**CLINICAL STUDY PROTOCOL**

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|---|---|
| **Title:** | Phase I, Randomized, Double-masked, Placebo-controlled Study (6 days) to Evaluate the Safety, Tolerability and Pharmacokinetics of Recombinant Human Nerve Growth Factor Eye Drops in Healthy Male and Female Volunteers of Japanese Ethnicity |
| **Study Number:** | NGF0117 |
| **IND Number:** | 115892 |
| **Investigational Product:** | rhNGF |
| **Phase of the study:** | Phase I |
| **Protocol Version - Date:** | **Version No. 1.0 – Final 26-Jan-2018** |

***STATEMENT OF CONFIDENTIALITY***

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## List Of Abbreviations and Definitions Of Terms

| Acronym | Definition |
|---|---|
| ADR | Adverse Drug Reaction |
| AE | Adverse Event |
| ALT | Alanine Transaminase |
| AST | Aspartate Transaminase |
| ATA | Anti-therapeutic antibody |
| AUC | Area under the curve |
| AUC | Area under the serum concentration-time curve |
| $AUC_{[0-24]}$ | Area under the serum concentration-time curve during 24 hours |
| $AUC_{0-tlast}$ | Area under the serum concentration-time curve calculated to the last quantifiable data point |
| BCDVA | Best Corrected Distance Visual Acuity |
| BP | Blood Pressure |
| BUN | Blood Urea Nitrogen |
| CFR | Code Of Federal Regulations |
| CFS | Corneal Fluorescein Staining |
| $C_{last}$ | Last measurable serum concentration |
| $C_{max}$ | Maximum observed serum concentration |
| CRA | Clinical Research Associate (Study Monitor) |
| CRO | Clinical Research Organisation |
| CSR | Clinical Study Report |
| CTCAE | Common Terminology Criteria For Adverse Events |
| $C_{trough}$ | Minimum observed serum concentration at the end of the dosage interval |
| D | Day |
| DMC | Data Monitoring Committee |
| DNA | Deoxyribonucleic acid |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| e-CRF/CRF | Electronic/Case Report Form |
| EDC | Electronic Data Capture |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EOE | External ocular examination |
| ETDRS | Early Treatment Diabetic Retinopathy Study |
| ETV | Early Termination Visit |
| EU | European Union |
| FDA | (United States) Food And Drug Administration |
| FO | Fundus Ophthalmoscopic examination |
| FSH | Follicle-Stimulating Hormone |
| GCP | Good Clinical Practice |
| GGT | Gamma-Glutamyl-Transferase |
| HIV | Human Immunodeficiency Virus |
| IB | Investigator Brochure |
| ICF | Informed Consent Form |
| ICH | International Conference Of Harmonization |
| IEC | Independent Ethics Committee |
| IMP | Investigational Medicinal Product |
| IND | Investigational New Drug |
| IOP | Intraocular Pressure |
| IRB | Independent Review Board |
| ITT | Intention To Treat |
| LNGFR | low-affinity NGF receptor |

| Acronym | Definition |
|---|---|
| LOT | Local Ocular Tolerability |
| mNGF | murine Nerve Growth Factor |
| MVPVU | Materiovigilance And Pharmacovigilance Unit |
| NGF | Nerve Growth Factor |
| NK | Neurotrophic Keratitis |
| NOAEL | No-Observed-Adverse-Effect Level |
| p75NTR | p75 neurotrophin receptor |
| PCI | Percutaneous Coronary Intervention |
| PI | Principal Investigator |
| PK | Pharmacokinetics |
| RBC | Red Blood Cell |
| rhNGF | recombinant human Nerve Growth Factor |
| RP | Retinitis Pigmentosa |
| SAE | Serious Adverse Effect |
| SAS | Statistical Analysis System |
| SCG | Superior Cervical Ganglia |
| SID | Subject Identification Number |
| SIRC | Statens Serum Institut Rabbit Cornea Cells |
| SLE | Slit Lamp Examination |
| ST | Schirmer's Test |
| SUSAR | Serious Unexpected Suspected Adverse Reaction |
| TFBUT | Tear Film Break-Up Time |
| $t_{lag}$ | Time delay between the time of dosing and time of appearance of concentration in the sampling compartment |
| $t_{last}$ | Time to last measurable serum concentration |
| $t_{max}$ | Time to reach Cmax |
| TMF | Trial Master File |
| TrkA | Tropomyosin receptor kinase A |
| VAS | Visual Analog Scale |
| WBC | White Blood Cell |
| WMA | World Medical Association |
| $\lambda_z$ | Terminal disposition rate constant/terminal rate constant |

# 1 STUDY SYNOPSIS

| | |
|---|---|
| **Study Number** | NGF0117 |
| **Title of Study** | Phase I, Randomized, Double-masked, Placebo-controlled Study (6 days) to Evaluate the Safety, Tolerability and Pharmacokinetics of Recombinant Human Nerve Growth Factor Eye Drops in Healthy Male and Female Volunteers of Japanese Ethnicity |
| **IND No** | 115892 |
| **Study Centers (Country)** | 1 center in US (enrolling subjects of Japanese ethnicity) |
| **Development Phase** | Phase I |
| **Objectives:** | The primary objective of this study is to assess the safety and tolerability of a single short-term and a multiple dose scheme of rhNGF when administered as eye drops in healthy subjects of Japanese ethnicity.<br>The secondary objective of this study is to assess the pharmacokinetics of single and multiple doses of rhNGF when administered as eye drops in healthy subjects of Japanese ethnicity.<br>The immunogenicity will be evaluated by determination of the anti-therapeutic antibodies. |
| **Study Design:** | This is a Phase I, randomized, double-masked, placebo-controlled eye drops administration study of rhNGF in healthy male and female subjects of Japanese Ethnicity. |
| **Investigational medicinal product (IMP):** | **rhNGF 20 µg /mL (formulation containing containing L-methionine as excipient ):**<br>Study Eye (For subjects randomized to rhNGF group)<br>Day 1: One drop instilled into study eye (35 µL, corresponding to 0.70 µg of rhNGF).<br>Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into study eye (210 µL, corresponding to 4.20 µg of rhNGF).<br>Total dose in the study eye will be 31 drops (1085 µL, equivalent to 21.7 µg rhNGF) over 6 days. |
| **Reference Product** | **Vehicle (Vehicle control containing L-methionine as excipient):**<br>Study Eye (For subjects randomized to vehicle group)<br>Day 1: One drop instilled into study eye (35 µL, corresponding to 0 µg of rhNGF].<br>Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into study eye (210 µL, corresponding to 0 µg of rhNGF).<br>Total dose of placebo vehicle in the study eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days.<br>Fellow (Non-Study) Eye for all subjects<br>Day 1: One drop instilled into fellow eye (35 µL, corresponding to 0 µg of rhNGF).<br>Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into fellow eye (210 µL, corresponding to 0 µg of rhNGF).<br>Total dose of placebo vehicle in the fellow eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days. |
| **Concomitant Medications** | Not allowed, except medication for the treatment of Adverse Events. |
| **Study Population:** | Healthy subjects will be included. |

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| | A total of 30 subjects will be included in the study. |
| **Study Duration** | Treatment duration is 6 days.<br>Maximum total study duration for a subject is 63 days (9.0 weeks) from screening visit to the final follow-up visit at 35 to 42 days.<br>The subject will be resident in the Phase I clinic for 9 days (Baseline (D-1) to Follow-Up Visit 2 (D8).<br>Study Duration is expected to be 4 months (first visit of the first subject to the last visit of the last subject). |

**Inclusion Criteria**

To be eligible for inclusion into this study, each subject must fulfil the following inclusion criteria. Each subject must meet all of the following inclusion criteria at the pre-study Screening visit (within 20 days prior to admission in the Unit for the dosing period) in order to participate in this study.

1. Male or female subjects of Japanese ethnicity, aged between 18 and 60 years inclusive, who must have all four Japanese grandparents who were born in Japan.
2. Subject has to be able to communicate well with the investigator, understands and complies with the requirements of the study, and understands and signs the written volunteer informed consent form.
3. Subject's systemic and ocular medical history must be considered normal in the opinion of the investigator at the Screening and Baseline visits.
4. Best corrected distance visual acuity (BCDVA) score ≤ 0.00 LogMAR (≥83 ETDRS letters, 20/20 Snellen or 1.0 decimal fraction) in each eye at the Screening and Baseline visits.
5. Normal anterior segment on external and slit lamp examination in both eyes at the Screening and Baseline visits.
6. Normal posterior segment on fundus ophthalmoscopic examination in both eyes at the Screening and Baseline visits.
7. Subject must be considered in good systemic health in the opinion of the investigator at the Screening and Baseline visits, as determined by:
    a. Subject's body mass index is between 18.5 and 30.4 kg/m$^2$ inclusive
    b. A pre-study physical examination with no clinically significant abnormalities.
    c. Vital signs within clinically acceptable ranges for the purposes of the study (sitting systolic blood pressure [BP] ≥ 90 mmHg and ≤ 150 mmHg; diastolic BP ≥ 50 mmHg and ≤ 95 mmHg; heart rate ≥ 40 and ≤ 100 beats per minute; oral body temperature ≥ 35.5°C and ≤ 37.5°C).
    d. An ECG with no clinically significant abnormalities.
    e. Pre-study clinical laboratory findings within normal range or not deemed clinically significant in the opinion of the investigator if outside of the normal range
8. Woman subject who meets the criteria for post-menopausal stage (post menopause is defined as the period following peri-menopause, i.e. postmenopausal after 12 months without a menstrual period and with a serum FSH value within the reference range for postmenopausal females at Screening) or permanently sterilised (e.g. tubal occlusion, hysterectomy, bilateral salpingectomy) or woman subject using oral, injected or implanted hormonal methods of contraception or with a double barrier methods of contraception: condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. A female condom and a male condom should not be used together as friction between the two can result in either product failing.
9. Male subjects with female partners of child-bearing potential must use 2 different forms of highly effective contraception throughout the study and for a further 3 months after the follow-up visit and all male subjects must be willing to avoid donating sperm during this time. The following methods of contraception are considered to be highly effective: established use of oral, injected or implanted hormonal contraception; placement of an intrauterine device or intrauterine system; use of a barrier method of contraception (condom or occlusive cap with use of a spermicide); male sterilisation (post-vasectomy documentation of the absence of sperm in the ejaculate must be provided).

**Exclusion criteria:**

Subjects meeting any of the following criteria at Screening will be excluded from entry into the study:

1. Subject has had a clinically significant illness in the 6 weeks before screening in the opinion of the investigator.
2. Subject is not suitable to participate in the study in the opinion of the investigator
3. Subject has participated in any clinical study with an investigational drug/device within 3 months prior to the first day of dosing.
4. Subject has had a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or has a clinically significant allergy to drugs, foods or other materials (in the opinion of the investigator).
5. Administration of any topical ocular (prescription or over the counter including artificial tears) or systemic medication including herbal product or fish oil preparations within 14 days before the first dose of study drug. Vitamins and mineral supplements not containing other substances are allowed until 96 hours before each dose if considered by the Investigator unlikely to interfere with the study results. Paracetamol at doses of at most 2 grams per day and ibuprofen at doses of at most 1200 mg per day for no more than 3 consecutive days or 6 non-consecutive days are allowed. Oral, injectable and implantable hormonal contraceptives are allowed without restrictions for female subjects. Longer exclusion periods apply for:
    a. amiodarone and hydroxychloroquine (210 days),
    b. monoclonal antibodies/ immunoglobulins/ other therapeutic proteins (120 days)
    c. Experimental drugs with a half life known to the Study Unit: Five half lives plus 2 weeks
    d. Experimental drugs with a half life unknown to the Study Unit: 120 days
    e. chloroquine and flunarizine (100 days)
    f. fluoxetine (75 days),
    g. benzodiazepines different from midazolam, lorazepam and triazolam, chlorpromazine, mephenytoin, nortryptyline, phenobarbital, primidone, carbamazepine, phenytoin and phenprocoumone (35 days).
6. Subject has a significant history of drug/solvent abuse or a positive drugs of abuse test at any time during the study.
7. Subject has a history of alcohol abuse or currently drinks in excess of 28 units per week or has a positive alcohol breath test at any time during the study.
8. Subject is a smoker or has smoked in the 6 months prior to dosing.
9. Subject who has a positive human immunodeficiency virus (HIV) screen, hepatitis B screen or hepatitis C screen.
10. Subject has donated blood or blood products (e.g., plasma or platelets) within the 3 months prior to screening.
11. Subject has a partner who will be pregnant or breastfeeding during the study
12. Pregnant or breastfeeding female or those with a positive pregnancy test or who will not use a medically acceptable contraceptive method from selection and during the study
13. Subject having used corticosteroid sporadically in the last 30 days whichever the route of administration, or any medication by ocular or nasal administration route
14. Subjects diagnosed with any ocular disease other than refractive error
15. Subject with history of ocular surgery, including laser refractive surgery
16. Subject using a contact lens within 7 days prior administration of the first dose
17. Intraocular pressure (IOP) ≥ 22 mmHg in either eye at screening or baseline
18. Presence of any corneal opacity or corneal fluorescein staining >0.5 grade using the modified Oxford scale in either eye at screening or baseline
19. Schirmer's test without anesthesia ≤ 9 mm/5 minutes in either eye at screening or baseline
20. Tear film break up time (TFBUT) < 8 seconds in either eye at screening or baseline

Note: Alcoholic beverages should not be taken from 48 h before first drug administration until discharge from the Study center.

