary)</li> <li>➤ Artificial tears use (diary)</li> <li>➤ Recording any new or changes in concomitant medications (diary)</li> <li>➤ Recording any unusual medical conditions - AE monitoring (diary)</li> </ul> <p>Data will be recorded by the patient on the patient's diary.</p>

	Day	Procedures/Assessments
<b>Week 4 Visit</b>	<i>Day 28±2</i>	<ul style="list-style-type: none"> <li>➤ Assessment of compliance to treatment (from patient diary and IMP reconciliation from returned weekly boxes)</li> <li>➤ Current medical conditions</li> <li>➤ Concomitant medications</li> <li>➤ AE monitoring</li> <li>➤ Ocular examination of both eyes:               <ol style="list-style-type: none"> <li>1. Assessment by Symptom Assessment iN Dry Eye (SANDE)</li> <li>2. Slit lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Oedema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber</li> <li>3. External Ocular Examination</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <ol style="list-style-type: none"> <li>4. Tear Film Break Up Time (TFBUT)</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <ol style="list-style-type: none"> <li>5. Ocular surface staining (NEI score)</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> </li> <li>➤ Frequency of patient’s own artificial tear use (to be reported in eCRF)</li> <li>➤ Pregnancy test for female patients.</li> <li>➤ Study Drug Dispensation</li> </ul> <p><i>Note: At the study visit the Investigator will provide each patient with diary and one monthly box of the study drug for the following 4 weeks together with an adequate number of adaptors and syringes.</i></p> <p><i>After visit completion the patients will continue study treatment as per instructions and will self-administer at home the IMP. Patients will return to the clinical site on Day 56 ± 2 (Week 8 Visit).</i></p> <p><i>The patients will be instructed to fill in the diary with time of self-administration, potential AE occurrence, concomitant medication intake and the quantity of artificial tears used.</i></p>
<b>At home</b>	<i>Days 28±2 -56±2</i>	<ul style="list-style-type: none"> <li>➤ Self-administration at home of the IMP, six times daily every 2 h (diary) in every study eye</li> <li>➤ Artificial tears use (diary)</li> <li>➤ Recording any new or changes in concomitant medications (diary)</li> <li>➤ Recording any unusual medical conditions - AE monitoring (diary)</li> </ul> <p>Data will be recorded by the patient on the patient’s diary.</p>

	Day	Procedures/Assessments
<b>Week 8 Visit</b>	Day 56±2  Or  Early termination (if applicable)	<p>In case of study early discontinuation (at any time) patients will undergo the assessments foreseen at Week 8 visit.</p> <ul style="list-style-type: none"> <li>➤ Compliance to treatment (from patient diary and control of returned weekly boxes)</li> <li>➤ current medical conditions</li> <li>➤ concomitant medications</li> <li>➤ AE monitoring</li> <li>➤ Ocular examination in both eyes:               <ol style="list-style-type: none"> <li>1. Assessment by Symptom Assessment iN Dry Eye (SANDE)</li> <li>2. Assessment of best corrected distance visual acuity (BCDVA)</li> <li>3. Slit lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Oedema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber</li> <li>4. External Ocular Examination</li> <li>5. Corneal Confocal Microscopy (Only for patients who underwent LASIK surgery)</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>6. Tear Film Break Up Time (TFBUT)</li> <p style="margin-left: 20px;"><b>Five minutes break</b></p> <li>7. Corneal sensitivity (Cochet-Bonnet)</li> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>8. Ocular surface staining (NEI score)</li> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> </li> <li>➤ Frequency of patient’s own artificial tear use (to be reported in eCRF)</li> <li>➤ Pregnancy test for female patients.</li> </ul> <p><i>Note:</i> The Investigator will deliver to the patients the diary. Patients will return to the clinical site on Day 84 ± 2 (Week 12 Visit). The patients will be instructed to fill in the diary with AE occurrence, concomitant medication intake and the quantity of artificial tears used.</p>
<b>At home</b>	Days 56±2-84±2	<ul style="list-style-type: none"> <li>➤ Artificial tears use (diary)</li> <li>➤ Recording any new or changes in concomitant medications (diary)</li> <li>➤ Recording any unusual medical conditions - AE monitoring (diary)</li> </ul> <p>Data will be recorded by the patient on the patient’s diary.</p>
<b>Week 12 Visit Follow-Up</b>	Day 84±2	<ul style="list-style-type: none"> <li>➤ Current medical conditions</li> <li>➤ Prior/concomitant medications</li> <li>➤ AE monitoring</li> <li>➤ Ocular examination in both eyes:               <ol style="list-style-type: none"> <li>1. Assessment by Symptom Assessment iN Dry Eye (SANDE)</li> <li>2. Assessment of best corrected distance visual acuity (BCDVA)</li> <li>3. Slit lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Oedema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber</li> <li>4. External Ocular Examination</li> </ol> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>5. Tear Film Break Up Time (TFBUT)</li> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> <li>6. Ocular surface staining (NEI score)</li> <p style="margin-left: 20px;"><b>Ten minutes break</b></p> </li> <li>➤ Frequency of patient’s own artificial tear use (to be reported in eCRF)</li> </ul>

