Dompé farmaceutici S.p.A.

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

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Statistical Analysis Plan

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1 SOPs to be followed

The statistical analysis will be carried out according to the following CROMSOURCE standard operation procedures (SOPs):

SOP number	SOP title
SOP-ST-03	Statistical Analysis Plan
SOP-ST-04	SAS Programming and Validation
SOP-ST-05	Data Review Meeting
SOP-ST-06	Study Unblinding for Analysis
SOP-ST-08	Trial Statistics File

2 Abbreviations

Acronym	Definition
ADAM	Analysis Data Model
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATA	Anti-therapeutic antibody
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
BDRM	Blind Data Review Meeting
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CDISC	Clinical Data Interchange Standards Consortium
CFS	Corneal Fluorescein Staining
C_{last}	Last measurable serum concentration
C_{max}	Maximum observed serum concentration
CSR	Clinical Study Report
C_{trough}	Minimum observed serum concentration at the end of the dosage interval
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
EOE	External ocular examination
ETDRS	Early Treatment Diabetic Retinopathy Study
ETV	Early Termination Visit
FO	Fundus Ophthalmoscopic examination
FSH	Follicle-Stimulating Hormone
FU	Follow-Up
FUAE	Follow-Up Adverse Event

Acronym	Definition
GGT	Gamma-Glutamyl-Transferase
HBsAg	Hepatitis B antigen
HCV	Hepatitis C antibodies
HEENT	Head Eyes Ears Nose Throat
HIV	Human Immunodeficiency Virus
IOP	Intraocular Pressure
LOT	Local Ocular Tolerability
MedDRA	Medical Dictionary for Regulatory Activities
MOS	Modified Oxford Scale
NEI	National Eye Institute
PK	Pharmacokinetics
PKS	PK-Set
PR	The interval from the beginning of the P wave to the beginning of the QRS complex on an electrocardiogram
PT	Preferred Term
QRS	The combination of three of the graphical deflections seen on a typical ECG
QT	Measure of the time between the start of the Q wave and the end of the T wave
QTc	QT interval corrected
QTcB	QT interval corrected for heart rate
QTcF	QT interval corrected for cube-root of the difference between QRS complexes
RAND	Randomized Subjects Set
RBC	Red Blood Cell
rhNGF	Recombinant Human Nerve Growth Factor
SAE	Serious Adverse Event
SAF	Safety Set
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SI	International System of Units
SLE	Slit Lamp Examination
SOC	System Organ Class

Acronym	Definition
SOP	Standard Operation Procedure
ST	Schirmer's Tear Test
TEAE	Treatment Emergent Adverse Event
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
TLF	Tables, Listings and Figures
t_{max}	Time to reach Cmax
TOT	Total Set
VAS	Visual Analogue Scale
WBC	White Blood Cell
WHO-ATC	World Health Organization Anatomical Therapeutic Chemical
WHO-DRL	WHO-drug reference list
λz	Terminal disposition rate constant/terminal rate constant

3 Study Protocol

This NGF0117 study is conducted under the sponsorship of Dompé farmaceutici S.p.A. The clinical monitoring, data management, statistical analysis and medical writing are performed by CROMSOURCE under contract and in collaboration with Dompé farmaceutici S.p.A.

This Statistical Analysis Plan (SAP) provides a complete, expanded and detailed description of the planned statistical methods outlined in the study protocol Final Version 1.0 of 26 January 2018 and clarified in the Protocol Clarifications of 21 May 2018. Text that has been copied from the protocol is formatted in italics to indicate that it is identical to the protocol, which will ease the review and will avoid unnecessary alterations to text approved in the protocol.

It lists all Tables, Listings and Figures (TLFs), which will be produced by CROMSOURCE for the final analyses and which are used for inclusion in the Clinical Study Report (CSR).

3.1 Study 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.

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 evaluated by determination of the anti-therapeutic antibodies.

3.2 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.*

Treatment duration is 6 days. The following study treatment will be applied to the study eye:

- Day 1: One drop study medication (35 µL).
- Day 2, 3, 4, 5, 6: One drop of study medication six times a day (every 2h).

Total dose in the study eye will be 31 drops of study medication over 6 days.

For the fellow eye, vehicle will be administered in parallel to all subjects following the identical schedule as for the study eye.

The study will consist of a screening period of up to 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 (to 42).

3.3 Study Schedule

The protocol visit plan and specification of study procedures and examinations per visit is summarized in Table 1 below.

Table 1: Schedule of Assessments

Visit Day (D)	Drug/ Placebo	AE/ Conmed	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 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 and one drop of vehicle six times a day (every 2 h) 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 - 42 (FU 4)		X					X							

ATA – Anti-therapeutic antibodies. BCDVA - Best corrected distance visual acuity (ETDRS). Drugs Abuse/Alcohol breath test/pregnancy. 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). LOT always to be applied before the SLE FO and/or IOP (if applicable). Physical exam. 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 and tear film break-up time (TFBUT). ST - Schirmer's test (ST) without anesthesia. Vital Signs - blood pressure, pulse rate, respiratory rate and oral body temperature. A detailed view of the evaluations is provided in protocol Appendix 3 – Detailed List of Procedures

3.4 Primary and Secondary 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 parameters systolic and diastolic blood pressure, pulse rate, respiratory rate and* oral *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 haematology parameters over all visits.*
  Baseline is defined as the Laboratory assessment of Baseline (D-1) Visit

- *Shift in overall interpretation 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.*

Pharmacokinetics endpoints

The kinetics of single dose administration as well as multiple dose assessment will be evaluated. Two validated ELISA tests will be used for PK measurements.

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}/λ_z*

Immunogenicity

Anti-Therapeutic Antibodies (ATA) serum levels will evaluated for immunogenicity. *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.*

A more detailed description on all study endpoints on derivation of study endpoints is provided in section 7.2 of the SAP.

3.5 Interim Analyses

No interim analysis is planned.

3.6 Changes in the Conduct of Study or Planned Analysis compared to Protocol

The following changes of the study conduct did affect the planned analysis:

- Study protocol section 8.2 describes the assessment of “daily consumption of caffeine”. This was not collected on the eCRF and is therefore not presented in the analysis.
- Study protocol section 8.2 describes the assessment of “daily consumption of alcohol”. However, assessment of usual alcohol consumption was change to weekly assessment to facilitate verification of exclusion criterion 7.
- Study protocol section 7 describes the assessment of body weight for all scheduled assessment times of vital sign. However, body weight was assessed on the eCRF only at Screening Visit. As a consequence, no presentation of post-baseline body weight will be provided.
- No clinical interpretation are recorded for urinalysis including the endpoint “overall interpretation of urinalysis results”. As a consequence, this endpoint will not be analyzed.
- In the study protocol section 8.2, assessment of the horizontal diameter of the cornea is specified as part of the slit lamp evaluation. However, this was not collected in the eCRF and is therefore not part of the analysis.
- For Fundus Ophthalmology, the site did perform additional unscheduled assessments in an not-dilated manner at D1 8hr and D2 pre-dose. These assessments were done without any specific safety reason, so results were not entered in the eCRF as unscheduled assessments and are therefore not included in the data listings.