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| **Study Procedures:** | The study will consist of a screening period of upto 20 days before admission [i.e., Day –21 to Day -1], a one day pre-baseline phase (Day -1) before eye drop application on Days 1 to 6, with follow-up visits on Days 7, 8, 16 ± 2, and 35 (to 42).<br>A summary of the evaluations is provided in § 1.1. |
| **Sample Size** | No formal sample size calculation was made. Sample size considerations for this type of study are dependent on the common understanding on how many subjects one needs to demonstrate safety, tolerability and pharmacokinetics for the dose level investigated and the different ethnicity.<br>Thirty (30) subjects randomized 2:1 (rh-NGF : vehicle) could be considered acceptable. Twenty (20) subjects on active (one eye active, one eye vehicle) and 10 on vehicle (both eyes) will be treated. |
| **Statistical analysis** | **Data analysis:**<br>Descriptive statistics will include median, maximum and minimum values, arithmetic mean with standard deviation, geometric mean with coefficient of variation and confidence intervals for the geometric mean. All statistical calculations will be made in SAS.<br>Pharmacokinetic evaluation will be done using Phoenix WinNonlin 6.4 or later (Pharsight Corporation, Palo Alto, CA, USA). |

## 1.1 Schedule Of Evaluations

**Table 1: Schedule of Assessments**

| Visit Day (D) | Drug/ Placebo | AE/ Conmed | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CF STFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Screen** | | | | | | | | | | | | | | |
| D –21 to -2 | Informed Consent, Inclusion/Exclusion Criteria, Demography, Medical History/current medical conditions, Prior medication, Physical Exam, Serology, Drugs Abuse/pregnancy | | | | | | | | | | | | | |
| | | | | X | X | X | | | X | X | X* | X | X | X |
| **Baseline** | | | | | | | | | | | | | | |
| D -1 | Inclusion/Exclusion Criteria, Current Medical Conditions, Adverse Events, Concomitant Medication, Physical Exam, Drugs Abuse/Pregnancy | | | | | | | | | | | | | |
| | | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Treatment Phase** | | | | | | | | | | | | | | |
| **Day 1: One drop of either rhNGF or vehicle instilled into study eye (35 µL: 0.70 µg of rhNGF) and one drop of vehicle instilled into the fellow (non-study) eye** | | | | | | | | | | | | | | |
| D1 | X | X | X | X | X | | | X | X | | | X | | X |
| **Day 2,3,4,5,6: One drop of either rhNGF or vehicle six times a day (every 2h) instilled into study eye (210 µL: 4.20 µg of rhNGF) and one drop of vehicle instilled into the fellow (non-study) eye** | | | | | | | | | | | | | | |
| D2 | X | X | X | X | X | | | X | X | | | X | | X |
| D3 | X | X | X | | X | | | X | X | | | X | | X |
| D4 | X | X | X | | X | | | | | | | | | |
| D5 | X | X | X | | X | | | | | | | | | |
| D6 | X | X | X | X | X | | | X | X | | | X | | X |
| **Follow-up** | | | | | | | | | | | | | | |
| D7 (FU 1) | Physical Exam | | | | | | | | | | | | | |
| | | X | X | | X | X | | X | X | | | X | | X |
| D8 (FU 2) | Physical Exam | | | | | | | | | | | | | |
| | | X | X | | X | Y | | X | X | X | X | X | | X |
| D16 ± 2 (FU 3) | | X | X | | X | Y | | X | X | X | X | X | | X |
| D35 to 42 (FU 4) | | X | | | | | X | | | | | | | |

ATA – Anti-therapeutic antibodies. BCVDA - Best corrected distance visual acuity (ETDRS). Drugs Abuse/pregnancy - Drugs of Abuse including Alcohol breath test, pregnancy test for females. ECG – Electrocardiogram. EOE - External ocular examination (motility and eyelids). FO –Fundus Ophthalmoscopic examination (*dilated FO only at Screening). IOP – Introcular Pressure. LAB - Laboratory Safety Tests - Hematology, Biochemistry, Urinalysis. ATA – Anti-therapeutic antibodies. LOT - Local Ocular Tolerability - Visual Analogue Scale (VAS): foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia. If it is to be applied at a same visit as an FO or IOP examination the VAS for ocular tolerability is to be applied before the SLE FO and/or IOP examination. Physical exam – Physical examination, body weight, height (height is only required at screening). PK – Pharmacokinetics. Serology: Hepatitis/HIV and for postmenopausal females FSH Laboratory Safety Tests. SLE/CFS/TFBUT - Slit lamp examination (SLE): eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens with the instillation of fluorescein to evaluate corneal fluorescein staining (CS, modified Oxford scale) and tear film break-up time (TFBUT). ST - Schirmer's test (ST) without anesthesia. Vital Signs - blood pressure, pulse rate, respiratory rate and ear body temperature. PK timepoints: Baseline D-1: 0, 0.5, 2, 4, 8, 9, 10, 11, 12, 14, 16 hours; Treatment D1 0 (pre-dose) 0.5, 2, 4, 8, 9, 10, 11, 12, 14, 16 hours; Treatment D2: 0 (pre-dose) 0.5, 2, 4, 8, 9, 10, 11, 12, 14, 16 hours; Treatment D3: 0 (pre-dose) 2, 4, 6, 8. 10 hours; Treatment D4 and Treatment D5: 0 (pre-dose); Treatment D6: 0 (pre-dose), 2, 4, 6, 8, 10, 10.5, 11, 12 hours; Follow-up D7: (FU1) 0 hours; Follow-up D8: (FU2) 0, 8 hours; Follow-up D16 +-2 (FU3): 0 hours.

A detailed view of the evaluations is provided in Appendix 3-Detailed List And Timing Of Procedures (§ 15.3).

# 2 BACKGROUND INFORMATION

## 2.1 Nerve Growth Factor - Overview

Nerve growth factor (NGF) is a polypeptide essential for the survival and growth of sympathetic and sensory neurons, and for differentiation of neurons in the central nervous system. It binds with at least two classes of receptors: high-affinity tropomyosin receptor kinase A (TrkA), a transmembrane tyrosine kinase, and low-affinity NGF receptor (LNGFR), also known as p75 neurotrophin receptor (p75NTR).

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

## 2.2 Chemical And Formulation Data

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

## 2.3 Preclinical Pharmacokinetics

In Study A1122, Wistar rats were treated with rhNGF eye drops at ascending doses of 0.2, 0.4, 0.6, 0.8, and 1.2 mg/mL (3 times within one day, 3 hours apart) with a 3-day wash-out period between all administrations.

At the 0.2 mg/ml concentration, which is 10 fold that intended for clinical use, the results suggest systemic absorption following ocular administration. Disproportionately greater degrees of systemic exposure are seen at the higher doses used in animal toxicology studies.

In Study A1119, rhNGF was administered via eye drops (into both eyes) to one male and one female New Zealand White rabbit at ascending doses of 0.2, 0.4, 0.6, 0.8, and 1.2 mg/mL (3 times within one day, 3 hours apart) with 3-day wash-out periods between all administrations.

Overall the systemic absorption in rabbit was very marginal and not dose-proportional.

In the toxicokinetic analysis of the four-week ocular toxicity study in rats (Study A1129), only two from the potential three peak concentrations could be observed due to insufficient time-points.

Relative bioavailability of topical ocular NGF vs. an i.v. dose was estimated as follows:

- 12.8% – 20.7% at 0.6 mg/ml NGF.
- 21.5% - 35.7% at 0.8 mg/ml NGF.
- 22.8% - 70.9% at 1.2 mg/ml NGF (22.8% - 32.5% for females; 64.9% - 70.9% for males).

The Tmax range was 0.50 – 8.0 h. The reliable terminal half-life t½,z ranged between 3.0 and 4.0 h.

rhNGF exposure tended to be more than dose-proportionally increased at incrementing doses for male rats. For female rats, the exposure increase approached the dose-proportional increase with variability of the data.

No gender difference could be identified: the female / male exposure ratios were however variable.

After 4-week repeated administration, rhNGF exposure tended to be decreased or to be stable compared with day 1: the AUC0-t week 4 / day 1 ratios ranged between 0.30 and 0.98. No accumulation of the test item was therefore observed.

## 2.4 Primary Pharmacodynamics

rhNGF has been investigated in a range of in vitro and in vivo pharmacology studies. In the human Erythroleukemia cells TF-1 cell proliferation assay it was found to be more active than murine NGF. On the basis of the rabbit Statens Serum institut Rabbit Cornea cells (SIRC) proliferation assay it was concluded that rabbit SIRC cells are responsive only to mNGF, no effect being observed in response to rhNGF.

The results of the rat superior cervical ganglia (SCG) hypertrophy test indicate that treatment with rhNGF and mNGF increases SCG weight dose-dependently. Morphological and histological analysis revealed a direct correlation between the increase of SCG weight and the extent of neuronal tissue.

## 2.5 Toxicology Summary

In accordance with Scientific Advice from the European Medicines Agency, no safety pharmacology studies such as telemetry investigation have been performed; however, a modified Irwin Test was included in the 4-week rat toxicity study by eye drop administration.

The toxicology programme to date has included short-term intravenous studies, short-term ocular studies and four-week ocular studies, again in these species in rat and rabbit.

No causes for concern emerged from these toxicology studies. The systemic (intravenous) no-observed-adverse-effect level (NOAEL) in the rats and rabbits was shown to be 2.4 mg/kg while the local NOAEL in the same species was determined to be 1.2 mg/mL (equivalent to 6 µg per eye, three times daily).

## 2.6 A Summary Of Clinical Data

rhNGF has been already studied in healthy volunteers and in different subject populations.

In the completed Phase I study in healthy volunteers (study NGF0112), a total of 58 subjects (out of 74 enrolled) were treated with rhNGF. Furthermore, as of January 2016, in the Phase I/II and Phase II in subjects with NK (REPARO study NGF0212 and study NGF0214) a total of 174 and 48 subjects respectively have completed the two months treatment and in the Phase I/II in subjects with RP (LUMOS study NGF0113) 50 subjects have completed the six months treatment; furthermore, in the pilot Phase II study in subjects with dry eye (study NGF0213) 40 patients have completed the one month treatment with rhNGF.

The dose that is proposed for this study (20 µg /mL) has been already tested both in healthy volunteers for five consecutive days and subjects for up to six months. Besides higher doses of rhNGF (up to 180 µg /mL) were administrated for 168 days to retinitis pigmentosa subjects. Table 2 below summarizes the subjects exposed to date at different rhNGF eye drops concentrations:

In healthy volunteers no SAEs were recorded. Mild and transient ocular AEs possibly related to rhNGF administration were reported. rhNGF did not accumulate in serum after multiple doses and was detectable only in 6 subjects. Evaluation of vital signs, electrocardiograms, blood chemistry, urinalyses, and ocular

parameters indicated no evidence of rhNGF treatment effects. It can be concluded that at doses up to 180 µg/ml, rhNGF eye drops were well tolerated with no detectable clinical evidence of systemic AEs. Most of the ocular AEs were mild in severity and transient in nature.

In all studies rhNGF was well tolerated and the reported ocular AEs were generally transient and mostly mild and moderate in intensity during the controlled treatment period and the follow-up period.

Only moderate and transient ocular AEs possibly related to rhNGF administration were reported.

**Table 2: Doses of rhNGF used in previous clinical studies in Europe and US**

| **Doses of rhNGF in Human Healthy Volunteers** | | | | |
|---|---|---|---|---|
| **Dose per 35 µL drop** | **Concentration** | **Frequency of Dose** | **Total Dose per Day** | **Total Dose** |
| **Part 0, Ascending Single Dose:** 1 drop, 1 treatment day | | | | |
| 0.0175 µg | 0.5 µg/mL | once | 0.0175 µg | 0.0175 µg |
| 0.175 µg | 5 µg/mL | once | 0.175 µg | 0.175 µg |
| 0.7 µg | 20 µg/mL | once | 0.7 µg | 0.7 µg |
| **Part A, Ascending Fractionated Single Dose:** 1 drop, q4h (three times a day), 1 treatment day | | | | |
| 0.7 µg | 20 µg/mL | q4h, 1 day | 2.1 µg | 2.1 µg |
| 2.1 µg | 60 µg/mL | q4h, 1 day | 6.3 µg | 6.3 µg |
| 6.3 µg | 180 µg/mL | q4h, 1 day | 18.9 µg | 18.9 µg |
| **Part B, Ascending Multiple Fractionated Dose:** 1 drop, q4h (three times a day), 5 treatment days | | | | |
| 0.7 µg | 20 µg/mL | q4h, 5 days | 2.1 µg | 10.5 µg |
| 2.1 µg | 60 µg/mL | q4h, 5 days | 6.3 µg | 31.5 µg |
| 6.3 µg | 180 µg/mL | q4h, 5 days | 18.9 µg | 94.5 µg |
| **Doses of rhNGF in Neurotrophic Keratitis Subjects** | | | | |
| ***Phase II:** 1 drop, q2h (six times a day) one eye, 56 treatment days | | | | |
| 0.35 µg | 10 µg/mL | q2h, 56 days | 2.1 µg | 117.6 µg |
| 0.7 µg | 20 µg/mL | q2h, 56 days | 4.2 µg | 235.2 µg |
| **Doses of rhNGF in patients after cataract and refractive surgery** | | | | |
| ***Phase II:** 1 drop, q2h (six times a day) one eye, 56 treatment days | | | | |
| 0.7 µg | 20 µg/mL | q2h, 56 days | 4.2 µg | 235.2 µg |
| **Doses of rhNGF in Dry Eye pilot:** 1 drop, q12h (two times a day) both eyes, 28 treatment days | | | | |
| 0.14 µg | 4 µg/mL | q12h, 28 days | 0.6 µg | 15.7 µg |
| 0.7 µg | 20 µg/mL | q12h, 28 days | 2.8 µg | 78.4 µg |
| **Doses of rhNGF in dry eye** | | | | |
| ***Phase II:** 1 drop, q2h (six times a day) one eye, 56 treatment days | | | | |
| 0.7 µg | 20 µg/mL | q2h, 56 days | 4.2 µg | 235.2 µg |
| **Doses of rhNGF in Retinitis Pigmentosa:** 1 drop, q6h (three times a day) both eyes, 168 treatment days, | | | | |
| 2.1 µg | 60 µg/mL | q6h, 168 days | 12.6 µg | 2116.8 µg |
| 6.3 µg | 180 µg/mL | q6h, 168 days | 37.8 µg | 6350.4 µg |

***Note.** 174 subjects treated formulation without methionine and 48 subjects treated with formulation containing methionine

## 2.7 A Summary Of Toxicology Data

In accordance with Scientific Advice from the European Medicines Agency, no safety pharmacology studies such as telemetry investigation have been performed; however, a modified Irwin Test was included in the 4-week rat toxicity study by eye drop administration.

The toxicology programme to date has included short-term intravenous studies, short-term ocular studies and four-week ocular studies, again in these species in rat and rabbit.

No causes for concern emerged from these toxicology studies. The systemic (intravenous) no-observed-adverse-effect level (NOAEL) in the rats and rabbits was shown to be 2.4 mg/kg while the local NOAEL in the same species was determined to be 1.2 mg/mL (equivalent to 6 µg per eye, three times daily).

For additional information regarding the development of rhNGF, consult the current Investigator Brochure (IB) [Reference: 4].

## 2.8 Risk - Benefit Evaluation

In the present study, potential risks of multiple rhNGF applications to healthy male and female volunteers of Japanese ethnicity are not expected to surpass the frequency of adverse reactions and untoward effects previously reported in the Phase I/II studies.