## **7 DESCRIPTION OF SPECIFIC PROCEDURES**

### **7.1 Ophthalmological Evaluations**

Ocular evaluations will be performed on both eyes even if just one eye is eligible (determined as study eye). The assessments must always be performed according the sequence detailed in Par 6.1. The assessment will be performed at Day 0 Baseline Visit, Week 4 Visit, Week 8 Visit and at Week 12 Visit. 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 anaesthetic 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 millimetres 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). **Note: the AVERAGE value of severity and frequency scores will be used for verifying inclusion criterion #2 at Day 0 visit.**

#### **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. Refraction should be conducted prior to visual acuity testing to obtain best-corrected vision as described below. Best-corrected visual acuity is measured at Day 0 Baseline Visit, Week 8 Visit and at Week 12 Visit 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 metre 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 metre 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 metres 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 centre 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 metre 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 centred 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 metres first, even if the refraction was performed at 1 metre.

The patient should be seated comfortably directly in front of the chart so that the eyes remain at the 4 metre 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 metres, the patient is tested at 1 metre. The distance from the patient to the chart should be measured again using the rigid one metre stick. The distance is measured from the outer canthus to the centre 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 metre. 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 metres the visual acuity score for that eye is recorded as the number of letters correct at 4 metres plus 30 (refer to the VA worksheet). The patient gets credit for the 30 letters at 1 metre even though they did not have to read them. Otherwise, the visual acuity score is the number of letters read correctly at 1 metre plus the number, if any, read at 4 metres. If no letters are read correctly at either 4.0 metres or 1 metre, then the visual acuity score is recorded as “0.”

#### **7.1.4 Slit-lamp examination (SLE)**

The slit lamp examination 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. Eyelids, lashes, conjunctiva, cornea, lens, iris and anterior chamber will be evaluated.

### **7.1.5 Tear Film Break Up Time (TFBUT)**

TFBUT will be measured by determining the time to tear break-up. The TFBUT will be performed after instillation of 5 µl of 2% 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 Corneal sensitivity**

For the assessment of corneal sensation the Luneau Cochet-Bonnet aesthesiometer (Western Ophthalmics Corporation©) should be used.

The Cochet-Bonnet aesthesiometer contains a thin, retractable, nylon monofilament that extends up to 6 cm in length. Variable pressure can be applied to the cornea by adjusting the monofilament length. The monofilament length ranges from 6 to 0.5 cm. As the monofilament length is decreased the pressure increases from 11 mm/g to 200 mm/g.

Corneal sensation will be measured in both eyes in each of the four quadrants of the cornea using the Cochet Bonnet aesthesiometer before the instillation of any dilating or anesthetic eye drops.

Steps for using the Cochet Bonnet aesthesiometer: extend the filament of the aesthesiometer to full length of 6 cm. Slowly advance the monofilament towards the eye until the tip perpendicularly touches the corneal surface. Continue to exert a slight pressure until obtaining an inflection deviation of the filament of about 4% (i.e. the first visible inflection). If a positive reaction to the touch of cornea is elicited proceed to step 3. If a positive reaction is not achieved proceed to step 2. (NOTE: A positive response is any action indicating corneal sensation whether communicated verbally or physically).

Retract the filament incrementally in 0.5 cm and repeat this step until the patient gives a positive reaction indicating that the contact of the monofilament on the cornea has been sensed. (NOTE: The shorter filament lengths indicate decreased corneal sensation).

Record the length of the filament in cm at which the patient sensed the contact with the cornea.

Repeat steps 1-3 in each of the 4 corneal quadrants (superior nasal, inferior nasal, superior, temporal and inferior temporal) in the study eye(s) and in each of the 4 corneal quadrants of the other eye (not the study eye, if applicable).

To clean the instrument, please refer to your national standards for devices that contact the eye and tears or follow the suggested manufacturer's recommendations.