The following refinements of the pre-planned analysis in the study protocol were implemented in this SAP:

- For a better perceivability, the primary safety endpoints are rephrased to Incidence of Treatment Emergent Adverse Events during Single/Multiple Dose Scheme, respectively.
- In the eCRF, no start time of Adverse Event has been recorded. During SAP creation, this was considered as required for a precise assignment of AEs to Single-Dose or Multiple Dose Scheme. and multiple dose treatment phase. As a consequence, the start times of an AE was entered in the eCRF as free-text comment associated to the AE start date field.
- Fundus Ophthalmology was performed in a dilated manner at Screening Visit, whereas a non-dilated Fundus Ophthalmological assessment was done for all other study visits. As a consequence, data listings will clearly indicate the mode of fundus ophthalmological assessments.
- Study protocol section 8.6 describes the ECG assessments heart rate, PR interval, QRS duration, QT interval as well as QTc (Fridericia and Bazett corrections), whereas in section 10.1 ventricular rate, PR interval, QRS duration and QT interval were specified as secondary ECG endpoints. For the secondary endpoint analysis, all recorderd parameters mentioned in section 8.6 were considered as secondary safety endpoints.
- For Corneal Staining, the study protocol states in headers that a modified Oxford Scale is to be assessed. However, the details of assessment in section 8.12 and the study endpoints as described in protocol section 10.1 describes the NEI grading for corneal and conjunctival staining, which has been applied to the site and were analyzed accordingly. To accommodate for regulatory purposes, a post-hoc assessment of modified Oxford Scale (MOS) is performed by the investigator based on documented NEI source data before the BDRM.

  The MOS assessments will be used for a verification of Exclusion Criterion 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*" during the BDRM.

  For the analysis, NEI scales will be analysed as planned in the study protocol. The collected MOS values will be mapped into SDTM version only.

4 General Definitions

4.1 Report Language

The TLFs as output of the analyses will be prepared in English.

4.2 Analysis Software

The statistical analysis will be performed using the SAS® statistical software package (Statistical Analysis System, Version 9.3 or later).

Pharmacokinetic derivations will be done using Phoenix WinNonlin 6.4 or later (Pharsight Corporation, Palo Alto, CA, USA) by a subcontractor

5 Data Preparation

5.1 Data Handling and Medical Coding

For data quality control and medical coding, please refer to the Data Management Plan, including the Data Validation Plan in its most recent version.

5.2 CDISC

All output as defined in the SAP will be generated based on CDISC ADaM datasets following ADaM implementation Guide 1.0, as per contract with Dompé farmaceutici S.p.A. SDTM programming will follow SDTM version 1.3 together with SDTM implementation guide 3.1.3.

Specifications for the ADaM datasets (as well as SDTM datasets) are described in a separate document.

5.3 SAS-Programming Quality Level

The following quality level of programming deliverables will be applied, as per contract with Dompé Farmaceutici S.p.A. All statistical output will receive a tailored Quality Control approach by:

- Full independently double programmed reproduction of CDISC:
  - SDTM datasets
  - ADaM datasets
  - Tables and Figures
- Listings will not be double programmed.
- All tables, figures and listings will undergo comparison with specification (i.e. SAP and templates), cross checking with other tables and listings, a sensibility review and SAS-log review.

6 Analysis Sets and Subgroups

6.1 Analysis Sets

The Total Set (TOT) will include all subjects that have signed informed consent.

The Randomized Subjects Set (RAND) will include all subjects, which have been randomized to a study treatment.

If a subject is re-screened, the subject is formally dropped out of the study as screening failure and will be re-included with a new screening number. Details on re-screened subjects will be documented during the BDRM based on a detailed file note provided by clinical operations.

The Safety Set (SAF) will consist of all subjects randomized into the study and receiving a dose of study medication in at least one eye.

The PK-Set (PKS) will consist of all subjects who did not show serious protocol deviations or non-compliance and 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 (BDRM).

For the analysis of the SAF and PKS, summaries by treatment group (and eye) will follow the treatment a subject received.

Further analysis sets might be implemented during the BDRM, if deemed necessary as a consequence of treatment related protocol deviations.

6.2 Subgroup Definitions

No pre-planned subgroups.

7 Definition of Time Points and Analysis Variables

7.1 Definition of Time Points

The study consists of 12 scheduled clinical evaluation visits as described in Table 2.

The ***Date of First Study Treatment*** will be identified as the (earliest) date documented on the eCRF page "Administration of Study Drug" at D1 Visit, irrespective of the applied eye.

The ***Treatment Day*** of an event/assessment will be calculated relative to the First Study Treatment.

The Treatment Day of events/assessments occurring before the First Study Treatment will be calculated as:

- Treatment Day = (Date of assessment/event - Date of First Study Treatment).

For events/assessments occurring on or after First Study Treatment Administration, Treatment Day will be calculated as:

- Treatment Day = (Date of assessment/event - Date of First Study Treatment) + 1.

Relative Assessment Time of assessments is derived as the difference of the 24hr clock time on a respective day minus the time of the First Study Treatment at D1 Visit in hours utilizing 1 decimal. For example, if the time of First Study Treatment at D1 Visit was on 8:00 hr and the time of Baseline 0.5 hour was on 8:30 hr, then the Relative Assessment time will be 0.5 hours. So, the Relative Assessment Time is presenting the relative time within a visit day.

Relative Treatment Time Single Dose Scheme (hr) will be derived for all PK assessments and is defined as difference of the Date/Time of the assessment minus the time of First Study Treatment at D1 Visit in hours utilizing 1 decimal. For example, if the time of First Study Treatment at D1 Visit was on 8:00 hr and the time of D2 Pre-Dose was on 07:00 hr on the next day, then the Relative Treatment Time Single Dose Scheme will be 23.0 hours. So, the Relative Treatment time Single Dose Scheme presents the Time to First Study Treatment.

Relative Treatment Time Multiple Dose Scheme (hr) will be derived for all assessments on or after D2 and is defined as difference of the Date/Time of the assessment minus the time of First Study Treatment at D2 Visit in hours utilizing 1 decimal. For example, if the time of First Study Treatment at D2 Visit was on 8:00 hr and the time of D3 Pre-Dose was on 07:00 hr on the next day, then the Relative Treatment Time Single Dose Scheme will be 23.0 hours. So, the Relative Treatment time Multiple Dose Scheme presents the Time to First Study Treatment of the Multiple Dose Scheme.

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.

All assessments except event type data will be analyzed according to the nominal visit identifier on the eCRF page, irrespective of meeting the time window as specified in the study plan and of having the procedures performed over 1 or 2 consecutive days.

This rule does not hold for subjects, who terminate study prematurely. For these subjects, the records collected at the Early Termination Visit will be shifted to the next applicable visit assessment according to Table 2. If this is not possible, the last record will be treated as Unscheduled Assessments based on the rules below.

Unscheduled Assessments will not be considered for by-visit analyses but all collected data will be listed. Additional assessments at a given visit will be labelled under the respective Visit label. However, the respective timepoint will be labelled as "xx hr U1", "xx hr U2", etc. with xx indicating the number of the last scheduled assessment time before the unscheduled assessment plus a consecutive sequence number.

Unscheduled Assessments which were not taken on a scheduled visit will be labelled as "Unscheduled Visit x.01 U1", "Unscheduled Visit x.02 U1", "Unscheduled Visit y.01 U1" etc. indicating the number of the last preceding visit plus a consecutive sequence number. If more than one unscheduled assessment is done at the same day, the assessments will be labeled consecutively "Unscheduled Visit x.01 U1", "Unscheduled Visit x.01 U2".

The ***Treatment Period*** starts with the first intake of study medication and ends before the first assessment of the D7 visit. Any assessment before the first intake of study medication is allocated to the ***Screening Period***. The ***Follow-Up Period*** of the study will start with the first assessment on the D7 visit.

Moreover, the Treatment Period is divided into ***Treatment Period of the Single Dose Scheme***, which ends before the first intake of study medication on D2 and the ***Treatment Period of the Multiple Dose Scheme***, which starts with the first intake of study medication on D2.

If not stated otherwise in subsequent sections, Baseline is defined as the first assessment of the D-1 Visit.