The results of this study will allow development of rhNGF for use in the treatment of eye conditions in subjects with Japanese ethnicity.

A Full Marketing Authorization for the treatment of stage 2 and 3 NK has been granted to rhNGF eye drops in July 2017 by the European Medicines Agency (EMA) in the European Union (EU), and the drug has been put on the European market in November 2017.

## 2.9 Description Of The Investigational Product

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

The matching placebo vehicle consists of a sterile isotonic solution containing L-methionine as excipient.

Further information is given in § 5.

# 3 OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

## 3.1 Study Objectives

### 3.1.1 Primary Objective

The primary objective of this study is to assess the safety and tolerability of a single short-term and a multiple dose scheme of rhNGF when administered as eye drops in healthy subjects of Japanese ethnicity.

### 3.1.2 Secondary Objective

The secondary objective of this study is to assess the pharmacokinetics of single and multiple doses of rhNGF when administered as eye drops in healthy subjects of Japanese ethnicity.
The immunogenicity will be valuated by the determination of the anti-therapeutic antibodies.

## 3.2 Study Administrative Structure

This study will be performed at one study center located in in US, enrolling subjects of Japanese ethnicity. At the study center, the Principal Investigator (PI) will be responsible for ensuring that the investigation is conducted according to the signed Investigator agreement, the protocol, ICH-GCP guidelines [Reference: 1], and local regulations.

The PI at the study center will be responsible for the management of the study, which will consist of maintaining the study file and the subject records, corresponding with the IRB/IEC, and completing the case report forms (CRFs) and reporting SAEs within 24 hours of initial awareness.

## 3.3 Overall Study Design

This is a Phase I, randomized, double-masked, placebo-controlled eye drops administration study of rhNGF in healthy male and female subjects of Japanese ethnicity.

A total of 30 subjects will be randomized in a masked manner: 20 subjects on rhNGF group and 10 on placebo. A study eye will be assigned to each subject by random. In the study eye, the subjects will be treated with:

- rhNGF (active) in the rhNGF group.
- vehicle (placebo) in the vehicle group.

In parallel, the fellow-eye (non-study eye) will receive vehicle (placebo) treatment.

### 3.3.1 Rationale for Selection of dose, control group and treatment schedule in the study

The dose that is proposed for this study (20 µg /mL) has been already tested both in healthy volunteers for five consecutive days and subjects for up to six months.

This dose (20 µg /mL, 1 drop in each eye, six times daily) has also been used for 56 consecutive days in patients with Stage 2 and 3 Neurotrophic Keratitis (NGF0214), dry eye (study NGF0216) and in patients after cataract and refractive surgery (NGF0116). The same dose and duration has been used in the First In Human study with rhNGF in healthy volunteers (NGF0112).

Higher doses of rhNGF (up to 180 µg /mL) were administrated for 168 days to retinitis pigmentosa subjects (Study NGF0113).

# 4 SELECTION OF STUDY POPULATION

The Inclusion/Exlusion are intended to identify healthy male and female volunteers of Japanese ethinicity. At least 6 males as well as 6 females will be included in the study to ensure, that a sufficient number of each gender are present in each treatment group.

## 4.1 Inclusion Criteria

To be eligible for inclusion into this study, each subject must fulfil the following inclusion criteria.

Each subject must meet all of the following inclusion criteria at the pre-study Screening visit (within 20 days prior to admission in the Unit for the dosing period) in order to participate in this study.

1. Male or female subjects of Japanese ethnicity, aged between 18 and 60 years inclusive, who must have all four Japanese grandparents who were born in Japan.
2. Subject has to be able to communicate well with the investigator, understands and complies with the requirements of the study, and understands and signs the written volunteer informed consent form.
3. Subject's systemic and ocular medical history must be considered normal in the opinion of the investigator at the Screening and Baseline visits.
4. Best corrected distance visual acuity (BCDVA) score ≤ 0.00 LogMAR (≥83 ETDRS letters, 20/20 Snellen or 1.0 decimal fraction) in each eye at the Screening and Baseline visits.
5. Normal anterior segment on external and slit lamp examination in both eyes at the Screening and Baseline visits.
6. Normal posterior segment on fundus ophthalmoscopic examination in both eyes at the Screening and Baseline visits.
7. Subject must be considered in good systemic health in the opinion of the investigator at the Screening and Baseline visits, as determined by:
    a. Subject's body mass index is between 18.5 and 30.4 kg/m$^2$ inclusive
    b. A pre-study physical examination with no clinically significant abnormalities.
    c. Vital signs within clinically acceptable ranges for the purposes of the study (sitting systolic blood pressure [BP] ≥ 90 mmHg and ≤ 150 mmHg; diastolic BP ≥ 50 mmHg and ≤ 95 mmHg; heart rate ≥ 40 and ≤ 100 beats per minute; oral body temperature ≥ 35.5°C and ≤ 37.5°C).
    d. An ECG with no clinically significant abnormalities.
    e. Pre-study clinical laboratory findings within normal range or not deemed clinically significant in the opinion of the investigator if outside of the normal range
8. Woman subject who meets the criteria for post-menopausal stage (post menopause is defined as the period following peri-menopause, i.e. postmenopausal after 12 months without a menstrual period and with a serum FSH value within the reference range for postmenopausal females at Screening) or permanently sterilised (e.g. tubal occlusion, hysterectomy, bilateral salpingectomy) or woman subject using oral, injected or implanted hormonal methods of contraception or with a double barrier methods of contraception: condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. A female condom and a male condom should not be used together as friction between the two can result in either product failing.
9. Male subjects with female partners of child-bearing potential must use 2 different forms of highly effective contraception throughout the study and for a further 3 months after the follow-up visit and all male subjects must be willing to avoid donating sperm during this time. The following methods of

contraception are considered to be highly effective: established use of oral, injected or implanted hormonal contraception; placement of an intrauterine device or intrauterine system; use of a barrier method of contraception (condom or occlusive cap with use of a spermicide); male sterilisation (post-vasectomy documentation of the absence of sperm in the ejaculate must be provided).

## 4.2 Exclusion Criteria

Subjects meeting any of the following criteria at Screening will be excluded from entry into the study:

1. Subject has had a clinically significant illness in the 6 weeks before screening in the opinion of the investigator.
2. Subject is not suitable to participate in the study in the opinion of the investigator
3. Subject has participated in any clinical study with an investigational drug/device within 3 months prior to the first day of dosing.
4. Subject has had a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or has a clinically significant allergy to drugs, foods or other materials (in the opinion of the investigator).
5. Administration of any topical ocular (prescription or over the counter including artificial tears) or systemic medication including herbal product or fish oil preparations within 14 days before the first dose of study drug. Vitamins and mineral supplements not containing other substances are allowed until 96 hours before each dose if considered by the Investigator unlikely to interfere with the study results. Paracetamol at doses of at most 2 grams per day and ibuprofen at doses of at most 1200 mg per day for no more than 3 consecutive days or 6 non-consecutive days are allowed. Oral, injectable and implantable hormonal contraceptives are allowed without restrictions for female subjects. Longer exclusion periods apply for:
    a. amiodarone and hydroxychloroquine (210 days),
    b. monoclonal antibodies/ immunoglobulins/ other therapeutic proteins (120 days)
    c. Experimental drugs with a half life known to the Study Unit: Five half lives plus 2 weeks
    d. Experimental drugs with a half life unknown to the Study Unit: 120 days
    e. chloroquine and flunarizine (100 days)
    f. fluoxetine (75 days),
    g. benzodiazepines different from midazolam, lorazepam and triazolam, chlorpromazine, mephenytoin, nortryptyline, phenobarbital, primidone, carbamazepine, phenytoin and phenprocoumone (35 days).
6. Subject has a significant history of drug/solvent abuse or a positive drugs of abuse test at any time during the study.
7. Subject has a history of alcohol abuse or currently drinks in excess of 28 units per week or has a positive alcohol breath test at any time during the study.
8. Subject is a smoker or has smoked in the 6 months prior to dosing.
9. Subject who has a positive human immunodeficiency virus (HIV) screen, hepatitis B screen or hepatitis C screen.
10. Subject has donated blood or blood products (e.g., plasma or platelets) within the 3 months prior to screening.
11. Subject has a partner who will be pregnant or breastfeeding during the study

12. Pregnant or breastfeeding female or those with a positive pregnancy test or who will not use a medically acceptable contraceptive method from selection and during the study
13. Subject having used corticosteroid sporadically in the last 30 days whichever the route of administration, or any medication by ocular or nasal administration route
14. Subjects diagnosed with any ocular disease other than refractive error
15. Subject with history of ocular surgery, including laser refractive surgery
16. Subject using a contact lens within 7 days prior administration of the first dose
17. Intraocular pressure (IOP) ≥ 22 mmHg in either eye at screening or baseline
18. Presence of any corneal opacity or corneal fluorescein staining >0.5 grade using the modified Oxford scale in either eye at screening or baseline
19. Schirmer's test without anesthesia ≤ 9 mm/5 minutes in either eye at screening or baseline
20. Tear film break up time (TFBUT) < 8 seconds in either eye at screening or baseline

Note: Alcoholic beverages should not be taken from 48 h before first drug administration until discharge from the Study center. There are also restrictions with regard to chewing gum, heavy exercise and smoking (See § 7.6).

# 5 STUDY MEDICATION

## 5.1 Presentation, Storage, Packaging Of The Investigational Medicinal Product

| | |
|---|---|
| TEST PRODUCT | |
| IMP | Recombinant human nerve growth factor (rhNGF), containing L-methionine as excipient<br>- 20 µg/mL vials |
| Manufacturer active substance | Dompé Farmaceutici S.p.A., Italy |
| Manufacturer finished product | Bulk drug product is manufactured by Patheon Italia S.p.A-Italy.<br>Packaging and labelling is performed by PCI Inc. USA |
| Pharmaceutical form | Sterile buffered aqueous solution |
| Dose | Study Eye (For subjects randomized to rhNGF group)<br>**Day 1:** One drop instilled into study eye (35 µL, corresponding to 0.70 µg of rhNGF).<br>**Day 2, 3, 4, 5, 6:** One drop six times a day (every 2h) into study eye (210 µL, corresponding to 4.20 µg of rhNGF).<br>Total dose in the study eye will be 31 drops (1085 µL, equivalent to 21.7 µg rhNGF) over 6 days . |
| Administration route | Ophthalmic |

## 5.2 Presentation, Storage, Packaging Of The Vehicle Product

| | |
|---|---|
| REFERENCE PRODUCT | |
| IMP | Placebo vehicle, containing L-methionine as excipient<br>- Vehicle vials |
| Manufacturer active substance | Dompé Farmaceutici S.p.A., Italy |
| Manufacturer finished product | Bulk drug product is manufactured by Patheon Italia S.p.A-Italy.<br>Packaging and labelling is performed by PCI Inc. USA |
| Pharmaceutical form | Sterile buffered aqueous solution |
| Dose | Study Eye (For subjects randomized to vehicle group)<br>Day 1: One drop instilled into study eye (35 µL, corresponding to 0 µg of rhNGF].<br>Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into study eye (210 µL, corresponding to 0 µg of rhNGF).<br>Total dose of placebo vehicle in the study eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days.<br>Fellow (non-study) Eye for all subjects<br>Day 1: One drop instilled into fellow eye (35 µL, corresponding to 0 µg of rhNGF).<br>Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into fellow eye (210 µL, corresponding to 0 µg of rhNGF).<br>Total dose of placebo vehicle in the fellow eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days. |
| Administration route | Ophthalmic |

## 5.3 Formulation And Packaging

The Investigator will be provided with a Subject box containing vials of frozen IMP solutions (-20 ± 5°C) containing:

- rhNGF, at concentrations of 20 µg /mL, for dosing the right or left eye (study eye), according to the randomization list, or
- vehicle (placebo) for dosing the right or left eye (study eye) and for dosing the non-study eye according to the randomization list.

For subjects randomized to the rhNGF treatment group, each Subject box will contain a a box of 6 vials of rhNGF (for one eye) and a box containing 6 vials of Vehicle for the fellow (non-study) eye.

For subjects randomized to the Vehicle treatment group, each Subject box will contain a box of 6 vials of Vehicle (for one eye) and a box of 6 vials of Vehicle for the fellow (non-study) eye.

A total of 60 kits (containing the treatment for right and left eye) will be supplied to the Pharmacy of the Clinical Unit.

Syringes and adaptors will be provided separately in single sterile polyethylene packages and may be kept at room temperature.

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

The Investigator 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

### 5.3.1 Labeling

**Primary packaging: Label for vial: single panel (rh-NGF or Vehicle):**

Label for vial: single panel

| Line No. | Text | Comments |
|---|---|---|
| 1 | NGF0117 | |
| 2 | Dompé farmaceutici s.p.a | |
| 3 | rhNGF 20 µg /ml or Vehicle | |
| 4 | Administration eye: RIGHT or LEFT | Active/placebo or placebo/placebo according to the randomization list stratified per eye and per subject |
| 5 | Eye drops (1 ml) | |
| 6 | Batch: <vvvv> | Codified batch |
| 7 | Kit XXXX | Format of kit no.: "XXXX" |
| 8 | **CAUTION: New Drug-Limited by Federal (or United States) law to investigational use** | |

* The number of KIT is reported according to the randomization list that will be generated and balanced by gender, study eye and treatment

**Secondary packaging: Six day kit (containing 6 vials): tear-off panel**

| Line No. | Text | Comments | Tear Off part of the label |
|---|---|---|---|
| 1 | Protocol no.: NGF0117 | | To be enclosed |
| 2 | Sponsor: Dompé farmaceutici s.p.a, Via Santa Lucia, 6 - 20122 Milano, Italy | | To be enclosed |
| 3 | rhNGF 20 µg /ml or Vehicle | | To be enclosed |
| 4 | Eye drops solution (1 ml per vial) | | |
| 5 | Contains: 6 multidose vials | | |
| 6 | Directions for use: refer to dosing instructions | | |
| 4 | Administration eye: RIGHT or LEFT | Active/Placebo or Placebo/Placebo according to the randomization list stratified per eye and per subject | To be enclosed |
| 7 | Batch: <vvvv>\ | Codified batch | To be enclosed |
| 8 | Kit XXXX | Format of kit no.: "XXXX" | To be enclosed |
| 9 | Subj. Screening #: S☐☐☐ | | To be enclosed |
| 10 | Subj. Randomization #: ☐☐☐☐ | | To be enclosed |
| 11 | Store in a freezer at -20°C at the Investigational site | | |
| 12 | Store in a refrigerator at 2-8 °C (36°F to 46°F) during 6 days treatment period | | |
| 13 | Do not shake | | |
| 14 | Keep out of the reach of children | | |
| 15 | For clinical trial use only | | |
| 16 | **CAUTION: New Drug-Limited by Federal (or United States) law to investigational use** | | |

### 5.3.2 Supply, Storage and Handling of IMP

rhNGF must be stored at -20°C in an appropriate locked room accessible only to the pharmacist, the Investigator, or a duly designated person.