The length of the filament in cm at which the patient corneal sensation was observed for the tested area of the cornea will be entered into the eCRF.

Corneal sensitivity findings will be entered in the eCRF.

#### **7.1.7 Ocular surface staining (NEI score)**

As grading scale of the corneal and conjunctiva damage, the NEI/Industry Workshop guidelines will be used. 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.

Ideally the grading should be performed between one and four minutes after staining. 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

#### **7.1.8 Corneal confocal microscopy (only for patients who underwent LASIK surgery)**

In vivo scanning confocal microscopy will be performed bilaterally in the central cornea to more closely examine the corneal epithelium, sub-basal nerve plexus, stroma and endothelium with particular attention given to the presence and density of corneal nerves. Prior to the procedure patients will be treated with anesthetic drops and a viscoelastic liquid gel will be applied to the cornea as an immersion substance for the microscope lens.

## **8 ASSIGNMENT OF STUDY TREATMENT**

### **8.1 Randomization**

After obtaining informed consent a consecutive screening number will be assigned to each patient according to the sequence of study entry, from S001 onwards (S002, S003 etc.). After confirming eligibility for the study, patient will be randomized (2:1 to rhNGF eye drops solution at 20 µg/ml (120 patients) or vehicle eye drops solution (60 patients) to be administered 6 times per day per study eye (or both eyes if applicable) for 8 weeks) and allocated with a consecutive randomization number of the randomization list starting with 001 onwards (002, 003 etc.).

### **8.2 Treatment allocation**

After successful completion of screening each eligible patient will be assigned a patient number (randomization number) according to the sequence of study entry (randomization), from 001 to 180. Drop outs will not be replaced after randomization.

### **8.3 Masking**

For the whole duration of the trial treatments will be unknown to the patient, the Investigator and the site staff. 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 (data base lock) except in case of specific events that will require unmasking of the patient.

The vials of rhNGF (20 µg/ml) and the vials containing the vehicle of rhNGF will be identical in appearance and the contents of the vials will be indistinguishable. All staff who is 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. If there is sufficient time, the Investigators are encouraged to contact the CRO staff to discuss individual case before unmasking concerned patient.

## **9 EVALUATION PARAMETERS**

### **9.1 Study variables**

#### **9.1.1 Primary variables**

- Treatment-emergent adverse events (TEAEs), assessed throughout the study
- Symptom Assessment in Dry Eye (SANDE) – mean changes from baseline will be evaluated for severity and frequency (for primary week 8 endpoint analysis, LOCF will be imputed for early end-of treatment data)

#### **9.1.2 Co-primary variable**

- Cornea vital staining with fluorescein (National Eye Institute [NEI] scales) – mean changes from baseline will be evaluated at week 8 for Co-primary endpoint analysis, LOCF will be imputed for early end-of treatment data)

#### **9.1.3 Secondary variables**

- Conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales)
- Tear Film Break-Up Time (TFBUT)
- Cochet-Bonnet corneal aesthesiometry
- Nerve count and morphology at scanning laser in vivo corneal confocal microscopy (only patients who had LASIK surgery)

Evaluations will be performed on week 4, week 8 and 4 weeks after discontinuation of treatment (week 12).

## **10 STATISTICAL METHODS**

The data documented in this study will be summarized using descriptive summary statistics, i.e. arithmetic mean, SD, CV (%), minimum, median and maximum values for quantitative variables, and frequencies for qualitative variables. In case a patient has two study eyes, its quantitative efficacy data will be regarded as within subject duplicates, allowing more precise patient values as well as the splitting of the SD into a within-subject and between-subject component.

The statistical analysis will be performed using SAS<sup>®</sup> version 9.1.3 Service Pack 4 for Windows<sup>®</sup> or higher.

A statistical analysis plan (SAP) will be issued before database lock and de-masking. Final statistical analysis on the study primary, co-primary and secondary 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 meets all the inclusion criteria and does not fulfil any of the exclusion criteria for at least one eye (defined as study eye). 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.
- Safety Set (SAF): all enrolled patients who receive at least one dose of the investigational medicinal product(s) at the study eye(s). This analysis set will be used for demographic, baseline and background characteristics and the safety analysis
- Full Analysis Set (FAS): all patients in the SAF, who have at least one post-baseline efficacy measurement. This analysis set will be used for the primary and co-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 and co-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, SAF, FAS and PP. Patients will be evaluated according to the treatment 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 any study eye
- lack of any efficacy data post enrolment
- failure to satisfy major inclusion/exclusion criteria (eligibility violations). Patients who fail to satisfy an inclusion/exclusion criterion may be excluded from the analysis without the possibility of introducing bias only under the following circumstances:
  - the inclusion/exclusion criterion was measured prior to enrolment
  - the detection of the relevant eligibility violations can be made completely objectively
  - all detected violations of the particular inclusion/exclusion criterion are excluded