Change from Baseline is defined as the difference between an assessment and the respective first assessment at the Baseline Visit.

Change from Multiple Dose Baseline is defined as the difference between an assessment and the respective Pre-Dose assessment at the D2 Visit.

A ***Time-Matched Change from Baseline*** is defined as the difference between an assessment and the respective assessment at the corresponding time-point at the Baseline Visit.

Table 2: Study Visits

Scheduled Visit	Scheduled Visit Label	Scheduled Relative Assessment Times within Visit	Comments
Screening Visit	Screening	Before Baseline Visit	Acceptable time window for screening visit: Treatment Day -21 to Treatment Day -2
Baseline Visit	D-1	0 hr 0.5 hr 2 hr 4 hr 8 hr 9 hr 10 hr 11 hr 12 hr 14 hr 16 hr	Acceptable time window: Day before first dose
Treatment Day 1	D1	Pre-Dose 0 hr 0.5 hr 2 hr 4 hr 8 hr 9 hr 10 hr 11 hr 12 hr 14 hr 16 hr	Dosing should occur at 0 hr Time of dosing should be identical to respective time point at baseline visit.
Treatment Day 2	D2	Pre-Dose 0 hr 0.5 hr 2 hr 4 hr 6 hr 8 hr 10 hr 10.5 hr 11 hr 12 hr 13 hr 14 hr 16 hr	Dosing should occur at 0, 2, 4, 6, 8, 10 hr. Time of dosing should be identical to respective time points at baseline visit.

Scheduled Visit	Scheduled Visit Label	Scheduled Relative Assessment Times within Visit	Comments
Treatment Day 3	D3	Pre-Dose 0 hr 2 hr 4 hr 6 hr 8 hr 10 hr	Dosing should occur at 0, 2, 4, 6, 8, 10 hr. Time of dosing should be identical to respective time points at baseline visit.
Treatment Day 4	D4	Pre-Dose 0 hr 2 hr 4 hr 6 hr 8 hr 10 hr	Dosing should occur at 0, 2, 4, 6, 8, 10 hr. Time of dosing should be identical to respective time points at baseline visit.
Treatment Day 5	D5	Pre-Dose 0 hr 2 hr 4 hr 6 hr 8 hr 10 hr	Dosing should occur at 0, 2, 4, 6, 8, 10 hr. Time of dosing should be identical to respective time points at baseline visit.
Treatment Day 6	D6	Pre-Dose 0 hr 2 hr 4 hr 6 hr 8 hr 10 hr 10.5 hr 11 hr 12 hr 13 hr 14 hr 16 hr	Dosing should occur at 0, 2, 4, 6, 8, 10 hr. Time of dosing should be identical to respective time points at baseline visit.
Follow-up Visit 1	FU1	0 hr	Acceptable time window: treatment day 7 Assessment times should be identical to respective time points at baseline visit.
Follow-up Visit 2	FU2	0 hr 8 hr	Acceptable time window: treatment day 8 Assessment times should be identical to respective time points at baseline visit.

Scheduled Visit	Scheduled Visit Label	Scheduled Relative Assessment Times within Visit	Comments
Follow-up Visit 3	FU3	0 hr	Acceptable time window: treatment day 14-18 Assessment times should follow respective time point at baseline visit.
Follow-up Visit 4	FU4	0 hr	Acceptable time window: treatment day 35-42 Assessment times should follow respective time point at baseline visit.
Early Termination	ETV	-	Early termination visit.

7.2 Analysis Variables

This section describes all variables that are used for analysis as well as their source data variables. For each variable it is specified how missing values will be handled, if applicable.

7.2.1 Disposition and Protocol Deviations

Disposition parameters were recorded on the eCRF Page “End of Study”.

Study discontinuations were recorded for all subjects who provided informed consent.

A subject discontinued the study prematurely, if the question “Did the subject complete the study” was answered as ‘No’. A subject is considered as study completer, if the question “Did the subject complete the study” was answered as ‘Yes’.

Primary Reason for Study Discontinuation is documented on the eCRF Page “End of Study” in categories ‘Adverse Event’, ‘Lost to Follow-Up’, ‘Decision unrelated to an Adverse Event’, ‘NonCompliance’, ‘Study terminated by the sponsor’, ‘Study terminated by the investigator’, ‘Other’.

If the eCRF question “Was the emergency envelope opened during the study?” was answered ‘Yes’, the subject will be considered as unmasked.

The eligibility of all subjects for entry into the study will be assessed at the Screening visit and confirmed at the Baseline Visit on the eCRF Pages “Inclusion Criteria” and “Exclusion Criteria”.

Protocol deviations were collected during the study and reviewed during the BDRM according to a separate BDRM plan. During the BDRM, identification of protocol deviations and further categorization of deviations for TLF output will be performed. For the analysis, all protocol deviations as documented in the BDRM minutes will be presented.

7.2.2 Demographics and Other Baseline Characteristics

Demographics

Demographic characteristics were recorded on the eCRF page “Demography”.

Gender (‘Male’, ‘Female’), Ethnicity (‘Japanese’, ‘Not Japanese’), All 4 Grandparents born in Japan (‘Yes’, ‘No’) will be analyzed as recorded on the eCRF.

Age (years) at Screening is calculated as:

- INT((date of informed consent - date of birth)/365.25).
  In case of incomplete dates, missing days will be set to 1st and missing months will be set to July.

Other Background Information

Body height and weight are recorded on the eCRF page “Vital Signs” at the Screening Visit.

Based on this, the BMI (kg/m^2) at Screening is derived as Weight at Screening (kg) / (Height at Screening [cm] * 0.01)^2. BMI will be presented with 1 decimal of precision.

Smoking habits and alcohol consumption are recorded on the eCRF page “Demography” at Screening Visit.

For the analysis, the number of alcohol units will be presented. For this, subjects who answered the question “Is the subject an alcohol consumer” with ‘No’ will be imputed by 0 alcohol units.

An alcohol breath test is performed at Screening and Baseline Visit and is recorded on the eCRF page “Alcohol Breath Test”.

An urine drug screening is performed at Screening and Baseline Visit and is recorded on the eCRF page “Drugs of Abuse”.

Medical history and other medical conditions

The study eye (‘Left Eye’, ‘Right Eye’) as randomized will be presented according to the assigned randomization number on the eCRF page “Randomization”.

Schirmer’s tear test assessing the wetting distance at 5 min (mm) is performed at Screening and Baseline Visit and is recorded on the eCRF page “Schirmer Tear Test”.

Medical history is recorded on the “Medical history” eCRF page. Medical history contains information about conditions that a subject might have suffered prior to Screening Visit, or conditions that are ongoing at the time of the Screening Visit. The medical history terms as specified by the investigator will be coded to a Preferred Term (PT) and a System Organ Class (SOC) using the Medical Dictionary for Regulatory Activities (MedDRA) thesaurus.

If no coding information will be available for a specific medical history record, the record will be presented as ‘Uncoded Record’. However, for final TLFs it is anticipated that all medical/surgical history records are coded.

For females, a serum pregnancy test was performed at Screening and a urine pregnancy test is performed at Baseline Visit. Both tests are documented on the eCRF pages “Serum/Urine Pregnancy Test”, respectively.

At Screening Visit, a test for Hepatitis B antigen (HB_sAg) and Hepatitis C antibodies (HCV) as well as a combined screening test for HIV1 and HIV2 was performed in blood serum and documented on eCRF page “Serology”.

7.2.3 Prior/Concomitant Medication

Prior and Concomitant medication data will be collected throughout the study on the “Prior / Concomitant Medications” eCRF page.

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. 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.