Daily vials containing rhNGF are thawed at 2 to 8°C in the carton and protected from light. Because rhNGF does not contain preservatives, the contents of each vial are for daily-use only and opened vials must be maintained at 2 to 8°C and used within 12 hours. Agitation of vials may cause foaming and/or particle formation.

**Protect vials from light. Do not shake vials.**

Administration

Drug preparation and administration instructions will be provided separately in the Pharmacy Manual for the study.

### 5.3.3 Replacement Kits

Thirty additional kits will be prepared as replacements. Assignment of subject and kit number is described in § 6.1).

## 5.4 Dose, Route And Schedule Of Imp Administration

### 5.4.1 Administration route

The Administration route is ophthalmic, to be applied by investigator at site.

### 5.4.2 Dose regimen

In all subjects both eyes will be treated; with one eye as the study eye.

- All subjects will receive either rhNGF or vehicle in the study eye (right or left) according to the randomization list.
- The fellow (non-study) eye will receive vehicle.

Both the subjects and the investigator/ophthalmologist will be masked to the study treatment and study eye randomization (see § 6.3)

The exact times of study drug administration, and comments, will be recorded on the appropriate page of the eCRF.

**rhNGF 20 µg /mL:**

For subjects randomized to rhNGF group:

Study Eye

- **Day 1**: One drop instilled into study eye (35 µL, corresponding to 0.70 µg of rhNGF).
- **Day 2, 3, 4, 5, 6**: One drop six times a day (every 2h) into study eye (210 µL, corresponding to 4.20 µg of rhNGF).

Total dose in the study eye will be 31 drops (1085 µL, equivalent to 21.7 µg rhNGF) over 6 days .

**Vehicle:**

For subjects randomized to vehicle group:

Study Eye

- **Day 1**: One drop instilled into study eye (35 µL, corresponding to 0 µg of rhNGF].
- **Day 2, 3, 4, 5, 6**: One drop six times a day (every 2h) into study eye (210 µL, corresponding to 0 µg of rhNGF).

Total dose of placebo vehicle in the study eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days.

Fellow (non-study) Eye

For all subjects (subjects randomized to rhNGF group and subjects randomized to vehicle group):

- Day 1: One drop instilled into fellow eye (35 µL, corresponding to 0 µg of rhNGF).
- Day 2, 3, 4, 5, 6: One drop six times a day (every 2h) into fellow eye (210 µL, corresponding to 0 µg of rhNGF).

Total dose of placebo vehicle in the fellow eye will be 31 drops (1085 µL, 0 µg rhNGF) over 6 days.

## 5.5 Criteria For Schedule Adjustment/Dose-Modification/ Discontinuation Of Investigational Product

No schedule adjustment or dose adjustments are allowed.

## 5.6 Accountability Of The Investigational Product

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

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

An accurate drug disposition record will be kept, specifying the date and amount dispensed to each subject.

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

At the end of the study, unused IMP will be returned to Dompé for disposal.

## 5.7 Concomitant Medication

As a general rule, no medication other than study drug will be given during the course of the study, i.e., from the initial day of Screening until all of the final study evaluations have been completed.

Following the first dose of study drug, the exceptions are the medications allowed in § 4.2 Exclusion Criteria, under Exclusion Criterion 5:

- Vitamins and mineral supplements not containing other substances are allowed until 96 hours before each dose if considered by the Investigator unlikely to interfere with the study results.
- Paracetamol at doses of at most 2 grams per day and ibuprofen at doses of at most 1200 mg per day for no more than 3 consecutive days or 6 non-consecutive days are allowed.
- Oral, injectable and implantable hormonal contraceptives are allowed without restrictions for female subjects.

Decisions regarding subjects requiring concomitant medication will be discussed with the sponsor on a case-by-case basis. The administration of any such medication (including over-the-counter medications and vitamins) will be clearly documented on the Prior and Concomitant Medications eCRF page.

### 5.7.1 Prior and concomitant medications

Before the administration of the first dose of study drug, the following medications have the following exclusion periods specified in § 4.2 Exclusion Criteria, under Exclusion Criterion 5 and 12.

Within 96 hours before each dose:

Vitamins and mineral supplements not containing other substances are allowed if considered by the Investigator unlikely to interfere with the study results.

Within 14 days before the first dose of study drug:

Administration of any topical ocular (prescription or over the counter including artificial tears) or systemic medication including herbal product or fish oil preparations.

Within 30 days

Subject has corticosteroid sporadically, whichever the route of administration

Any medication by ocular or nasal administration route.

Exclusion periods apply for the following prior medications:

a. amiodarone and hydroxychloroquine (210 days),
b. monoclonal antibodies/ immunoglobulins/ other therapeutic proteins (120 days)
c. Experimental drugs with a half life known to the Study Unit: Five half lives plus 2 weeks
d. Experimental drugs with a half life unknown to the Study Unit: 120 days
e. chloroquine and flunarizine (100 days)
f. fluoxetine (75 days),
g. benzodiazepines different from midazolam, lorazepam and triazolam, chlorpromazine, mephenytoin, nortryptyline, phenobarbital, primidone, carbamazepine, phenytoin and phenprocoumone (35 days).

# 6 ASSIGNMENT OF SUBJECT NUMBER

After obtaining informed consent a 3 digit consecutive screening number will be assigned to each subject according to the sequence of study entry, starting from from S001.

## 6.1 Randomisation

A total of 30 subjects will be randomized: 20 subjects on rhNGF treatment group and 10 on Vehicle treatment group.

Eligible subjects will be randomized at the Baseline Visit in a 2:1 ratio to a respective treatment groupaccording to the randomization code and determined by the allocated randomization number. Randomization will be stratified by gender to ensure a balanced (2:1) gender distribution between treatment groups. With at least 6 subjects be recruited for each gender, it is ensured to have at least 4 male and 4 female subjects in the rhNGF group and 2 male and 2 female subjects in the Vehicle group.

Moreover, the study eye will be randomized for each subject to left and right eye.

During randomization, each subject will be allocated with randomization number. Together with the randomization number, the randomized study eye and associated kit number will be provided.

Randomization list and study eyes as well as associated kit numbers will be be generated by a member of Cromsource SAS programming group not involved in the conduct of the study. A total of 60 kits (containing the treatment for right and left eye) will be supplied to the Pharmacy of the Clinical Unit.

Subjects 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.

## 6.2 Treatment Allocation

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

## 6.3 Masking

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

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

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

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.

The investigator and Dompé Pharmacovigilance contact will be provided with Code Breakers, containing the treatment allocation, to be opened only in case of a medical emergency. Therefore, a copy of the sealed

code envelopes will be filed at the clinic, in a secure, locked place. The integrity of these Code Breakers will be checked by the monitor at the end of the study.

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

# 7 STUDY PROCEDURE AND ASSESSMENTS

The schedule of study procedures and assessments is tabulated by visit in the Schedule of Evaluations in § 1.1.

A detailed view of the evaluations, including timings, is provided in Appendix 3-Detailed List And Timing Of Procedures (§ 15.3).

The descriptions of the procedures to be performed at each visit are provided below. Note: the order detailed in the lists below does not reflect the order of assessment.

## 7.1 Screening Visit

The pre-study Screening visit is to take place within 20 days prior to admission in the Unit for the dosing period (Baseline Day -1).

During Screening:

- Informed Consent
- Assesment of Inclusion and Exclusion Criteria
- Demography
- Medical History / Current medical conditions
- Prior / Concomitant medication
- Physical examination and height
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.
- ECG (12 lead)
- Laboratory safety tests including: Hematology, Clinical chemistry, Urine analysis, Hepatitis screen, HIV screen
- Screening for drugs of abuse including alcohol breath test
- Pregnancy test for female subjects and serum FSH test for postmenopausal females.
- Ocular examination (both eyes) including:
  - Assessment of best corrected distance visual acuity (BCDVA)
  - External ocular examination (EOE): motility and eyelids
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens (Note: The SLE must be performed before IOP)
  - Corneal fluorescein staining (CFS; modified Oxford scale) following instillation of fluorescein
  - Tear film break-up time (TFBUT).
  - Schirmer test (ST) without anesthesia
  - Intraocular pressure (IOP)
  - Dilated Fundus Ophthalmoscopic (FO) examination to assess the vitreous, retina/macula/choroid, and optic nerve, including cup/disc ratio

## 7.2 Study Visits And Follow-Up Assessments

Baseline (Day -1)

- Review of inclusion/exclusion criteria
- Current Medical conditions
- Physical examination
- Adverse Events
- Concomitant medication
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.

- ECG (12 lead)
- Laboratory safety tests including: hematology, clinical chemistry and urine analysis
- Screening for drugs of abuse including alcohol breath test
- Pregnancy test for female subjects
- Blood samples for PK
- Blood sample for ATA
- Ocular examination (both eyes):
  - BCDVA Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - EOE: motility and eyelids
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens (Note: The SLE must be performed before IOP)
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
  - Schirmer test (ST) without anesthesia
  - Intraocular pressure (IOP)
  - Dilated Fundus Ophthalmoscopic (FO) examination to assess the vitreous, retina/macula/choroid, and optic nerve, including cup/disc ratio

Treatment Day 1

One drop of rhNGF or vehicle instilled into one eye on day 1 (35 µL).

- Adverse Events
- Concomitant Medication
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.
- ECG (12 lead)
- Ocular examinations (both eyes):
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
- Blood samples for PK
- Randomization
- Study drug administration

Treatment Day 2

One drop of rhNGF or vehicle instilled into one eye six times a day on 2,3,4,5,6 (every 2h; 210 µL, corresponding to 4.20 µg of rhNGF).

- Adverse Events
- Concomitant Medication
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.
- ECG (12 lead)
- Ocular examinations (both eyes):
  - BCVDA
  - EOE: motility and eyelids

  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
- Blood samples for PK
- Study drug administration

Treatment Day 3

One drop of rhNGF or vehicle instilled into one eye six times a day (every 2h; 210 µL, corresponding to 4.20 µg of rhNGF).

- Adverse Events
- Concomitant Medication
- ECG (12 lead)
- Ocular examinations (both eyes):
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
- Blood samples for PK
- Study drug administration

Treatment Day 4

One drop of rhNGF or vehicle instilled into one eye six times a day (every 2h; 210 µL, corresponding to 4.20 µg of rhNGF).

- Adverse Events
- Concomitant Medication
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.
- ECG (12 Lead)
- Blood samples for PK
- Study drug administration

Treatment Day 5

One drop of rhNGF or vehicle instilled into one eye six times a day (every 2h; 210 µL, corresponding to 4.20 µg of rhNGF).

- Adverse Events
- Concomitant Medication
- ECG (12 lead)
- Blood samples for PK
- Study drug administration

Treatment Day 6

One drop of rhNGF or vehicle instilled into one eye six times a day (every 2h; 210 µL, corresponding to 4.20 µg of rhNGF).

- Adverse Events
- Concomitant Medication
- Vital signs (body weight, blood pressure, pulse rate, respiratory rate and ear body temperature) measured after at least 3 minutes in the sitting position.
- ECG (12 lead)
- Ocular examinations (both eyes):
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
- Blood samples for PK
- Study drug administration

Follow Up Day 7 (FU 1)

- Adverse Events
- Concomitant Medication
- Physical examination
- ECG (12 lead)
- Laboratory safety tests including: Hematology, Clinical chemistry, Urine analysis
- Ocular examinations (both eyes):
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
  - CFS (modified Oxford scale) following instillation of fluorescein
  - TFBUT
- Blood sample for PK

Follow Up Day 8 (FU 2)

- Adverse Events
- Concomitant Medication
- Physical examination
- ECG (12 lead)
- Laboratory safety tests including: Hematology, Clinical chemistry, Urine analysis
- Blood samples for PK
- Ocular examination (both eyes) including:
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
    (Note: The SLE must be performed before IOP)

- CFS (modified Oxford scale) following instillation of fluorescein
- TFBUT
- Intraocular pressure (IOP)
- Fundus ophthalmoscopic (FO) examination to assess the vitreous, retina/macula/choroid, and optic nerve, including cup/disc ratio.

Follow Up Day 16±2 (FU 3)

- Adverse Events
- Concomitant Medication
- Physical examination
- ECG (12 lead)
- Laboratory safety tests including: Hematology, Clinical chemistry, Urine analysis
- Blood samples for PK
- Ocular examination (both eyes) including:
  - BCVDA
  - EOE: motility and eyelids
  - Assessment of LOT (VAS: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia)
  - SLE to assess the eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens
    (Note: The SLE must be performed before IOP)
  - CSF (modified Oxford scale) following instillation of fluorescein
  - TFBUT
  - Intraocular pressure (IOP)
  - FO to assess the vitreous, retina/macula/choroid, and optic nerve, including cup/disc ratio.

Follow Up Day 35 to 42 (FU 4)

- Adverse Events
- Blood sample for ATA

Early Termination Visit (ETV)

For any subject discontinuing the study, the Investigator ask the subject to undergo, as far as possible, a final medical visit to examine the subject's health conditions

a. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e., not clinically significant changes compared to screening). The assessments will be conducted as detailed in Follow Up Day 16±2 (FU 3).
b. Arrange for alternative medical care of the withdrawn subject, if necessary
c. Report in the eCRF date and time of the last dose administration, and date and primary reason of study discontinuation
d. Record in the eCRF any follow-up, if the subject is withdrawn for an AE.

Procedures conducted at the Early Termination Visit are at the discretion of the Investigator and may be among the study procedures or additional procedures not performed during the study but deemed necessary by the investigator.

## 7.3 Premature Discontinuation From The Study

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the primary reason for discontinuation will be recorded.