### **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 or co-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 at single eye level a mean difference from baseline mean SANDE scores of  $-30 \pm 20$  in the rhNGF groups versus  $-20 \pm 20$  in vehicle group (80% power,  $\alpha = 0.05$ ; 2-sided test), considering that the active comparator has already proven safe and effective in dry eye patients. The sample size has been calculated based on a 2:1 randomization by the following formula:

$$n_1 = (0.5) \times n \times (1 + k)$$

$$n_2 = (0.5) \times n \times (1 + (1/k))$$

with  $n_1$  = active treatment and  $n_2$  = vehicle and  $n = 63$  and  $k = n_1 / n_2 = 2/1 = 2$ . Therefore  $n_1 = 94,5$  and  $n_2 = 47,25$ . Assuming a 5% drop out rate, a total of 100 patients in the active arm and 50 in the vehicle arm are required. This calculation is based on one study eye per patient. Whereas also two study eyes per patient may occur, that will increase power to above 80%).

However, in order to provide the mean of detecting a significant difference also on a clinical sign of the effect of the rhNGF therapy (namely Co-primary Efficacy Endpoint cornea vital staining with fluorescein (National Eye Institute [NEI] scales)), sample size calculation was also performed by assuming, at single eye level, a mean difference from baseline in Cornea NEI scale scores of  $-3.9 \pm 2.2$  in the rhNGF groups versus  $-2.9 \pm 2.2$  in vehicle group (80% power,  $\alpha = 0.05$ ; 2-sided test), considering that the active comparator has already proven safe and effective in dry eye patients. The sample size has been calculated based on a 2:1 randomization by the following formula:

$$n_1 = (0.5) \times n \times (1 + k)$$

$$n_2 = (0.5) \times n \times (1 + (1/k))$$

with  $n_1$ = active treatment and  $n_2$ = vehicle and  $n= 76$  and  $k= n_1 / n_2 = 2/1=2$ . Therefore  $n_1= 114$  and  $n_2=57$ . Assuming a 5% drop out rate, a total of 120 patients in active arm and 60 in the vehicle arm are required. This calculation is based on one study eye per patient. Whereas also two study eyes per patient may occur, that will increase power to above 80%).

Considering the two calculations performed to determine the sample size, in this study will be enrolled 180 patients (120 patients enrolled in active arm and 60 in the vehicle arm) in order to detect both changes at the same time in the variables.

### **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:

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

Gross non compliance will be defined as compliance lower than 80% or greater than 120% and in case of 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(s) 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 all study eye(s) together using descriptive statistics for quantitative variables, and frequencies for qualitative variables by treatment and evaluation time point. Changes from baseline (Day 0 Baseline Visit assessment) will be presented as well, if applicable.

No adjustment for multiplicity has been made considering that rhNGF efficacy is to be evaluated on the contemporaneous change of both variables (see ICH-E9).

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).

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

Individual AEs/TEAEs will be listed in patient data listings. TEAEs will be summarized by treatment. 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 EVALUATION OF ADVERSE EVENTS AND SAFETY INFORMATION**

### **11.1 Definition and handling of AEs and SAEs**

#### **11.1.1 Adverse Events**

An Adverse Event (AE) is defined as any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product, which does not necessarily have a causal relationship with the study treatment (Directive 2001/20/EC). An AE can therefore be any unfavourable 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.

#### **11.1.2 Adverse Drug Reaction**

An Adverse Drug Reaction (ADR) is any noxious and unintended response to an Investigational Medicinal Product (IMP) which is a reasonably likely to have been caused by the drug, at any dose administered.

For the purposes of reporting to the concerned Authorities, “reasonable possibility” means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event (reference: par. 11.4 and 11.5).

#### **11.1.3 Serious Adverse Event (SAE)**

A serious adverse event (SAE) is defined in line with Directive 2001/20/EC as any adverse experience that 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 out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils 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, diarrhoea, 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 well-being 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. 11.5.2). 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, the event shall be reported as SAE and cause of death shall always be investigated and specified as soon as known, as applicable.