All medications will have a calculated start and stop day to enable categorization of the medications as 'Prior' or 'Concomitant':

- Start Day is defined as:
  - Start Date of Medication – Date of First Study Treatment + 1

    If the Start Date of the Medication is greater than or equal to the Date of First Study Treatment (see section 7.1)
  - Start Date of Medication – Date of First Study Treatment

    If the Start Date of the Medication is less than the Date of First Study Treatment.
- Stop Day is defined as:
  - Stop Date of Medication – Date of First Study Treatment + 1

    If the Stop Date of the medication is greater than or equal to the Date of First Study Treatment
  - Stop Date of Medication – Date of First Study Treatment

    If the Stop Date of the Medication is less than the Date of First Study Treatment.

'Prior medications' are medications that were started before and stopped before or on the day of First Study Treatment Administration.

'Concomitant medications' include all medications that a subject used during the Treatment or Follow-Up Period of the study.

Any medication started prior to first study treatment, which was not discontinued on/before Date of First Study Treatment, will be considered as 'Concomitant'. Moreover, any medication started at any time after the First Study Treatment will be considered 'Concomitant'.

Medications started and stopped on the day of First Study Treatment will be considered to be 'Concomitant'.

The Investigator Terms (Medication Name, and Indication) will be coded to a World Health Organization Anatomical Therapeutic Chemical (WHO-ATC) Drug class, a WHO-ATC Drug number and a WHO Drug Name (Preferred Term) using the WHO-DRL Drug Dictionary.

<u>Missing Codes or Medication Dates Imputation:</u>

In the event that coding information will not be available for a specific concomitant medication, the concomitant medications will be presented as 'Uncoded Medication'. However, for final TLFs it is anticipated that all medication records are coded.

Missing and/or incomplete dates for prior and concomitant medications will be imputed for calculating relative start and stop days only. Dates will be listed as missing/incomplete [with "-" replacing missing information] but the Start/Stop Day listed between square brackets to denote it was calculated based on missing data (i.e. [-28], [1], [Ongoing]).

Missing and/or incomplete dates will be imputed in a manner that assumes the worst case scenario.

Technically, incomplete stop dates will be imputed as follows:

- For a missing day (but month and year is available), it will be assumed that medication have been stopped on the last day of the respective month.
- For a missing month (but year is available), it will be assumed that medication have stopped on 31st December of the respective year.
- For a completely missing stop date, the medication will be assumed to be ongoing.

Similarly, incomplete start dates will be imputed as follows:

- For a missing start day (but month and year is available), onset is assumed on the first day of the respective month.
- For a missing start month (but year is available), onset is assumed on 1st January of the respective year.
- For a completely missing start date, no imputation is performed. However, the medication will be considered as concomitant, unless indicated different by stop date.

7.2.4 Measurements of Exposure and Treatment Compliance

Exposure

Exposure will be presented by displaying Individual Study Duration, Number of Drops Single Dose Scheme in Study Eye/Non-Study Eye, Number of Drops Multiple Dose Scheme in Study Eye/Non-Study Eye, which will be derived as follows

- Individual Study Duration:
  Date of Study Discontinuation/Completion (as recorded on eCRF Page “End of Study”) – Date of Informed Consent (as recorded on eCRF Page “Informed Consent”) + 1.
- Number of Drops Single Dose Scheme in Study Eye:
  Number of records on eCRF Page “Administration of Study Drug” at D1 Visit, which are indicating an administration of study medication in the study eye according to the assigned randomization number documented on the eCRF Page “Randomization”; 0 if no administration is indicated (i.e. no data record found or box ‘Not Done’ is ticked). For the single dose scheme only 1 drop should be applied according to the protocol.

- Number of Drops Single Dose Scheme in Non-Study Eye:
  Number of records on eCRF Page “Administration of Study Drug” at D1 Visit, which are indicating an administration of study medication in the non-study eye according to the assigned randomization number documented on the eCRF Page “Randomization”; 0 if no administration is indicated (i.e. no data record found or box ‘Not Done’ is ticked). For the single dose scheme 1 drop should be given according to the protocol.

- Number of Drops Multiple Dose Scheme in Study Eye:
  Number of records on all eCRF pages “Administration of Study Drug” at D2, D3, D4, D5, D6 visits, which are indicating an administration of study medication in the study eye according to the assigned randomization number documented on the eCRF Page “Randomization”; 0 if no administration is indicated at all visits (i.e. no data record found or box ‘Not Done’ is ticked). For the multiple dose scheme, 30 drops should be given according to the protocol.

- Number of Drops Multiple Dose Scheme in Non-Study Eye:

Number of records on all eCRF pages "Administration of Study Drug" at D2, D3, D4, D5, D6 visits, which are indicating an administration of study medication in the non-study eye according to the assigned randomization number documented on the eCRF Page "Randomization"; 0 if no administration is indicated at all visits (i.e. no data record found or box 'Not Done' is ticked). For the multiple dose scheme, 30 drops should be given according to the protocol.

Compliance

For the evaluation of compliance, the following derivations will be created:

- Compliance Single Dose Scheme in Study Eye (%):
  is defined as 100* Number of Drops Single Dose Scheme in Study Eye.
- Compliance Single Dose Scheme in Non-Study Eye (%):
  is defined as 100* Number of Drops Single Dose Scheme in Non-Study Eye.
- Compliance Multiple Dose Scheme in Study Eye (%):
  is defined as 100* Number of Drops Multiple Dose Scheme in Study Eye/ 30.
- Compliance Multiple Dose Scheme in Non-Study Eye (%):
  is defined as 100* Number of Drops Multiple Dose Scheme in Non-Study Eye/ 30.

For the analysis, the proportion of 100% compliant subjects will be presented per aspect based on these derivations.

7.2.5 Study Medication

Table 3: Study Treatments shows the study treatments and how they will be labelled in all TLF outputs.

Table 3: Study Treatments

Treatment arm label	*Associated Treatment*
rhNGF	*rhNGF 20 µg/m:single drop on D1, one drop six times a day on D2, D3, D4, D5, D6*
Vehicle	*Vehicle control: single drop on D1, one drop six times a day on D2, D3, D4, D5, D6*

7.2.6 Primary Safety Endpoints

For this study, the incidence of the following events will serve as primary safety endpoints:

- Treatment emergent adverse events (TEAEs) as defined in section 7.2.8.
- TEAEs during single dose scheme (TEAEs Single Dose):
  these comprise all TEAEs, which start after first dose of study treatment and before the date/time of administration of the first dose at Treatment Day 2. In case of missing time, a TEAEs with a start date of the day of D1 Visit will be considered as TEAEs Single Dose.
- TEAEs during multiple dose scheme (TEAEs Multiple Dose):
  these comprise all TEAEs during the treatment period, which start after the date/time of first dose of study treatment at D2 Visit. In case of missing time, a TEAEs with a start date on the day of D2 up to D6 Visit will be considered as TEAEs Multiple Dose.

7.2.7 Primary and Secondary Tolerability Endpoints

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.*

The VAS Ocular tolerability scores will be collected on the eCRF page "Visual Analogue Scale Ocular Tolerability" per eye for the symptoms:

- Foreign body sensation
- Burning/stinging
- Itching
- Ocular pain
- Sticky feeling
- Blurred vision
- Photophobia

The assessments should be performed at Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, Fu3 0hr.

The Overall VAS Ocular Tolerability Score acts as a global discomfort score and is defined as the mean of all VAS Ocular Tolerability symptom scores per visit per eye. In case of missing symptom scores, the mean will be calculated on the remaining VAS scores if at least 5 out of 7 scores are non-missing.

The primary tolerability endpoints for the assessment of the single dose scheme are defined as Change from Baseline of the D2 Pre-Dose value in the study eye. Baseline is defined as the corresponding assessment at Baseline Visit 0hr. This will be derived for the Overall VAS Ocular Tolerability Score as well as for each single symptom scale.