### 7.3.1 Primary Reason For Discontinuation From The Study

- **Adverse event (AE)**: Any significant AE that, in the opinion of the Investigator or concerned subject, is not compatible with study continuation. For the definition of AE, please refer to § 9.1
- **Death**: the absence of life or state of being dead
- **Lost to follow-up**: the loss or lack of continuation of a subject to follow-up
- **Non-compliance with study drug**: an indication that a subject 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 subject
- **Pregnancy**: pregnancy is the state or condition of having a developing embryo or fetus in the body (uterus), after union of an ovum and spermatozoon, during the period from conception to birth (please see § 9.6
- **Protocol violation**: an event or decision that stands in contrast to the guidelines set out by the protocol
- **Study terminated by the Sponsor**: an indication that a clinical study was stopped by its Sponsor
- **withdrawal by subject**: study discontinuation requested by a subject for whatever reason
- **other**: different than the ones previously specified.

### 7.3.2 Discontinuation procedures

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

- ask the subject to undergo, as far as possible, a final medical visit (ETV) to examine the subject's health conditions This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e., not clinically significant changes compared to screening)
- arrange for alternative medical care of the withdrawn subject, 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 subject is withdrawn for an AE (as per § 9.5).

### 7.3.3 Replacement

Subjects that drop out prior to receiving the first dose will be replaced.

## 7.4 Study Duration

The study will consist of a screening period of upto 20 days before admission [i.e., Day –21 to Day -1], a one day baseline phase (Day -1) before eye drop application on Days 1 to 6, with follow-up visits on days 7, 8, 16±2, and 35±7 (ranging from 28 to 42) days.

Treatment duration is 6 days.

The subject will be resident in the Phase I clinic for 9 days [Baseline (D-1) to Follow-Up Visit 2 (D8].

Maximum total study duration for a subject is 63 days (9.0 weeks) from screening visit to the follow up visit at 35 to 42 days.

Study Duration is expected to be 4 months (first visit of the first subject to the last visit of the last subject).

## 7.5 End Of Study

For the purpose of this trial, the End of Study is defined as the date of the last visit of the last subject.

## 7.6 Domiciling And General Study Conditions

The subjects will be confined to the study center from two days before the administration of study drug (Day -2) until 56 hours after last dosing (Day 8).

**Alcoholic beverages** should not be taken from 48 h before first drug administration until discharge from the Study center. This is only a precaution for the general safety of the subjects.

In order to avoid undesired influences on the objectives of the study, the following instructions are given:

- **Chewing gums** are not allowed at study site.
- **Heavy exercise** (meaning unusual exercise for the degree of conditioning of the individual subject) has to be avoided from 4 days before the start of the treatment period and while confined to the study center.
- **Smoking** is not allowed while confined to the study center. (Note: being a smoker, or having smoked in the 6 months prior to dosing. is an exclusion criteria).

### 7.6.1 Dietary and Fluid Restrictions

From the Screening visit to the treatment period, the subjects shall keep their normal dietary habits. Strenuous exercise should be avoided throughout the study.

On the evening before the Baseline (Day -1) assessments (i.e. the domiciling of the subjects), subjects will get a standardized dinner. All first doses in a dosing day will be given after an overnight fast (only tap water allowed from the dinner on the day before). Breakfast will be given 2 h after the first dose on a dosing day.

In the dosing days, **5 hours** after the administration of the first dose of study drug in the day, a lunch will be served. This will be followed by a light snack after **10 hours** and dinner **13 hours** after the administration of the first dose of study drug in the day.

Beverages containing xanthine or food such as coffee, tea, cola, cocoa, or chocolate are not allowed at the study site. Alternatively, after lunch, fruit tea decaffeinated coffee, or non-carbonated water can be consumed. Alcoholic beverages are not allowed in the Unit.

The composition of meals and fluids will be provided by the study center and will be similar for all subjects while at the study center.

When meal and blood draw times coincide, blood will be drawn BEFORE the meal is provided.

### 7.6.2 Physician coverage

The investigator or designee will personally supervise the subjects during the drug administrations. A physician shall be available by pager at all other times throughout the study.

# 8 EVALUATIONS AND PROCEDURES

The following evaluations and procedures will be required in this study (a schedule of these assessments is summarised in § 1.1 and a detailed schedule can be found in Appendix 3-Detailed List And Timing Of Procedures). If several assessments have to be performed at the same time, the sampling for pharmacokinetic analyses always has priority. In case multiple assessments are foreseen for a given time point, these assessments should always be performed in the same order.

## 8.1 Inclusion And Exclusion Criteria

Subject selection is to be established by examination of the inclusion/exclusion checklist (§ 4).

## 8.2 Background Information

This includes a complete medical history including current medical conditions, age, gender, ethnic origin, height (in cm), body mass index (in kg/m2 with one decimal), daily consumption of caffeine and alcohol, and history of smoking.

## 8.3 Prior And Concomitant Medication

All medications (including over-the-counter drugs, herbal products, vitamins, and antacids) taken within 4 weeks prior to the start of and throughout the study must be recorded on the case report form. Medication entries should be specific to product name (if a combination drug product) and spelled correctly. The dose, unit, frequency, route of administration, start date, discontinuation date, and indication should also be recorded. For medications administered only one time, the frequency column may reflect "once".

The use of the medications with exclusion periods before dosing longer than 4 weeks will only be checked at Screening for eligibility but would need not to be otherwise recorded.

## 8.4 Physical Examination

This evaluation will include an examination of general appearance, head-eyes-throat-nose-ears, skin, thorax/lungs, cardiovascular system, abdomen, lymph nodes, musculoskeletal system, neurological system and other examinations as necessary.

Clinically significant abnormalities (or a clinically significant worsening of a physical condition), which were observed the first time during study will be reported as adverse event.

## 8.5 Vital Signs

This evaluation will include body weight (in kg with one decimal), and sitting assessments of systolic and diastolic blood pressure and pulse rate as well as respiratory rate and body temperature. Blood pressure and pulse rate as well as respiratory rate will be recorded after the subject has rested in the **sitting position** for at least 3 minutes. Blood pressure should be assessed on the same arm for each time of determination using an automated measurement device.

Body temperature will be assessed in the ear (normal below 37.5$^{o}$C).

Clinically significant changes (or a clinically significant worsening) in vital signs, which were observed the first time during study will be reported as adverse event.

## 8.6 Electrocardiographic Evaluations

ECG testing is planned based on the previous study in health volunteers (NGF0112), to cover a potential rapid absorption of rhNGF (0.5 h after dose) and to cover the multiple dose treatment during the study.

A 12-lead standard ECG tracing with a rhythm strip will be used.

If the QTC is greater than 450ms, the ECG will be repeated 3 times.

The following ECG measures at each time point will be transferred in eCRF:

- Heart rate
- PR interval
- QRS duration
- QT interval
- QTc (Fridericia and Bazett corrections)

The tracings will be interpreted, dated and signed prior to submission to the sponsor. Original tracings will accompany the original eCRF. The subject's number and initials as well as the date and actual time of the tracing must appear on the printout of the tracing.

All occurring abnormalities will be identified and listed by subject and evaluation time point.

Clinically significant changes (or a clinically significant worsening) in ECG, which were observed the first time during study will be reported as adverse event.

## 8.7 Laboratory Safety Tests

The following laboratory evaluations will be performed at the study site:

The subject need not be in fasting condition. Examinations marked with Y instead of X in Appendix 3- Detailed List And Timing Of Procedures (§ 15.3) may be done at any time on the examination day.

**Hematology:** hemoglobin, hematocrit, RBC count, reticulocyte count, WBC with differential platelet count.

**Clinical Chemistry:** sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, total bilirubin, AST, ALT, alkaline phosphatase, GGT, FSH (only for postmenopausal females at Screening to confirm post-menopausal status), creatinine, BUN(urea-N), glucose, uric acid, cholesterol and high sensitivity C-reactive protein.

**Urinalysis** Stix: pH, density, glucose, protein, blood (free Hb), bilirubin, urobilinogen, ketones, nitrites, leukocytes and erythrocytes. A midstream urine sample (approx. 30 mL) will be obtained, in order to avoid contamination and allow a proper assessment.

**Pregnancy test (females only):** Performed in serum at Screening and in Urine during the Baseline period

For all laboratory values, date and time of laboratory sample will be documented on the eCRF. Laboratory results will be categorized either Normal, abnormal not clinically significant, abnormal clinical significant by the investigator. Each laboratory value determined in the study as well as the clinical assessments will be entered on the eCRF.

Clinically significant changes (or a clinically significant worsening) in laboratory safety assessments, which were observed the first time during study will be reported as adverse events.

Values which are considered clinically significant and/ or study drug related will be noted on the eCRF. The investigator will record his/ her medical opinion of the clinical significance of each laboratory value outside of the reference range in the eCRF. This decision shall be based upon the nature and degree of the observed abnormality. The investigator may choose to repeat any abnormal result ONCE, in order to rule out laboratory error. Clinically relevant deviations of laboratory test results occurring during or at post study examination of the study must be reported and discussed with the sponsor's Medical Expert. Repeated evaluations are mandatory until their normalization or until the time course and reason of the underlying process can clearly be assessed. In case of doubt, the sponsor's Medical Expert must be contacted.

The investigator will provide the sponsor with a copy of each laboratory's certification and a tabulation of the normal ranges.

## 8.8 Drug Screen

Subjects who are found to have positive urine results for any substances of abuse (Amphetamines, methamphetamines, benzodiazepines, barbiturates, cocaine, cannabinoids, opiates, methadone and phencyclidine) at Screening or at baseline will be excluded from study entry.

## 8.9 Serology

### Hepatitis Screen

All subjects will be screened for hepatitis B surface antigen ($HB_sAg$) at Screening. Screening for hepatitis C will be based on HCV antibodies. Any subject who has a positive test result will be excluded from the study and referred to a specialized outreach clinic.

### HIV Screen

Evaluation for HIV 1 and 2 will consist of an ELISA. Any subject who has a positive test result will be excluded from the study and referred to a specialized outreach clinic.

## 8.10 Pharmacokinetic Analysis

Serum Collection for NGF Analysis

The concentration versus time profiles of NGF will be assessed from individual serum samples of all subjects.

All blood samples will be taken by either direct stick or an indwelling cannula inserted in a forearm vein. On the days of pharmacokinetic profiling, a cannula will be inserted. Any missing blood draws must be reported on the respective eCRF section.

Two validated ELISA tests performed at Dompé farmaceutici s.p.a. will be used for PK measurements. The original validated method will be the same applied to the NGF determination in serum samples taken from studies NGF0112 and NGF0212. A new additional validated ELISA method will be performed with the intention of improving the sensitivity in the determination of NGF serum levels.

The processing, storage and shipment instructions for the pharmacokinetic samples obtained in this Study will be provided in the Research Specimen Manual.Briefly: each blood (5 mL) sample will be transferred to a polypropylene test tube and kept undisturbed at room temperature (approximately 15-30 minutes) to allow coagulation. The samples will then be centrifuged at 1900 g for 15 minutes at 20 ± 5ºC and the serum obtained from each sample will be distributed to two fresh polypropylene test tubes. two aliquots of 1 mL each of serum will be immediately frozen in dry ice and then stored at -70 ± 10ºC or at -20 ± 5°C until shipment to Dompé farmaceutici s.p.a for NGF determination by ELISA methods.

The aliquots will be sent in frozen condition on dry ice by a specialized courier. The temperature will be tracked by temperature log during shipment.

The quantification of rhNGF in the serum samples for pharmacokinetics will be performed at:

Biotech Process & Analytical Development Laboratory
Dompé farmaceutici s.p.a.
Via Campo di Pile
67100 L'Aquila
Italy.

The remaining aliquots of serum samples will be stored up to one year after Clinical Study Report (CSR) finalization.

## 8.11 Anti-Therapeutic Antibodies (ATA) Analysis

Serum Collection for Anti-therapeutic Antibodies (ATA) Analysis

Blood samples at baseline, and at between 35 to 42 days after the first dose of rhNGF will taken from all subjects for the ATA determination.

The concentration of ATA will be assessed from individual serum samples of all subjects.

All blood samples will be taken by either direct stick or an indwelling cannula inserted in a forearm vein. On the days of ATA determination, a cannula will be inserted. Any missing blood draws must be reported on the respective eCRF comment sheet.

A validated ELISA assay performed by the provider will be used for anti-therapeutic antibodies (ATA) detection:

Swiss BioQuant AG
Kägenstrasse 18
4153 Reinach, Switzerland

The processing, storage and shipment instructions for the immunocnicity samples obtained in this Study will be provided in the Research Specimen Manual.

Briefly: each blood (7.5 mL) sample will be transferred to a polypropylene test tube and kept undisturbed at room temperature (approximately 15-30 minutes) to allow coagulation. The samples will then be centrifuged at 1900 g for 15 minutes at 20 ± 5ºC and the serum obtained from each sample (at least 3.5 mL) will be distributed to two fresh polypropylene test tubes. two aliquots of 1.7 mL each of serum will be immediately frozen in dry ice and then stored at -70 ± 10ºC until shipment to the Analytical LAB for the determination by ELISA validated method.

The aliquots will be sent to the bioanalytical laboratory using a specialized provider (e.g. Wourld Courier) in frozen condition . The temperature will be monitored by temperature log during the shipment period.

The serum concentrations of ATA will be provided by the bioanalytical laboratory in a separate report and incorporated into the final CSR as an appendix.

The remaining aliquots of serum samples will be stored up to one year after CSR finalization.

### 8.11.1 Total volume of blood to be taken from each subject

The total volume of blood to be taken from each subject during the course of the study is 400 mL, as follows:

- Laboratory Assessments (including hepatitis and HIV screen): 20 mL x 5 timepoints = 100 mL

- PK determinations 5 mLx 57 time points = 285 mL
- ATA 7.5 mL x 2 time points = 15 mL.

## 8.12 Ophthalmological Evaluations

Opthalmological evaluations will be performed on both eyes and will include the following, depending on the study day (See Appendix 3-Detailed List And Timing Of Procedures):

**Local Ocular Tolerability (LOT) score:**

- A global ocular discomfort score will be assessed by the subject using a self-administered 100 mm visual analogue scale (VAS) on which 0 means no symptoms and 100 means the worst possible discomfort. This evaluation is to be performed **before** any ophthalmic assessment at a given study visit.
- Specific ocular symptoms to be assessed with the VAS by the subject include:
    - foreign body sensation,
    - burning/stinging,
    - itching,
    - pain,
    - sticky feeling
    - blurred vision
    - photophobia

### Best corrected distance visual acuity (BCDVA) measured using the ETDRS score

Subjects with a BCDVA score ≤ 0.00 LogMAR (≥83 ETDRS letters, 20/20 Snellen or 1.0 decimal fraction) in each eye at the Screening and Baseline visits are eligible for enrollment in the study.