A Non-Serious Adverse Event/Non-Serious Adverse Drug Reaction is an adverse event or adverse drug reaction that does not meet the criteria listed above for a serious adverse event/serious adverse drug reaction.

#### **11.1.4 Suspected serious unexpected adverse reaction**

A Suspected Serious Unexpected adverse reaction (SUSAR) is defined as an adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction.

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

#### **11.2 Adverse Events 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):

- Adverse Events 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

- Adverse Events that caused a decrease in visual acuity to the level of Light Perception or worse lasting >1 hour
- Adverse Events 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
- Adverse Events associated with severe intraocular inflammation (i.e., 4+ anterior chamber cell/flare or 4+ vitritis)
- Adverse Events that, in the opinion of the Investigator, may require medical intervention to prevent permanent loss of sight.

### **11.3 Adverse Events (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 medication(s) that is the result of an untoward (unfavourable 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 (unfavourable 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.

### **11.4 Recording**

#### Adverse Events:

All AEs (non-serious and serious) which 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) until completion of the follow up period, should be specified in the eCRF. Subsequent to signing an informed consent form, all untoward medical occurrences that occur during the course of the study must be documented on the 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.

#### 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 11.4.1 Relationship of the Adverse Event to the Investigational Product**

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
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

Severity of AEs

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

**Table 11.4.2 Intensity (Severity) of the Adverse Event**

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

## 11.5 Serious Adverse Event Reporting

### 11.5.1 Reporting Procedure for Investigators to Sponsor or CRO

The Investigator must record all SAEs, including sight-threatening events, occurring at any time during the study regardless of presumed causal relationship, both in the dedicated section of eCRF and on a specific Non-Carbon Repeat Serious Adverse Event form (included in the Investigator's Site File) within 24 hours of learning of the event. Within 24 hours from first knowledge of the SAE, the Investigator shall send the filled in and signed SAE form to the CRO and to the Sponsor at the following addresses:

**Dompé Drug Safety**

Email to: [farmacovigilanza@dompe.com](mailto:farmacovigilanza@dompe.com)

or Fax: +39.02.36026913

**CromSource Drug Safety**

Email to: [NGF0116-PV@cromsource.com](mailto:NGF0116-PV@cromsource.com)  
or Fax: +39 045 82 50 574

**Dompé Medical Expert**

Flavio Mantelli – Chief Medical Officer Ophthalmology,  
Email to: [flavio.mantelli@dompe.com](mailto:flavio.mantelli@dompe.com)  
or Fax: +39.02.58383324

**CromSource Medical Monitor**

Email to: [NGF0116-MM@cromsource.com](mailto:NGF0116-MM@cromsource.com)  
or Fax: +39 045 82 50 574

**Dompé Clinical Development**

Paolo Battigello – Clinical Development Senior Specialist  
Email to: [paolo.battigello@dompe.com](mailto:paolo.battigello@dompe.com)  
or Fax: +39.02.58383324

If assistance is needed with the reporting of a SAE, contact details for the CRO/Sponsor are provided in the section “Contact Information”.

Serious adverse events will be managed directly by the Dompé Drug Safety department, with CRO support for Follow Up requests.

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

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 relatedness of the observed event(s) with the investigational medication.

In line with CT3 Detailed Guidance and ICH E2A provisions, although the Investigator does not usually need to actively monitor patients for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a patient after that patient has ended his/her participation in the study (whether study completion or withdrawal), the SAE should be reported by the Investigator to Dompé Drug Safety. Such “post-study cases” should be regarded for expedited reporting purposes as though they were study reports. Therefore, a causality assessment and determination of expectedness are needed for a decision on whether or not expedited reporting is required.

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

### **11.5.2 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 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, abnormal lab values or test results that do not induce clinical signs and/or symptoms and require intervention/therapy, i.e. are clinically significant shall not be considered SAE:

### **11.5.3 Reporting Procedure to IEC and to Regulatory Authorities**

During the course of the clinical trial, Dompé shall report any serious unexpected suspected adverse reaction (SUSAR) to Eudravigilance Clinical Trial Module and to the concerned IEC which approved the protocol as soon as possible and in no event later than:

- (a) seven calendar days after becoming aware of the information if the event is fatal or life threatening; and
- (b) fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

Dompé shall, within eight days after having informed the IEC/Competent Authority under paragraph (a) and within fifteen days under paragraph (b), submit a complete report in respect of that information that includes an assessment of the importance and implication of any findings, made on the basis of follow up information provided by the Investigator.