The primary tolerability endpoints for the assessment of the multiple dose scheme are defined as Change from Multiple Dose Baseline, i.e. the difference between the considered VAS score in the study eye at D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, Fu3 0hr and the D2 Pre-Dose value in the study eye. This will be derived for the Overall VAS Ocular Tolerability Score as well as for each single symptom scale.

Secondary tolerability endpoints are:

- the raw VAS ocular tolerability scores in Study Eye and Non-Study Eye at all scheduled visits.
- the intra-individual differences in VAS ocular tolerability scores between Study Eye and Non-Study Eye at all study visits.

7.2.8 Secondary Safety Endpoints – Adverse Events

General Definitions

AE data are collected on the "Adverse Events" eCRF page. The AE Description (Investigator term) will be analyzed on PT and SOC level using MedDRA.

Based on the information provided on the "Adverse Events" eCRF page, the following definitions will be utilized:

- An ocular event will be identified, if the question “Is this an ocular event?” is answered ‘Yes’.
- An AE with missing severity will be counted as severe.
- A Serious Adverse Event (SAE) is any adverse event where the question “Is the event serious?” has been answered as ‘Yes’. If this question is not answered the event will be considered as ‘Serious’ for analysis purposes.
- *For this study medically important events comprise the following sight threatening events, which are considered to be of special interest* (AESI) *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.*

  For analysis purposes, AESI will be identified as all ocular event, with a ticked box ‘Medically significant or important medical condition’ in the eCRF section “SAE criteria”.

  This definition will be verified during the BDRM.
- An AE leading to premature withdrawal of the study treatment is defined as an AE where in the eCRF section “Action taken with study treatment” the boxes ‘Withdrawn’ and ‘Permanently’ are ticked.
- An AE leading to study treatment interruption is defined as an AE where in the eCRF section “Action taken with study treatment” the boxes ‘Withdrawn’ and ‘Temporarily’ are ticked.
- An AE leading to study discontinuation is defined as an AE where in the eCRF section “Action regarding Subject” the box ‘Subject Withdrawn’ is ticked.
- An AE is classified as ‘Related’ to Study Treatment if the relationship to study medication recorded as ‘Possible’ or ‘Probable’ or ‘Highly Probable’. An AE will be classified as unrelated to study medication if the relationship to study medication was recorded as ‘None’ or ‘Unlikely’. AEs with missing relationship to study treatment will be counted as ‘Related’ to Study Treatment.
- A Fatal AE is defined as an AE where the outcome is recorded as ‘Fatal’.

These definitions will apply accordingly also for the subsequent sub-classes of AEs.

Non Treatment-emergent adverse events (Non-TEAEs)

A Non-TEAE is defined as any AE, which started before the first administration of study treatment. An AE is considered as Non-TEAE, if the eCRF question “Did the event occur:” was answered with ‘Before Treatment Period’.

Treatment-emergent adverse events (TEAEs)

A treatment-emergent adverse event is defined as an AE that started on or after the date of the First Study Treatment Administration. An AE is considered as TEAE, if the eCRF question "Did the event occurred:" was answered either with 'During Treatment Period' or 'During Follow-Up Period'.

TEAEs in Treatment Period

This is defined as a TEAE (as defined above) that started on or after the date of the First Study Treatment but on or before the D6 Visit. A TEAE in Treatment Period will be identified, if the eCRF question "Did the event occurred:" was answered with 'During Treatment Period'.

TEAEs in Follow-Up Period (FUAE)

This is defined as a TEAE with a start date on or after D7 Visit. A TEAE will be considered as TEAE in Follow-Up Period, if eCRF question "Did the event occur:" was answered with 'During Follow-Up Period'.

For listing purposes, treatment day of onset of AE will be derived according to the definition given in section 7.1.

Missing Codes or Incomplete AE start dates:

In the event that coding information will not be available for a specific AE, the event will be presented as 'Uncoded Event'. However, for final TLFs it is anticipated that all events are coded.

If the eCRF question "Did the event occur:" was not answered, AEs will be classified to sub-class Non-TEAE, TEAE in Treatment Period or In Follow-Up Period based on the AE onset date utilizing the definitions given above. If this is not possible, the event will be classified as TEAE in Treatment Period.

Missing and/or incomplete AE onset dates will be imputed for calculating relative start and stop days only. Dates will be listed as missing/incomplete [with "-" replacing missing information] but the onset Day listed between square brackets to denote it was calculated based on missing data (i.e. [1]).

Missing and/or incomplete dates will be imputed in a manner that assumes the worst case scenario:

- For a missing start day (but month and year is available), onset is assumed on the first day of the respective month.
- For a missing start month (but year is available), onset is assumed on 1st January of the respective year.
- For a completely missing start date, no imputation will be performed.

If for Non-TEAEs this procedure results in an onset date before date of informed consent, informed consent date will be used.

If for TEAEs in the Treatment Period this procedure results in an onset date before date of first study treatment, date of first study treatment will be used.

If for TEAEs in the Follow-Up Period this procedure results in an onset date before date of Week 8 visit, date of Week 8 visit will be used.

Secondary Safety Endpoints

The incidence of FUAEs as well as the Incidence of ocular TEAEs occurring in study eye and non-study eye will serve as secondary study endpoints following the definitions specified above.

7.2.9 Secondary Safety Endpoints – ECG

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

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

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

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

ECG was assessed at Screening Visit, D-1 0hr, D-1 0.5hr, D-1 2hr, D-1 4hr, D-1 8hr, D1 Pre-Dose, D1 0.5hr, D1 2hr, D1 4hr, D1 8hr, D1 14hr, D2 Pre-Dose, D2 0.5hr, D2 4hr, D2 8hr, D2 10hr, D2 10.5hr, D2 12hr, D3 Pre-Dose, D4 Pre-Dose, D4 4hr, D4 8hr, D5 Pre-Dose, D6 Pre-Dose, D6 4hr, D6 8hr, D6 10hr, D6 10.5hr, D6 12hr, FU1 0hr, FU2 0hr. FU3 0hr and is documented on eCRF Page "ECG".

For the secondary evaluations the documented parameters ECG Heart Rate (bpm), PR interval (msec), QRS duration (msec), QT interval (msec), QTcB and QTcF (msec) will be described as well as corresponding Changes from Baseline (D-1 0hr).

7.2.10 Secondary Safety Endpoints – Vital Signs

Vital Signs *evaluation included 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* oral *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 were assessed orally (normal below 37.5ºC).

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.

Vital Signs (with the exception of body weight) were assessed at Screening Visit, D-1 0hr, D-1 0.5hr, D-1 2hr, D-1 4hr, D-1 8hr, D1 0.5hr, D1 2hr, D1 4hr, D1 8hr, D1 14hr, D2 Pre-Dose, D2 0.5hr, D2 4hr, D2 8hr, D2 10hr, D2 10.5hr, D2 12hr, D4 4hr, D4 8hr, D6 4hr, D6 8hr, D6 10hr, D6 10.5hr, D6 12hr and is documented on eCRF Pages "Vital Signs". Body weight is collected on the same eCRF at Screening Visit only.

For the secondary evaluations the documented parameters systolic and diastolic blood pressure (mmHg), pulse rate (bpm), respiratory rate (breaths/min), as well as oral body temperature (°C) will be described as well as corresponding Changes from Baseline (D-1 0hr).

7.2.11 Secondary Safety Endpoints – Laboratory Safety Data

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

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

Biochemistry: *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.*

The subject need not be in fasting condition.

Laboratory results will be categorized either as 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.