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

Refraction equipment required includes:

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

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

A distance of 4 meters is required between the subject's eyes and the visual acuity chart. With the box light off, not more than 15 foot-candles of light (161.4 Lux) should fall on the center of the chart. To measure the amount of light, the room is set up for visual acuity testing, but with the box light off. The light meter is placed at the fourth line from the top of the chart, with its back against the chart and the reading is taken. If more than one line available for testing visual acuity, the visual acuity of an individual subject 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 wallmounted or mounted on a stand. The light box should be mounted at a height such that the top of the third row letter is 49 + 2 inches from the floor.
The visual acuity light box is equipped with two General Electric 20-watt fluorescent tubes and ballast. Each tube is partly covered by a 14-inch fenestrated sleeve, which is centered on the tube and open in the back. This serves as a "baffle" to produce even illumination over the testing chart. Because the illumination of fluorescent tubes diminishes by 5 percent during the first 100 hours and by another 5 percent during the next 2000 hours, new tubes should be kept on for 4 days (96 hours) continuously, and should be replaced once a year.
A sticker should be placed on the back of the light box, indicating the date when the present tubes were installed. A spare set of burned in bulbs should be available on site.

Detailed instructions for VA assessment

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

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

The subject should be seated comfortably directly in front of the chart so that the eyes remain at the 4 meter distance. Testing always begins with the right eye. The fellow (non-study) 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 subject is asked to read the letters slowly, approximately one letter per second. The subject 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 subject is unsure about the identity of the letter, then the subject should be encouraged to guess.

The subject 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 subject 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 subject reaches a level where he/she cannot guess, the examiner may stop the test, provided that the subject has made errors on previous guesses, which is a clear indication that the best visual acuity has been obtained.

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

The examiner should not tell the subject if a letter was identified correctly. The subject 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 subject and the VA worksheet. If the subject has difficulty locating the next line to read, the examiner may go up to the chart and point briefly to the next line to be read, but then must move away from the chart.

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

Clinically significant changes (or a clinically significant worsening) in BCDVA, which were observed the first time during study will be reported as adverse event.

### External ocular examination (EOE): motility and eyelids

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

Clinically significant changes (or a clinically significant worsening) in EOE, which were observed the first time during study will be reported as adverse event.

### Slit-lamp examination (SLE)

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

<u>Eyelid - Meibomian glands</u>

Evaluation of the central ten Meibomian gland openings in the mid-portion of the upper eyelid:

0 = None (none are plugged).
1 = Mild (1 to 2 glands are plugged).
2 = Moderate (3 to 4 glands are plugged).
3 = Severe (All glands are plugged).

<u>Eyelid - Erythema</u>

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

<u>Eyelid - Edema</u>

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

<u>Lashes</u>

0 = Normal.
1 = Abnormal (specify).

<u>Conjunctiva – Erythema</u>

0 = None (normal).
1 = Mild (a flush reddish color predominantly confined to the palpebral or bulbar conjunctiva).

2 = Moderate (more prominent red color of the palpebral or bulbar conjunctiva). 3 = Severe (definite redness of palpebral or bulbar conjunctiva).

Conjunctiva - Edema

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

Lens

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

Iris

0 = Normal
1 = Abnormal.

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

0 = None (no Tyndall effect).
1 = Mild (Tyndall effect barely discernible).
2 = Moderate (Tyndall beam in the anterior chamber is moderately intense).
3 = Severe (Tyndall beam in the anterior chamber is severely intense).

Corneal findings of interest on the SLE include also the horizontal diameter of the cornea (measured in mm at the time of the SLE using a ruler).

Clinically significant changes (or a clinically significant worsening) in SLE, which were observed the first time during study will be reported as adverse event.

### Corneal fluorescein staining (modified Oxford scale)

As grading scale of the corneal and conjunctiva damage, the NEI/Industry Workshop guidelines will be used [Reference: 2]. The cornea is divided into five sectors (central, superior, inferior, nasal and temporal), each of which is scored on a scale of 0–3, with a maximal score of 15. Both nasally and temporally, the conjunctiva is divided into a superior paralimbal area, an inferior paralimbal area and a peripheral area with a grading scale of 0–3 and with a maximal score of 9 for the nasal and temporal conjunctiva.

For a better reading it is also essential not to use an intense illumination beam, which may reduce the contrast and lead to an underestimation of grading [Reference: 3].

Clinically significant changes (or a clinically significant worsening) in corneal fluorescein staining, which were observed the first time during study will be reported as adverse event.

### Tear Film Break-up Time (TFBUT)

Subjects with TFBUT < 8 seconds in either eye at the screening or baseline visit are NOT eligible for enrollment.

TFBUT will be measured by determining the time to tear break-up. The TFBUT will be performed after instillation of 5 µl of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. The subject 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.

Clinically significant changes (or a clinically significant worsening) in ECG, which were observed the first time during study will be reported as adverse event.

### Schirmer test without anesthesia

Schirmer test without anesthesia is only required at the screening visit. Subjects with a Schirmer test without anesthesia ≤ 9 mm/5 minutes in either eye at the screening visit are NOT eligible for enrollment.

This test will be performed to measure aqueous tear secretion prior to the instillation of any dilating or eye drops. Both eyes may be tested at the same time.

This test will be conducted in a dimly lit room. While the subject looks upwards, the lower lid will be drawn gently downwards and temporally. The rounded bent end of a sterile strip will be inserted into the lower conjunctival sac over the temporal one-third of the lower eyelid margin. The test should be done without touching directly the Schirmer test strip with the fingers to avoid contamination of skin oils. The subjects will be instructed to close their eyes gently. After 5 minutes have elapsed, the Schirmer test strip will be removed and the length of the tear absorption on the strip will be measured (millimeters/5 minutes).

The wetting distance at 5 minutes for each eye will be recorded in the eCRF.

### Fundus ophthalmoscopic (FO) examination

Fundus ophthalmoscopic (FO) examination will be performed to assess the vitreous, retina/macula/choroid, and optic nerve, including cup/disc ratio. Dilated FO will be performed at screening with non-dilated FO performed at other visits.

Clinically significant changes (or a clinically significant worsening) in FO, which were observed the first time during study will be reported as adverse event.

### Intraocular pressure (IOP)

Subjects with IOP ≥ 22 mmHg in either eye at the screening or baseline visit are NOT eligible for enrolment,

Intraocular pressure (IOP) will be determined using Goldmann applanation tonometry.

Clinically significant changes (or a clinically significant worsening) in IOP, which were observed the first time during study will be reported as adverse event.

# 9 EVALUATION OF ADVERSE EVENTS AND SAFETY INFORMATION

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

## 9.1 Definitions

Adverse Event

An **Adverse Event (AE)** is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment (CFR-Code of Federal Regulations Title 21 Sec. 312.32). 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.

Non-Serious Adverse Event (NSAE)

A **non-serious AE (NSAE)** is defined as any untoward change in a subject's medical conditions that does not meet serious criteria noted below (e.g., is not fatal, is not life-threatening, does not require hospitalization, does not prolong a current hospitalization, is not disabling and is not an important medical event).

Serious Adverse Event (SAE)

A **Serious Adverse Event (SAE)** is defined in line with CFR-Code of Federal Regulations Title 21 Sec. 312.32 as any adverse experience that meets any of the following criteria:

- results in death;
- is life-threatening;

NOTE: Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe

- requires inpatient hospitalization or prolongation of existing hospitalization;

NOTE: In general, hospitalization means that the individual remained at the hospital or emergency ward for observation and/or treatment (usually involving an overnight stay) that would not have been appropriate in the physician's office or an outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether “hospitalization” occurred, the event should be considered serious.

- results in persistent or significant disability/incapacity;

NOTE: The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption).

- results in a congenital anomaly/birth defect;
- is an important medical event

NOTE: An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the subject’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 subject hospitalization or the development of drug dependency or drug abuse.

Death shall always be reported as SAE: a death due to progression of disease would not have a causal relationship to the product. The investigator should report the event immediately to the sponsor, but the sponsor will not report the event as expedited to the regulatory authority. Cause of death shall always be specified when known or duly investigated.

Sight Threatening Event

For this study medically important events comprise the following sight threatening events, which are considered to be of special interest and by default are to be reported as SAEs:

- 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 cause a decrease in visual acuity to the level of Light Perception or worse lasting >1 hour
- Adverse Events that require 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.

Any serious adverse event or sight-threatening event occurring in a subject from the time of study medication administration through the last follow up visit must be reported to the Sponsor within 24 hours, even if the SAE or sight-threatening event does not appear to be drug-related. In the event of an SAE or sight-threatening event, the following steps should be taken;

1. report the SAE or sight-threatening event to Dompé pharmacovigilance contact by fax or e-mail using the SAE form.
2. report the SAE or sight-threatening event to the relevant IRB according to local specific requirements.

Adverse Drug Reaction

An **Adverse Drug Reaction (ADR)** is defined as an adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. . Adverse events are to be considered unsuspected if the relationship to the study drug as described in the table in § 9.2.1 is none or unlikely; whereas any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

Unexpected Adverse Event Or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered **unexpected** if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. (CFR-Code of Federal Regulations Title 21 Sec. 312.32). The determination of expectedness should be made on the basis of the current IB [Reference: 4].

Suspected Unexpected Serious Adverse Reaction

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is defined as an adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Reaction. SUSARs related to the IMP qualify for regulatory reporting.

## 9.2 Recording

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

All AEs (non-serious and serious) that occur during the course of the study, from the time the subject signs the ICF for the trial until last follow up visit, will be collected and recorded in the e-CRF. Any pre-existing medical conditions or signs/symptoms present in a subject prior to the start of the study (i.e., before informed consent is signed) should be specified in the eCRF. These conditions are considered AEs only if they increase either in frequency or severity once informed consent has been signed. 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. Thus, any untoward medical occurrences or unfavorable and unintended signs, symptoms, or diseases that occur in the pretreatment, in treatment, or post treatment period are to be considered AEs and/or SAEs, and consequently recorded and reported as such. Should a non-serious AE become serious, the Investigator will then follow the same reporting procedures as for SAEs.

Each AE will be described by:

- Its duration (start and stop dates).
- Severity grade, as defined in § 9.2.2.
- Assessment of the adverse event relationship to the study treatment
- Any action with study treatment taken as a result of the event.
- Outcome.

All AEs should be followed-up to determine outcome of the reaction. The Investigator should follow up the event until resolution or stabilization of the condition. It is the Investigator's responsibility to assure that the subjects experiencing an AE receive definite treatment for any AE, if required.

Serious Adverse Events:

The Investigator must record all SAEs, including sight-threatening events, occurring at any time during the study regardless of presumed causal relationship, on the Serious Adverse Event form in the eCRF of the Electronic Data Capture (EDC) system within 24 hours of learning of the event; information on the SAE must also be recorded on a specific Non-Carbon Repeat SAE form (included in the Investigator's Site File) and forwarded to Cromsource Drug Safety and Dompé Drug Safety, within 24 hours from first knowledge.

If the Investigator becomes aware of a related serious adverse event occurring to a subject after the treatment of that subject has ended, this event should be reported by the Investigator to Dompé Drug Safety, as further described in § 9.3.1.

### 9.2.1 Relationship of Adverse Events to the Investigational Product

The Investigator will assess the relationship between the AE and the investigational medication, according to the criteria in Table 3 below:

**Table 3: Relationship of the Adverse Event to the IMP**

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

### 9.2.2 Severity of Adverse Events

The Investigator will grade the severity of any AE using the definitions in Table 4 below. For each episode, the highest severity grade attained should be reported.

**Table 4: Severity of the Adverse Event**

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

## 9.3 Serious Adverse Event Reporting

### 9.3.1 Reporting Procedure for Investigators to Dompé Drug Safety Pharmacovigilance and to Cromsource

The Investigator must record and report all SAEs, regardless of presumed causal relationship, to Dompé Drug Safety and to Cromsource Medical Monitor, by e-mail (preferred) or fax within 24 hours from first knowledge of the SAE,to the following addresses:

- by fax through the e-fax number: +1 201 326 7710 (linked to Dompé Drug Safety and to Cromsource Medical Monitor).
- or, via e-mail to all following addresses:
  - farmacovigilanza@dompe.com;
  - DOMPE-NGF0117-PV@cromsource.com
  - DOMPE-NFGF0117-MM@cromsource.com

Contact details for safety matters are listed below:

Dompé Contact Information

Dompé Drug Safety
Laura Boga, Senior Safety Manager
Email: farmacovigilanza@dompe.com

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

Early Clinical Development Manager
Mauro P. Ferrari
Email: mauro.ferrari@dompe.com

Cromsource Contact Information

For Pharmacovigilance: DOMPE-NGF0117-PV@cromsource.com

For Medical Monitor: DOMPE-NFGF0117-MM@cromsource.com

The Investigator should also report information on SAEs that continue after subject has completed his/her participation in the study (whether study completion or withdrawal), unless subject has withdrawn his/her consent. The follow-up will continue with no timelines for related SAEs, while for unrelated SAEs the type and extent of follow-up undertaken will be determined for each individual case and will depend upon the nature, severity and medical significance of the event.

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

SAE reports will be managed directly by the Dompé Drug Safety department, with Cromsource Materiovigilance And Pharmacovigilance Unit (MVPVU) support for follow-up requests.

Follow-up reports (as many as required) should be completed and e-mailed / faxed following the same procedure above, marking a new SAE form, with follow up Number XX", duly filled in and signed, within 24 hours from awareness.

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.

Depending on the nature and seriousness of the AE, further information, including copies of appropriate medical records of the subject, as well as results of laboratory tests performed will need to be included in the subjects chart.

Note: If a SAE is considered as not related by Investigator but Dompé/Cromsource consider the event related and unexpected this needs to be reported to Investigator.

If the subject was hospitalized due to a SAE, a copy of the discharge summary is to be forwarded to Cromsource/Dompé as soon as it becomes available. In addition, a letter from the Investigator that summarizes the events related to the case as well as results of any relevant laboratory tests also may be requested. Further, depending upon the nature of the SAE, Dompé may request copies of applicable segments of the subject's medical records.

For pharmacovigilance purposes, all SAEs should be followed up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or subject is lost to follow-up. Follow-up may therefore continue until after the subject has completed the study up to 30 days after his/her discontinuation from the study for unrelated SAEs, and without timelines for related SAEs.

Up to the database lock, SAE reports should be reported to Dompé Drug Safety and to Cromsource (MVPVU). New serious adverse event occurring after the site is closed should be reported directly to Dompé Drug Safety.