Dompé shall be responsible for cross trial reporting, as necessary.

Dompé shall assess SAE report received for relationship with IMP and expectedness. Expectedness will be assessed with respect to the current Investigator's Brochure: due to the early phase of development, all SAEs will be considered unexpected.

For serious adverse event reported by the Investigator as not related that is subsequently revised to be related by the Sponsor, the Investigator will receive a notification.

Events considered "Possible", "Probable" and "Highly Probable" related to the IMP treatment will be reported to appropriate regulatory authorities.

Treatment will be unblinded by Sponsor Pharmacovigilance prior to submission of a SUSAR to Regulatory Authorities and European IECs and only cases referred to active treatment will be expeditable for regulatory reporting, in line with law requirements.

The Sponsor shall be responsible to prepare and submit periodical update reports as appropriate to Investigator and to Competent Authority and Ethics Committee.

#### **11.6 Follow-up of Patients with Adverse events**

The Investigator is responsible for adequate and safe medical care of patients during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. All AEs should be followed-up to determine outcome of the reaction or until 10 days after the final visit. 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.

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 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 enrolment 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, 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 (included in the Investigator's Site File) as soon as possible (within 24 hours of learning of the pregnancy to the CRO/Dompé Drug Safety contacts reported at Paragraph 11.5.1, even if no AE has occurred, and follow it to term. If, however, 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 11.5.1 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 occurring to a female patient leads to the immediate cessation of study treatment.

#### **11.8 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.

## **11.9 Overdose**

Cases of overdose (accidental or intentional) which result in serious adverse events 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's clinical research personnel and CRO by e-mail or fax within 24 hours using the SAE form, in order to have information about symptoms, corrective treatment and outcome of overdose.

## **12 DATA MANAGEMENT PROCEDURES**

### **12.1 Data collection – 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 subject.

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 for study site consists of the Sponsor study code (i.e. NGF0116), the 4-digit screening number (e.g. S001, S002, etc.) and, if applicable, the 3-digit subject study number (e.g. 001, 002, etc.).

Study code, screening number and subject study number are separated by slashes (e.g. "NGF0116/S001/001"). The last 5 digits of the unique subject identifier (randomized subjects), corresponding to the subject screening and subject study numbers separated by a slash, or the last 3 digits of the unique subject identifier (not randomized subjects), corresponding to the subject screening number, will appear as subject identifier in the individual listings and figures of the clinical study report and will be used to identify the subjects in in-text tables or wording (if applicable).

### **12.3 Database management**

Data management of all data captured within the eCRF will be performed by the CRO with the OmniComm TrialMaster™. 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 (SDTM 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.

## **13 STUDY MONITORING, QUALITY CONTROL AND QUALITY ASSURANCE**

### **13.1 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 authorised 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.

### **13.2 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).

### **13.3 Applicable SOPs**

The Sponsor, the clinical centre 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.

### **13.4 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.

### **13.5 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 authorised individuals to have access to all the study documentation.

## **14 ETHICAL CONSIDERATIONS**

### **14.1 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 Research Ethics Committees Authorities will be obtained before the start of the study.

Study notification to the Competent Authorities will be performed according to the Italian 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.

### **14.2 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 § 15.2). 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.

### **14.3 Insurance policy**

An insurance cover has been issued in favour 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.

### **14.4 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.

#### **14.4.1 Primary reason for discontinuation**

- **Adverse event:** Any significant adverse event that in the opinion of the Investigator or concerned patient is not compatible with study continuation. For the definition of AE, please refer to § 11.1.1.
- **death:** the absence of life or state of being dead
- **safety concerns related to IMP**
- **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 foetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth (please see § 11.7)
- **protocol violation:** an event or decision that stands in contrast to the guidelines set out by the protocol
- **study terminated by 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

#### **14.4.2 Discontinuation procedures**

For any patient discontinuing the study, the Investigator will:

- ask the patient to undergo, as far as possible, a final medical visit to examine the patient's health conditions. This examination will verify that all values tested at baseline have

remained within a clinically acceptable range (i.e. not clinically significant changes compared to baseline)

- 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

#### **14.4.3 Replacement**

Discontinued patients will not be replaced.

#### **14.5 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 and including the end of study examination.

## **15 ADMINISTRATIVE PROCEDURES**

### **15.1 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.

### **15.2 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.

### **15.3 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 eCRFs 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.

#### **15.4 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

#### **15.5 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.

#### **15.6 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.

#### **15.7 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.

#### **15.8 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.

## 16 REFERENCES

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