The investigator may choose to repeat any abnormal result ONCE, in order to rule out laboratory error. Repeated evaluations are mandatory until their normalization or until the time course and reason of the underlying process can clearly be assessed.

Laboratory Safety Assessments are performed at Screening Visit, Baseline Visit, FU1, FU2, FU3 and are documented on eCRF Pages “Haematology”. “Chemistry” and “Urinalysis”, respectively. These assessment may be done at any time on the scheduled examination day.

For the presentation of data, the clinical evaluation as documented on the eCRF page will be utilized, i.e. no derivation of Normal/Abnormal values will be done for analysis purposes. As a consequence, a missing clinical evaluation will be presented as missing.

For this single center study, the laboratory parameters will be presented in the units, in which they were collected, i.e. no transformation into SI units will be performed.

For the secondary evaluations, all observed hematology and biochemistry parameters (except FSH-test) will be described as well as corresponding Changes from Baseline (D-1).

The documented results for FSH and urinalysis will be listed.

7.2.12 Secondary Safety Endpoints – Intraocular Pressure

Intraocular Pressure testing will be performed per eye using Goldmann Tonometer and the resulting values (mmHg) are recorded on the eCRF page “Intraocular Pressure” at Screening Visit, Baseline 0hr, FU2 8 hr, FU3 0 hr.

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

The change from baseline will be derived per eye for all scheduled post-baseline visits as differences from Baseline (D-1 0hr).

7.2.13 Secondary Safety Endpoints – Best Corrected Distance Visual Acuity (BCDVA)

Vision must be measured for each eye separately using ETDRS visual acuity chart at 4 meters (13 feet) and, if indicated, also at 1 meter (i.e. when less than 20 letters were read at 4m).

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

In case both tables on the eCRF page have been completed (at 4 meter and at 1 meter) then the visual acuity score is the sum of the total number of correct letters from both tables. If only the table at 4 meter is completed and the overall number of letters read is 20 or above then the Visual Acuity Score is the number of total letters read at 4 meter plus 30.

If no letter were read at 4 and 1 meter, the best visual potential ability of the subject is selected from: a) count fingers; b) hand motion; c) light perception; or d) no light perception. The Visual Acuity Score is then set to 0.

The Snellen Equivalent is defined as the acuity equivalent for the smallest line with 1 or no error. The logarithm of the minimal angle of resolution (logMAR) is calculated as "- log (Snellen Equivalent)" with two decimals precisions. The logMAR ranges from 0.30 (20/10) to 1.60 (20/800) and an increase in logMAR reflects a worsening of visual acuity. If for a subject only ability for counting fingers is reported, logMAR will be set to 2.00; if for a subject only recognition of hand motion is reported, logMAR will be set to 3.00 (Holladay 1997).

Visual acuity is assessed for each eye at Screening Visit, Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, FU3 0hr and are documented on the eCRF page "Best Corrected Distance Visual Acuity".

The change from baseline will be derived for Visual Acuity Score and LogMAR per eye for all scheduled post-baseline visits as differences from Baseline (D-1 0hr).

7.2.14 Secondary Safety Endpoints – Tear Film Break Up Time

TFBUT will be measured per eye *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 TFBUT, which were observed the first time during study will be reported as adverse event.

TFBUT is assessed for each eye at Screening Visit, Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, F U2 8hr, FU3 0hr and is documented on the eCRF page "Tear Film Break Up Time".

For the secondary analysis, the mean TFBUT (sec) value will be derived from the 2 or 3 documented single values per eye and will be presented with same number of digits as done for the raw values (i.e. 2 digits). The calculated average as provided in the eCRF will not be used for the analysis.

The change from baseline will be derived for mean TFBUT (sec) per eye for all scheduled post-baseline visits as differences from Baseline (D-1 0hr).

7.2.15 Secondary Safety Endpoints – Ocular Surface Staining

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

For the investigation of the conjunctiva, the nasally and temporally 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, respectively.

Briefly, on the grading scale used grade 0 reflects normal/healthy situation, whereas grade 3 reflects a severe damage in each considered sector.

Corneal Staining will be derived as sum of scores of the five corneal sectors (central, superior, inferior nasal and temporal) ranging from 0 to 15. Corneal Staining score will only be calculated, if all 5 sector scores are available.

Conjunctival Staining will be derived as sum of scores of the conjunctival area (nasal-superior paralimbal, nasal-inferior paralimbal, nasal-peripheral, temporal-superior paralimbal, temporal-inferior paralimbal, temporal-peripheral) ranging from 0 to 18. Conjunctival Staining will only be calculated, if all 6 area scores are available.

Both scores will be derived for each eye separately. No imputation will be envisaged for these parameters.

Ocular Surface Staining is assessed for each eye at Screening Visit, Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, FU3 0hr and are documented on the eCRF page “Corneal Fluorescein Staining”.

The change from baseline in corneal staining as well as conjunctival staining will be derived per eye for all scheduled post-baseline visits as differences from Baseline (D-1 0hr).

7.2.16 Other Safety Variables

External Ocular Examination (EOE)

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.*

Motility of extraocular muscle and appearance/function of eye lids is assessed separately for each eye at Screening Visit, Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, FU3 0hr and is recorded on the eCRF pages “External Ocular Examination” and “Appearance and Function of Eyelids”.

For the analysis, the motility of the extraocular muscle will be evaluated by the field of movements Right and up (‘Normal’, ‘Abnormal’), Right (‘Normal’, ‘Abnormal’), Right and Down (‘Normal’, ‘Abnormal’), Left and Up (‘Normal’, ‘Abnormal’), Left (‘Normal’, ‘Abnormal’), Left and Down (‘Normal’, ‘Abnormal’) as documented at the eCRF. No imputation will be envisaged for these parameters.

Appearance and function of eyelids will be summarized by the following parameters:

- Incidence of proven eyelid deformity/abnormality, which will be considered as present, if the question “Evidence of eyelid deformity or abnormality” on the eCRF page “Appearance and Function of Eyelids” is answered ‘Yes’.

- Incidence of abnormal motor function of eyelids (i.e. upper lid elevation and forceful lid closure), which will be considered as present, if the question “Motor function of eyelids” on the eCRF page “Appearance and Function of Eyelids” is answered ‘Abnormal’.

- Incidence of corneal exposure in case of closed eyelids, which will be considered as present, if the question “Is there corneal exposure when the eyelids are closed?” on the eCRF page “Appearance and Function of Eyelids” is answered ‘Yes’.

- Incidence of proven punctal occlusion, which will be considered as present, if the question “Is there evidence of punctal occlusion?” on the eCRF page “Appearance and Function of Eyelids” is answered ‘Yes’.

- Incidence of punctal plugs, which will be considered as present, if the box “Punctal plugs” is ticked on the eCRF page “Appearance and Function of Eyelids”.

- Incidence of thermal or surgical occlusion, which will be considered as present, if the box “Thermal or surgical occlusion” is ticked on the eCRF page “Appearance and Function of Eyelids”.

- Incidence of Other punctal occlusion, which will be considered as present, if the respective “Other” box is ticked on the eCRF page “Appearance and Function of Eyelids”.

Slit Lamp Examination

Slit lamp examination was performed separately for each eye at Screening Visit, Baseline Visit 0hr, D1 8hr, D2 Pre-Dose, D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, FU3 0hr and is recorded on the eCRF page “Slit Lamp Examination (Biomicroscopy)”.