In line with ICH E2A provisions, although the Investigator does not usually need to actively monitor subjects for AEs once the trial has ended, if the Investigator becomes aware of a SAE occurring to a subject after that subject 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.

### 9.3.2 Conditions that should not be reported as serious adverse events

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

- Hospitalizations planned before entry into the clinical study which is part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition.
- Hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
- Hospitalization for treatments, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen.
- Hospitalization for general care not associated with any deterioration in condition.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of SAEs given above and not resulting in hospital admission.
- In addition, the following situation shall not be considered SAE:
- Anticipated Disease progression in oncology trials, as applicable.
- Trial end points
- AE that are considered expected as part of the trial treatment (such as standard side effects of chemotherapy in an oncology trial or anticipated AE related to study procedure, unless the frequency or intensity is unusual).
- Abnormal lab values or test results that do not induce clinical signs and/or symptoms and require intervention/therapy, i.e. are clinically significant.

### 9.3.3 Adverse events exemption

Not applicable.

### 9.3.4 Reporting Procedure to IRB/IEC and to Regulatory Authorities

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

In line with the Sponsor's procedure and defined timelines, Dompé and Cromsource shall perform an assessment of the expectedness and the causality of each SAE report received and assess regulatory reportability.

Expectedness will be assessed with respect to the current IB [Reference: 4]: due to the early phase of development, all SAEs will be considered unexpected.

For a SAE reported by the Investigator as not related that is subsequently revised to be related by the Dompé, 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.

In line with provisions set forth in 21CFR312, Dompé Drug Safety shall notify all participating Investigators in an IND safety report of any suspected adverse reaction that is both serious and unexpected and of potential serious risks, from any clinical trials or any other source, as soon as possible, but in no case later than:

(*a*) seven calendar days after becoming aware of the information if the event is fatal or life threatening;

(*b*) fifteen calendar days after becoming aware of the information if the event is neither fatal nor life threatening.

The investigator in turn shall notify the relevant IRB.

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

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

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

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

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

Periodic Reporting to Regulatory Authorities

Dompé Drug Safety shall be responsible to prepare and submit appropriate periodic safety updates (Development Safety Update Report – DSUR) to relevant Regulatory Authorities and IRB, if specifically requested.

## 9.4 Unmasking Of The Study Treatment

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

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

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

## 9.5 Follow-Up Of Subjects With Adverse Events (Aes)

The Investigator is responsible for adequate and safe medical care of subjects 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 subjects experiencing AEs receive definite treatment for any AE, if required.

## 9.6 Exposure To Investigational Product During Pregnancy

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

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

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours from awareness of the pregnancy) to Cromsource/Dompé Drug Safety contacts reported at § 9.3.1, even if no AE has occurred, and follow it to term.

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

If the pregnancy is associated with an SAE (eg, if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed as described in § 11.7 with the appropriate serious criterion (eg, hospitalization) indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE. Any pregnancy leads to the immediate cessation of the study treatment.

## 9.7 Adverse Events Causing Treatment Discontinuation

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

## 9.8 Overdose

Cases of overdose (accidental or intentional) shall be reported within 24 hours from the Investigator's knowledge of its occurrence to Dompé Drug Safety and Cromsource, even if not associated with adverse events. 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, the administration of more than 3 times the total daily dose on any given treatment day should be considered as overdose. Dompé Medical Expert or Cromsource Medical Monitor shall be contacted in case of medical concern.

## 9.9 Termination Or Halting Of The Study

In the event of two SAE, in separate subjects, with highly probable drug causality the study will be halted with immediate ceasation of dosing.

In the event of the study being halted an expert Safety Committee will be created comprising a group of experts and will include the Principal Investigator, an independent specialist Opthalmologist and an unmasked Statistician.

An operating procedure describing how the Safety Committee works and how it communicates with other study participants will be prepared.

The Committee will review blinded study information (quarterly at a minimum), which will include:

- List of any protocol violations
- Numbers of patient withdrawals/reason for withdrawal.
- Adverse/Serious Adverse Events
- Laboratory Data

The Safety Committee will also be able to request unblinding of patients. The operating procedure will document the planned flow of information in order to describe how the integrity of the study with respect to preventing dissemination of unblinded study information is assured.

Following review of the safety data, the Safety Committee will prepare a written report which will be forwarded to Dompé advising of any recommendations regarding modifications, continuation or termination of the study.

Where changes in the study conduct are recommended to Dompé, sufficient (blinded) information will be provided to allow Dompé to decide whether and how to implement these recommendations.

# 10 STATISTICS

## 10.1 Study endpoints

Primary safety endpoints are:

- Incidence of treatment emergent adverse events (TEAEs).
  TEAEs are defined as an adverse event (AE), which start after first dose of study treatment. These comprise AEs during the treatment and follow-up period.

- Incidence of treatment emergent adverse events during 1st dose schedule (TEAEs Dose 1).
  TEAEs Dose 1 are defined as TEAEs, which start after first dose of study treatment and before administration of the first dose at Treatment Day 2.

- Incidence of treatment emergent adverse events during 2nd dose schedule (TEAEs Dose 2).
  TEAEs Dose 2 are defined as TEAEs, which start on/after the first dose at Treatment Day 2 and before Follow Up Day 7 (FU1) visit.

Secondary safety endpoints are:

- Incidence of Follow-Up AEs (FUAE)
  FUAEs are defined as TEAEs which start on or after Follow Up Day 7 (FU1) visit visit.

- Incidence of ocular TEAEs by eyes.

- Course of ECG parameters ventricular rate, PR interval, QRS duration and QT interval over all visits.

- Course of vital signs parametes systolic and diastolic blood pressure, pulse rate, respiratory rate and body temperature over all visits.

- Changes from baseline in biochemistry parameters over all visits.
  Baseline is defined as the Laboratory assessment of Baseline (D-1) Visit

- Changes from baseline in hematology parameters over all visits.
  Baseline is defined as the Laboratory assessment of Baseline (D-1) Visit

- Shift in overall interpration of urinalysis over all visits. Laboratory assessment at Baseline (D-1) Visit is considered as reference for assessing the shift.

- Course of intraocular pressure by eye over all visits.

- Course of visual acuity score by eyes over all visits
  The visual acuity score will be derived as the total numbers of letters, which were read correctly at 4 meter plus the total number of letters read at 1 meter as collected on the eCRF page. When 20 or more letters are read at 4 meters the visual acuity score for that eye is recorded as the number of letters correct at 4 meters plus 30. The subject gets credit for the 30 letters at 1 meter even though they did not have to read them. If no letters are read correctly at either 4.0 meters or 1 meter, then the visual acuity score is recorded as “0.”

- Course of LogMAR by eyes over all visits.
  The logMAR will be derived as – log (Snellen Equivalent result)

- Course of TFBUT by eye over all visits.

- Course of overall NEI score by eye over all visits.

Primary tolerability endpoints are:

- Change from baseline in VAS ocular tolerability scores at Treatment Day 2 pre-dose in study eye (Tolerability of 1x0.70 µg rh-NGF per day).
  Baseline is defined as the pre-treatment assessment at the Baseline (D-1) visit.

- Change from baseline in VAS ocular tolerability scores at D2 8h, D3 pre-dose, D6 pre-dose, D6 8h, D7 (Follow-up Day 7), D8 (Follow-up Day 8), D16 (Follow-up Day 16) in study eye (Tolerability of 6x0.70 µg rh-NGF per day).
  Baseline is defined as the pre-treatment assessment at the Treatment Day 2 visit.

Secondary tolerability endpoints are:

- Course of VAS ocular tolerability scores by eye over all visits.

- Intraindividual change in VAS ocular tolerability scores between study eye and fellow (non-study) eye at all study visits.

### 10.1.1 Pharmacokinetic endpoints

The kinetics of single dose administration as well as multiple dose assessment will be evaluated.

Beyond the respective rhNGF plasma levels for both ELISA methods, the following parameters will only be investigated for each ELISA method, if the data warrant to do so:

- maximum observed serum concentration ($C_{max}$), time to reach $C_{max}$ ($t_{max}$), time elapsed between dosing and the first serum concentration that exceeds the assay quantification limit ($t_{lag}$) as measured without treatment based on assessments on D-1
- $C_{max}$, $t_{max}$. $t_{lag}$ of single dose regimen based on assessments on D1 and pre-dose of D2
- $C_{max}$, $t_{max}$. $t_{lag}$ of multiple dose regimen based on assessments atarting with pre-dose of D2
- serum concentration measured before the administration of the next dose ($C_{trough}$) as assessed at pre-dose visit on D2, D3, D4, D5, D6
- $AUC_{[0-24]}$ for the single dose regimen and the first day of multiple dose regimen. $AUC_{[0-24]}$ as area under the serum concentration versus time curve from time 0 h to pre-dose value of the next day calculated by the linear trapezoidal rule. Values below the level of quantitation will be set to 0. Values below the baseline concentration will be set to zero other than for the values for which no later values above the baseline concentration are available, which will be set to missing.
- $AUC_{0\text{-}tlast}$ for the multiple dose regimen. This is derived as area under the serum concentration versus time curve from time 0 h to the last data point $t_{last}$ after drug administration above the limit of quantitation and the baseline concentration, calculated by the linear trapezoidal rule.

  Values below the baseline concentration will be set to zero other than for the values for which no later values above the baseline concentration are available, which will be set to missing
- AUC for the multiple dose regimen. AUC will be derived as area under the serum concentration versus time curve extrapolated to infinity, calculated as $AUC = AUC_{0\text{-}tlast} + C_{last}/\lambda_z$

### 10.1.2 Immunogenicity endpoints

For the investigation of immunogenicity, ATA serum levels will be investigated.

## 10.2 Sample Size

No formal sample size calculation was made. Sample size considerations for this type of study are dependent on the common understanding on how many subjects one needs to demonstrate safety and tolerability for the dose level investigated and the different ethnicity.

Thirty (30) subjects randomized 2:1 (rh-NGF : vehicle) could be considered acceptable. Twenty (20) subjects on active (one eye active, one eye placebo) and 10 on placebo (both eyes) each will be treated.

## 10.1 Analysis Sets

The "Safety" Set will consist of all subjects randomized into the study and receiving a dose of study medication.

The "PK" Set will consist of all subjects who did not show serious protocol deviations or non-compliance or completed the PK sampling according to the study protocol.

All protocol deviations that occur during the study will be considered for their severity/impact and will be taken into consideration when subjects are assigned to analysis sets during a blind data review meeting. Details of subject assignment to the analysis populations will be listed.

## 10.2 Changes to the Statistical Plan

Any deviations from the original statistical plan will be described in the Clinical Study Report.

## 10.3 Data Analysis

All endpoints of this study will be summarized using descriptive summary statistics, i.e. arithmetic mean, standard deviation (SD), minimum, median and maximum for quantitative variables by treatment group. If applicable, geometric mean together with 95% confidence interval and coefficient of variation will be reported. For qualitative variables, absolute and relative frequencies will be reported.

All ocular assessments will be presented by treated and untreated eyes. No inferential analyses are forseen for this study.

For event-based variables (e.g. adverse events) the number of events as well as the incidences (i.e. number and percentage of affected subjects) will be provided.

All assessed data will be listed. For the analysis, no imputation of data is planned; all data will be analysed as collected.

All statistical calculations will be made in SAS.

A statistical analysis plan (SAP) will be developed and finalized before data base lock and unmasking describing in detail the planned statistical analysis.

No interim analysis is planned for this study.

### Evaluation of Pharmcokinetics

The serum concentrations of rhNGF will be provided by the bioanalytical laboratory. Pharmacokinetic evaluation will be done using Phoenix WinNonlin 6.4 or later (Pharsight Corporation, Palo Alto, CA, USA).

The analysis of pharmacokinetic parameters will be based on the difference between the measured rhNGF value after dosing and the value measured at the corresponding time on Day -1.

Individual pharmacokinetic raw data will be checked for consistency by comparison with the enclosed hard copies. Values below the limit of quantitation and missing values will be labeled and accounted for accordingly during evaluation.

Additional evaluations, such as compartmental analysis, may be performed with an exploratory purpose for the multiple dose regimen if the study data suggest that they are needed for a better understanding of the drug. Likewise, the pharmacokinetic results of this study may be later pooled with results of other studies for population pharmacokinetic and pharmacokinetic-pharmacodynamic analysis and modelling purposes.

**Anti-therapeutic Antibodies (ATA)**

ATA samples will be analyzed by a central bioanalytical laboratory (Swiss BioQuant AG).

The serum concentrations of ATA will be provided by the bioanalytical laboratory in a separate report and incorporated into the final CSR as an appendix.

# 11 ETHICAL CONSIDERATIONS

## 11.1 Institutional Review Board And Independent Ethics Committee

Approval by the Institutional Review Board (IRB) before the start of the study will be the responsibility of the Principal Investigator (PI). A copy of the approval letter will be supplied to the sponsor, along with a roster of IRB members or the US Department of Health and Human Services (DHHS) general assurance number. During the course of the study, the PI will provide timely and accurate reports to the IRB on the progress of the study, at intervals not exceeding 1 year (or as appropriate), and will notify the IRB of serious adverse events (SAEs) or other significant safety findings. The study protocol, informed consent form (ICF), information sheet advertisements, and amendments (if any) will be approved by the IRBs at the study centers in conformance with Code of Federal Regulations (CFR), Title 21, Part 56.

## 11.2 Ethical Conduct Of The Study

The study will be conducted in full compliance with FDA and ICH guidelines for good clinical practice (GCP) [Reference: 1] and in accordance with the ethical principles that have their origins in the Declaration of Helsinki and 21 CFR § 312.120.

## 11.3 Data Monitoring Committee

Not applicable – there is no Data Monitoring Committee for this study.

## 11.4 Subject Information And Consent

Subjects, after being given an explanation of the study, will give voluntary and written informed consent before participating in any study-related procedures. A copy of the Experimental Subject's Bill of Rights (Footnote 1) will provided to a subject prior to performing the consent process.

Each subject will read or be read (if he or she cannot read or write), assent understanding of, and sign or thumbprint an instrument of informed consent after having had an opportunity to discuss them with the PI before signing; each subject will be made aware that he or she may withdraw from the study at any time.

The informed consent statement contains all the elements of informed consent and contains all the core elements and mandatory statements as defined in the CFR. Signed copies of the ICF will be given to the subject, and both documents will be placed in the PI's study files. A unique subject identification (SID) number will be assigned according to § 6 at the time the subject signs the ICF.