The following assessments will be performed:

Eye Structure	Rating
Eyelid - Meibomian Glands	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)
Eye Lid – Erythema	0 = None (normal) 1 = Mild (redness localized to a small region of the lid(s) margin OR skin) 2 = Moderate (redness of most of all of lid margin OR skin) 3 = Severe (redness of most or all of lid margin AND skin) 4 = Very Severe (marked diffuse redness of both the lid margin AND skin)
Eye Lid – Edema	0 = None (normal) 1 = Mild (localized to a small region of the lid) 2 = Moderate (diffuse, most or all lid but not prominent/protruding) 3 = Severe (diffuse, most or all lid AND prominent/protruding) 4 = Very Severe (diffuse AND prominent/protruding AND reversion of fhe lid

Eye Structure	Rating
Lashes	0 = Normal 1 = Abnormal (specify)
Conjunctiva – Erythema	0 = None (normal) 1 = Mild (a flush reddish color predominantly confined to the palpebral or bulbar conjunctiva) 2 = Moderate (More prominent red color of the palpebral or bulbar conjunctiva) 3 = Severe (definite redness of palpebral or bulbar conjunctiva))
Conjunctiva – Edema	0 = None (normal) 1 = Mild (slight localized swelling) 2 = Moderate (moderate/medium localized swelling or mild diffuse swelling) 3 = Severe (severe diffuse swelling) 4 = Very severe (very prominent/protruding diffuse swelling)
Lens	0 = No Opacification (normal lens) 1 = Mild Lens Opacification 2 = Moderate Lens Opacification 3 = Severe Lens Opacification 4 = N/A Subject with Artificial Lens In addition there is also a field to specify Other findings
Iris	0 = Normal 1 = Abnormal
Anterior Chamber Inflammation	0 = None (no Tyndall Effect) 1 = Mild (Tyndall Effect barely discernable) 2 = Moderate (Tyndall beam in the anterior chamber is moderately intense) 3 = Severe (Tyndall beam in the anterior chamber is severely intense)
Other	Specification and assessment of other investigated eye structure

For each eye structure, the investigator will have the opportunity to tick also 'Not done/Not evaluable', which will be treated as 'Missing' for analysis purposes.

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

All grading assessments of different eye structures will be evaluated as collected on the eCRF. No imputation will be envisaged for these parameters.

No aggregated presentation is planned for other assessments done during Slit lamp evaluation.

Fundus Ophthalmoscopy

Fundus ophthalmoscopy to assess the eye structure will be performed and results recorded on the eCRF page "Fundus Ophthalmoscopy" by utilizing the following assessments.

Eye Structure	Rating
Vitreous	0 = Normal 1 = Abnormal (specify) 2 = Not done/Non-Evaluable
Retina / Macula	0 = Normal 1 = Abnormal (specify) 2 = Not done/Non-Evaluable
Choroid	0 = Normal 1 = Abnormal (specify) 2 = Not done/Non-Evaluable
Optic Nerve	0 = Normal 1 = Abnormal (specify) 2 = Not done/Non-Evaluable
Cup/Disc Ratio	0 = Normal 1 = Abnormal (specify) 2 = Not done/Non-Evaluable In addition, the Cup/Disc Ratio is recorded

Dilated FO will be performed at Screening Visit with non-dilated FO performed at Baseline Visit 0 hr, FU2 8hr and FU3 0hr. Clinically significant changes (or a clinically significant worsening) in FO, which were observed the first time during study will be reported as adverse event.

Physical Examination

Physical Examination includes an examination of general appearance, head-eyes-throat-nose-ears (HEENT), skin, thorax/lungs, cardiovascular system, abdomen, lymph nodes, musculoskeletal system, neurological system and other examinations as necessary.

The clinical evaluation ('Normal', 'Abnormal, Not Clinically Significant', 'Abnormal, clinically significant'). It is collected on eCRF Page "Physical Examination" at Screening Visit, Baseline Visit, FU1, FU2 and FU3.

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.

7.2.17 Pharmacokinetic Endpoints

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

Pharmacokinetics blood samples were taken on Baseline Visit 0 hr, 0.5 hr, 2 hr, 4 hr, 8 hr, 9 hr, 10 hr, 11 hr, 12 hr, 14 hr and 16 hr, for assessment of single dose scheme at D1 Pre-Dose, 0.5 hr, 2 hr, 4 hr, 8 hr, 9 hr, 10 hr, 11 hr, 12 hr, 14 hr and 16 hr, for multiple dose scheme at D2 Pre-Dose, 0.5 hr, 2 hr, 4 hr, 6hr, 8 hr, 9 hr, 10 hr, 10.5hr, 11 hr, 12 hr, 13hr, 14 hr and 16 hr, D3 Pre-Dose, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, D4 Pre-Dose, D5 Pre-Dose, D6 Pre-Dose, 4 hr, 8 hr, 10 hr, 10.5hr, 11 hr, 12 hr, 13 hr, 14 hr and 16 hr, FU1 0hr, FU2 0 hr and 8 hr, FU3 0hr.

The assessment times are documented on eCRF Pages “Pharmacokinetics – Anti-Therapeutic Antibodies”, the rhNGF serum concentration assessed via both ELISA methods will be provided as external Excel files by Dompé. To maintain masking, these files will be provided for BDRM purposes in a masked manner by utilizing a dummy subject identification. For the final analysis, the Excel Files will contain the correct subject identification number.

The concentration versus time profiles of NGF will be assessed from individual serum samples of all subjects. As a general principle, the nominal assessment times will be used.

For the rhNGF serum levels according to ELISA I and ELISA II time-matched changes from baseline will be presented for the PKS (excluding blood samples at 6 hr, 10.5hr and 13 hr).

For this purposes, values below the limit of detection will be set to a value of 1 pg/ml. However, for listings values below the limit of detection will be presented as “< 32 pg/ml’ for ELISA I levels and ‘< 15 pg/ml’ for ELISA II.

A binary variable ‘Presence of rhNGF’ will be derived for each ELISA with ’No’ indicating a value below the limit of detection and ‘Yes’ indicating a value above the limit of detection.

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 $AUC = AUC_{0\text{-}tlast} + C_{last}/\lambda_z$*

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

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.

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.

If required, more details on the PK evaluation will be provided in an appendix to the SAP, which will be finalized before unmasking of the study.

7.2.18 Immunogenicity Endpoints

ATA blood samples were taken on Baseline Visit 0 hr, and FU4 0hr.

The assessment times are documented on eCRF Pages “Pharmacokinetics – Anti-Therapeutic Antibodies”, the ATA levels will be provided as Excel files by external provider after the study has been unmasked for listing purposes.

8 Analysis Methods

8.1 General Methods

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 pharmacokinetic parameters*. For qualitative variables, absolute and relative frequencies will be reported.* Percentages will be based on the number of subjects in the respective treatment group.

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.

Ocular assessments will be generally be presented separately by Study Eye and Non-Study Eye. *All assessed data will be listed.*

8.2 Specific Methods for Primary Safety and Tolerability Analyses

No inferential analyses are foreseen for this study. However, 95% confidence intervals for the geometric mean are calculated for pharmacokinetic analysis.

8.3 Statistical/Analytical Issues

8.3.1 Adjustments for Covariates

Not applicable as no inferential analyses are foreseen.

8.3.2 Handling of Dropouts or Missing Data

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

8.3.3 Data Review

In order to perform the statistical analysis of the final analysis, a decision needs to be made on evaluability of subjects and visits prior to locking the database and breaking the code for the masked study medication. In particular, a review of protocol deviations is required to evaluate the validity of planned analysis needs to be identified and corresponding actions needs to be defined, if any. This will be done at the Blind Data Review Meeting.

Performance of the Blind Data Review Meeting will require a clean database, i.e. all data are available including masked PK data of both assays, coded and no data queries are open.

No data entry should be performed between the data base Soft Lock and database lock. Exception would be Queries, which raised during the BDRM requiring a solution before data base Lock.