## 11.5 Confidentiality

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

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1 The Protection of Human Subjects in Medical Experimentation Act (California Health and Safety Code 24170 – 24179.5) requires that a potential experimental subject (or subject's conservator, guardian, or other representative) be provided with a list of the rights of a subject in a medical experiment.

On the eCRFs, subjects will be identified ONLY by the assigned subject number. If subject names are included on copies of documents submitted to Dompé or Cromsource, the names will be obliterated or masked and the assigned subject number added to the document.

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

## 11.6 Compensation For Medicine-Induced Injury And Indemnification

Before the trial formally starts, Dompé will take out a study-specific insurance contract according to national laws for subjects/Investigators/Institutions participating in the clinical trial.

In case of questions about medical care, cost for medical care or insurance, subjects can talk to their Investigator. Contact details will be given in the Subject Informed Consent Document.

# 12 DATA HANDLING AND RECORD KEEPING

## 12.1 Case Report Forms

All data relating to the study will be recorded on eCRFs to be provided by Cromsource, through the EDC system. The PI is responsible for verifying that all data entries in the eCRFs are accurate and correct. The PI must sign the completed eCRF before its submission to the sponsor.

## 12.2 Data Management

Data collection will involve the use of an EDC system , to which only authorized personnel will have access. In addition to periodic monitoring occurring within the system by Sponsor/CRO Monitors, programmatic edit checks will be used to review the data for completeness, logic, and adherence to study protocol. As a result of this monitoring and these checks, queries may be electronically issued to the study centers and electronically closed by those study centers. The identifying information (assigned username, date, and time) for both the originator of the query (if created during the monitoring process) and the originator of the data change (if applicable), as well as the PI's approval of all changes performed on his or her subjects' data, will be collected.

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

## 12.3 Independent Reading Center (E.G. Independent Radiology Review)

Not applicable.

## 12.4 Documentation Required Prior To Initiation Of And During The Study

The following will be required from the Investigator prior to the initiation visit:

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/co-workers. Updates should be provided at least every two years (if applicable).
- Normal ranges of all laboratory tests to be performed at the study site and a recent certification or accreditation of established quality control (or other documentation of established quality control or external quality assessment or other validation). Updates should be provided as soon as any reference value has changed.
- A signed page of the final protocol and any amendments.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with Cromsource, including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- A financial disclosure agreement completed and signed by the PI and all Sub-Investigators listed on Form FDA 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.

## 12.5 Essential Document Retention

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

The essential documents include at least: the signed protocol, copies of the completed eCRFs, signed Subject Informed Consent Forms from all subjects who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

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

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

# 13 STUDY MANAGEMENT

The study will be performed in accordance with the protocol, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and ICH Harmonised Tripartite Guideline for Good Clinical Practice (ICH-GCP E6 (R2)) [Reference: 1] and any local regulations.

## 13.1 Monitoring

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

## 13.2 Access To Records

The Investigator will allow designated Cromsource representatives, including staff from the appointed Cromsource, and regulatory/ethics bodies to have direct access to the source documents to verify the data reported in the eCRFs. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the subject and substantiate the integrity of the data collected during the trial.

## 13.3 Audit And Inspection

The study site may be audited by Cromsource, Dompé or inspected by a regulatory agency on one or more occasions The Investigator may be informed in advance of such a visit.

## 13.4 Protocol Amendments

Any amendment to this protocol will be provided to the PI in writing by Dompé farmaceutici s.p.a. No protocol amendment may be implemented (with the exceptions noted below) before it has been approved by the IRB/IEC and the signature page, signed by the PI, has been received by Dompé farmaceutici s.p.a. If the protocol is amended to eliminate or reduce the risk to subjects, the amendment may be implemented before IRB/IEC review and approval. However, the IRB/EC must be informed in writing of such an amendment, and approval must be obtained within reasonable time limits. Deviating from the protocol is permitted only if absolutely necessary for the safety or clinical management of the subjects and must immediately be reported to Dompé farmaceutici s.p.a.

## 13.5 Discontinuation Of The Study

Dompé farmaceutici s.p.a. reserves the right to terminate the study in its entirety or at a specific study center at any time on the basis of new information regarding safety or efficacy, or if study progress is unsatisfactory, or for other valid administrative reasons.

### 13.6 Publications

All data generated in this study will be the property of Dompé farmaceutici s.p.a. Publication of the results by the PI will be subject to mutual agreement between the PI and Dompé farmaceutici s.p.a.

## 14 REFERENCES

1. ICH E6 (R2) Guideline for Good Clinical Practice: ICH E6 (R2) Good clinical practice. EMA/CHMP/ICH/135/1995, Published 15Dec2016, Effective from 14Jun2017.
2. Lemp MA (1995) Report of the National Eye Institute/Industry Workshop on clinical trials in dry eye. CLAO J 21: 221–232.
3. Foulks GN (2003) Challenges and pitfalls in clinical trials of treatments for dry eye. Ocul Surf 1: 20–30.
4. Dompé farmaceutici s.p.a. Recombinant Human Nerve Growth Factor (rhNGF): Investigator's Brochure Ed. No. 42.8/EN. July 31, 2017 or later version.

# 15 APPENDICES

## 15.1 Appendix 1-Sponsor Approval Page

Phase I, Randomized, Double-masked, Placebo-controlled Study (6 days) to Evaluate the Safety, Tolerability and Pharmacokinetics of Recombinant Human Nerve Growth Factor Eye Drops in Healthy Male and Female Volunteers of Japanese Ethnicity

Sponsor Medical Expert: _____ Date: _____/____/____

Flavio Mantelli, Chief Medical Officer **Biotech**

Sponsor Clinical Trial Manager: _____ Date: _____/____/____

Mauro P. Ferrari, Early Clinical Development Manager

Sponsor Development Director _____ Date: _____/____/____

Marcello Allegretti, Chief Scientify Officer

## 15.2 Appendix 2-Investigator's Signature Page

**Investigator's Statement**

I have read the study protocol: Phase I, Randomized, Double-masked, Placebo-controlled Study (6 days) to Evaluate the Safety, Tolerability and Pharmacokinetics of Recombinant Human Nerve Growth Factor Eye Drops in Healthy Male and Female Volunteers of Japanese Ethnicity

Name of Principal Investigator (block letters):_____

Signature: _____ Date: _____

## 15.3 Appendix 3-Detailed List And Timing Of Procedures

### Detailed view of the evaluations

**SCREEN (D -21 TO -2)**

| Day | Hours | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Screen** D–21 to -2 | Informed Consent, Inclusion/Exclusion Criteria, Demography, Medical History/current medical conditions, Prior / Concomitant medication, Physical Exam and Height, Serology, Drugs Abuse/pregnancy | | | | | | | | | | | | | |
| | | | | | X | X | X | | | X | X | X (dilated) | X | X | X |

**BASELINE (D -1)**

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **-Baseline D-1** | Inclusion/Exclusion Criteria, Current Medical Conditions, Concomitant Medication, Physical Exam, Drugs Abuse/Pregnancy | | | | | | | | | | | | | | |
| | 0 | | | X | X | X | Y | X | X | X | X | X | X | X | X |
| | 0.5 | | | X | X | X | | | | | | | | | |
| | 2 | | | X | X | X | | | | | | | | | |
| | 4 | | | X | X | X | | | | | | | | | |
| | 8 | | | X | X | X | | | | | | | | | |
| | 9 | | | X | | | | | | | | | | | |
| | 10 | | | X | | | | | | | | | | | |
| | 11 | | | X | | | | | | | | | | | |
| | 12 | | | X | | | | | | | | | | | |
| | 14 | | | X | | | | | | | | | | | |
| | 16 | | | X | | | | | | | | | | | |

**TREATMENT DAY 1: ONE DROP DAILY**

**Day 1: One drop of either rhNGF or vehicle instilled into study eye (35 µL: 0.70 µg of rhNGF) and one drop of vehicle instilled into the fellow (non-study) eye**

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 24h (Pre-dose | | | X | | X | | | | | | | | | |
| | 0 (dosing) | 0 | X | | | | | | | | | | | | |
| | 0.5 | 0.5 | | X | X | X | | | | | | | | | |
| | 2 | 2 | | X | X | X | | | | | | | | | |
| | 4 | 4 | | X | X | X | | | | | | | | | |
| | 8 | 8 | | X | X | X | | | X | X | | | X | | X |
| | 9 | 9 | | X | | | | | | | | | | | |
| | 10 | 10 | | X | | | | | | | | | | | |
| | 11 | 11 | | X | | | | | | | | | | | |
| | 12 | 12 | | X | | | | | | | | | | | |
| | 14 | 14 | | X | X | X | | | | | | | | | |
| | 16 | 16 | | X | | | | | | | | | | | |

**TREATMENT DAY 2 TO DAY 6: ONE DROP six TIMES DAILY**

**Day 2,3,4,5,6: One drop of either rhNGF or vehicle six times a day (every 2h) instilled into study eye (210 µL: 4.20 µg of rhNGF) and one drop of vehicle instilled into the fellow (non-study) eye**

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 (pre-dose) | 24 | | X | X | X | | | X | X | | | X | | X |
| | 0 (dose) | 24 | X | | | | | | | | | | | | |
| | 0.5 | 24.5 | | X | X | X | | | | | | | | | |
| | 2 | 26 | X | X | | | | | | | | | | | |
| | 4 | 28 | X | X | X | X | | | | | | | | | |
| | 6 | 30 | X | X | | | | | | | | | | | |
| | 8 | 32 | X | X | X | X | | | X | X | | | X | | X |
| | 10 | 34 | X | X | X | X | | | | | | | | | |
| | 10.5 | 34.5 | | X | X | X | | | | | | | | | |
| | 11 | 35 | | X | | | | | | | | | | | |
| | 12 | 36 | | X | X | X | | | | | | | | | |
| | 13 | 37 | | X | | | | | | | | | | | |
| | 14 | 38 | | X | | | | | | | | | | | |
| | 16 | 40 | | X | | | | | | | | | | | |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 (pre-dose) | 48 | | X | | X | | | X | X | | | X | | X |
| | 0 (dosing) | 48 | X | | | | | | | | | | | | |
| | 2 | 50 | X | X | | | | | | | | | | | |
| | 4 | 52 | X | X | | | | | | | | | | | |
| | 6 | 54 | X | X | | | | | | | | | | | |
| | 8 | 56 | X | X | | | | | | | | | | | |
| | 10 | 58 | X | X | | | | | | | | | | | |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 (pre-dose) | 72 | | X | | X | | | | | | | | | |
| | 0 (dosing) | 72 | X | | | | | | | | | | | | |
| | 2 | 74 | X | | | | | | | | | | | | |
| | 4 | 76 | X | | X | X | | | | | | | | | |
| | 6 | 78 | X | | | | | | | | | | | | |
| | 8 | 80 | X | | X | X | | | | | | | | | |
| | 10 | 82 | X | | | | | | | | | | | | |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 (pre-dose) | 96 | | X | | X | | | | | | | | | |
| | 0 (dosing) | 96 | X | | | | | | | | | | | | |
| | 2 | 98 | X | | | | | | | | | | | | |
| | 4 | 100 | X | | | | | | | | | | | | |
| | 6 | 102 | X | | | | | | | | | | | | |
| | 8 | 104 | X | | | | | | | | | | | | |
| | 10 | 106 | X | | | | | | | | | | | | |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 (pre-dose) | 120 | | X | | X | | | X | X | | | X | | X |
| | 0 (dosing) | 120 | X | | | | | | | | | | | | |
| | 2 | 122 | X | | | | | | | | | | | | |
| | 4 | 124 | X | X | X | X | | | | | | | | | |
| | 6 | 126 | X | | | | | | | | | | | | |
| | 8 | 128 | X | X | X | X | | | X | X | | | X | | X |
| | 10 | 130 | X | X | X | X | | | | | | | | | |
| | 10.5 | 130.5 | | X | X | X | | | | | | | | | |
| | 11 | 131 | | X | | | | | | | | | | | |
| | 12 | 132 | | X | X | X | | | | | | | | | |
| | 13 | 133 | | X | | | | | | | | | | | |
| | 14 | 134 | | X | | | | | | | | | | | |
| | 16 | 136 | | X | | | | | | | | | | | |

**FOLLOW-UP**

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 (FU1) | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 | 144 | | X | | X | X | | X | X | | | X | | X |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 (FU2) | Adverse Events/ Concomitant medication, Physical Exam, , | | | | | | | | | | | | | | |
| | 0 | 168 | | X | | X | | | | | | | | | |
| | 8 | 176 | | X | | | Y | | X | X | X | X | X | | X |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 +-2 (FU3) | Adverse Events/ Concomitant medication | | | | | | | | | | | | | | |
| | 0 | 360 | | X | | X | Y | | X | X | X | X | X | | X |

| Day | Hours (Day / Total) | | Drug/ Placebo | PK | Vital Signs | ECG | LAB | ATA | LOT | SLE/CFS /TFBUT | IOP | FO | BCDVA | ST | EOE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 to 42 (FU4) | Adverse Events | | | | | | | | | | | | | | |
| | 0 | 816-1056 | | | | | | X | | | | | | | |

ATA – Anti-therapeutic antibodies
BCVDA - Best corrected distance visual acuity (ETDRS)
Drugs Abuse/pregnancy - Drugs of Abuse including Alcohol breath test, pregnancy test for females
ECG – Electrocardiogram
EOE - External ocular examination (motility and eyelids)
FO –Fundus Ophthalmoscopic (FO) examination (dilated FO only at Screening Visit)
IOP – Introcular Pressure
LAB - Laboratory Safety Tests - Hematology, Biochemistry, Urinalysis
LOT - Local Ocular Tolerability - Visual Analogue Scale (VAS): foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision, photophobia. If it is to be applied at a same visit as an FO or IOP examination the VAS for ocular tolerability is to be applied before the SLE FO and/or IOP examination
Physical exam – Physical examination, height (height is only required at screening)
PK – Pharmacokinetics
Serology: Hepatitis/HIV and for postmenopausal females FSH Laboratory Safety Tests
SLE/CS/TFBUT - Slit lamp examination (SLE): eyelid margin, conjunctiva, cornea, anterior chamber, iris and lens with the instillation of fluorescein to evaluate corneal fluorescein staining (CS, modified Oxford scale) and tear film break-up time (TFBUT)
ST - Schirmer's test (ST) without anesthesia
Vital Signs – body weight, blood pressure, pulse rate, respiratory rate and ear body temperature
Examinations marked with Y instead of X may be done at any time on the examination day