The scope of the BDRM will be:

- Evaluation of Re-Screened subjects
- Check correct assignment of allocated treatment and study eye
- Check protocol deviations and define analysis sets
- Review and confirmation of AESIs (sight threatening events)

More details on scope and performance of data review meetings will be described in a separate BDRM plan.

After the BDRM was performed, all decisions were summarized in BDRM Minutes, which were approved before the database was locked for the analysis.

8.3.4 Multicenter Studies

Not applicable since this is a monocentric study.

8.3.5 Multiplicity of Endpoints

Not applicable as not inferential statistics will be performed.

9 Interim Analyses

No interim analysis is planned for this study.

10 Overview of Tables, Listings and Figures

In this section, TLFs are presented content wise. The full set of TLFs are tabulated in section 12, where it will also be indicated whether the item is unique (or first mentioned) or a repeat. Mock shells for unique TLFs are presented in a separate document.

10.1 Disposition of Subjects

Based on the Total Set, a disposition summary of subjects includes the number (n) and percentage (%) of subjects in all analysis sets defined in Section 6.1. All percentages will be based on the number of subjects randomized. For the Total Set only the number of subjects will be presented.

The primary reason for study discontinuation will be summarized for the SAF providing counts (n) and percentages (%). This summary will also include counts and percentages for unmasked subjects.

A listing will be present for the Total Set displaying the allocation to study treatment and study eye as well as the assignment of subjects to the respective analysis cohorts.

For the Total Set, a listing will be provided by summarizing study termination/completion information, date of informed consent (from eCRF page 'Informed Consent'), unmasking information and treatment day of study termination/completion.

10.2 Protocol Deviations

The number of subjects who had at least one major as well as minor protocol deviation and all categories of Protocol Deviations will be summarized by counts (n) and percentages (%) for the SAF. Percentages will be calculated based on the number of subjects in the SAF.

A listing presents all Protocol Deviation data for the SAF.

10.3 Inclusion and Exclusion Criteria

Two listings will be provided for all subjects in the SAF displaying status of violated Inclusion and Exclusion criteria, respectively.

10.4 Demographic and Other Baseline Characteristics

10.4.1 Demographic and other Background Information

Age at Screening Visit (years), Ethnicity ('Japanese', 'Non-Japanese'), BMI (kg/m²) and number of alcohol units will be summarized per treatment group and overall by summary statistics or absolute counts (n) and percentages (%) for the SAF.

All demographic data, body weight and height at Screening as well as other background information will be listed for the SAF.

10.4.2 Medical History and Other Medical Conditions

The selected study eye will be summarized per treatment group and overall by absolute counts (n) and percentages based on the SAF.

Medical History will be summarized per treatment group and overall by absolute counts (n) and percentages (%) for the number of subjects with at least one medical history event for the SAF. Percentages will be calculated based on the number of subjects in the population.

A listing will present all reported, derived and coded medical history data based on the SAF.

In addition, all data recorded and derived for Schirmer's tear test, pregnancy tests and serology assessments (HB_sAg, HCV, HIV1/2) will be listed for the SAF.

10.4.3 Prior/Concomitant Medications

Prior and Concomitant medications will be summarized by treatment group and overall for the SAF separately on a per-subject basis (i.e. if a subject reported the same medications repeatedly the medications will be counted only once at the specific level of display). Absolute counts (n) and percentages (%) will be presented for the number of subjects taking at least one medication and per WHO Drug class 2 (ATC therapeutic main class) and per WHO Drug Name (Preferred Term) within ATC Drug class 2. Percentages will be calculated based on the number of subjects in the SAF.

A Listing will present all documented medication data for the SAF and will include a flag for Prior medication.

10.5 Exposure and Compliance to Study Medication

For exposure, the variables Individual Study Duration, Number of Drops Single Dose Scheme in Study Eye/Non-Study Eye, Number of Drops Multiple Dose Scheme in Study Eye/Non-Study Eye will be summarized for the SAF per treatment group.

For compliance, the proportions of 100% compliant subjects in the SAF for the single dose as well as the multiple dose scheme will be summarized by treatment group separately for study eye and non-study Eye by absolute counts (n) and percentages (%).

A listing will present for all SAF subjects all records documented on the eCRF Page "Administration of Study Drug" as well as the corresponding derived parameters.

10.6 Analysis of Primary Safety and Tolerability Assessments

10.6.1 Primary and Secondary Safety Endpoints – Adverse Events

The incidences of the primary and secondary endpoints TEAEs, TEAEs Single Dose, TEAEs Multiple Dose, FUAEs, Ocular TEAEs in Study Eye, Ocular TEAEs in Non-Study Eye will be presented for the SAF by study treatment and overall displaying the number of events as well as the number and percentages of subjects with at least one event.

In addition a general overview will be provided for AEs, non TEAEs, TEAEs during Treatment Period, TEAEs leading to premature withdrawal of the study treatment. TEAEs leading to study discontinuation, serious TEAEs, Fatal TEAEs, treatment related TEAEs, treatment related serious TEAEs, Ocular TEAEs, TEAEs of special interest and Mild, Moderate and Severe TEAEs will be given by study treatment and overall for subjects in the SAF. For each event, the number and percentages of subjects with at least one event as well as the total number of events will be displayed.

Detailed adverse event descriptive tables on will be summarized separately for each study treatment and overall for the following events:

- TEAEs
- TEAEs Single Dose
- TEAEs Multiple Dose
- FUAEs
- Ocular TEAEs
- Ocular TEAEs in Study Eye
- Ocular TEAEs in Non-Study Eye
- TEAEs by severity.
- Treatment related TEAEs.
- Serious TEAEs (only if reported).
- Treatment related serious TEAEs (only if reported)
- TEAEs leading to premature withdrawal of the Study Treatment.
- TEAEs leading to study discontinuation.

They will be summarized on a per-subject basis (i.e. if a subject reported the same event repeatedly the event will be counted only once at the specific level of display). Absolute counts (n) and percentages (%) will be presented for the number of subjects with at least one AE, and per SOC and per PT within SOC, for the SAF. Percentages will be based on the number of subjects in the population. In addition number of events will be provided.

Descriptive tables will be ordered by descending frequency of the overall number of subjects within each SOC regardless of treatment group, and then ordered within each SOC by the overall number of subjects within each PT regardless of treatment group. In the event of equal frequencies tables will be ordered by active treatment frequency and then alphabetically.

Listings will present all Adverse Event data for the SAF by subject.

In addition specific listings will be created for:

- Ocular TEAEs
- TEAEs leading to premature withdrawal of study treatment
- TEAEs leading to study treatment interruption
- TEAEs related to study treatment
- Serious AEs (only if reported)
- Serious TEAEs related to study treatment (only if reported)
- Fatal AEs (only if reported)

10.6.2 Primary and Secondary Tolerability Endpoints

The primary endpoints Change from Baseline of VAS Tolerability Scores in Study Eye at D2 Pre-Dose Visit and Changes from Multiple Dose Baseline of VAS Tolerability Scores in Study Eye at D2 8hr, D3 Pre-Dose, D6 Pre-Dose, D6 8hr, FU1 0hr, FU2 8hr, Fu3 0hr visits will be summarized for the SAF by treatment group.

For secondary analyses, the VAS tolerability Scores will be summarized for all scheduled assessment times by treatment separately for Study Eye and Non-Study Eye as well as the intra-individual differences between the eyes in subjects included in the SAF.

All collected and derived tolerability data will be listed for the SAF.

10.7 Additional Secondary Safety Assessments

10.7.1 ECG

For secondary analyses, the raw values as well as the changes from baseline of the endpoints ECG Heart Rate, PR interval, QRS duration, QT interval, QTcB interval, QTcF interval will be summarized by treatment for the SAF.

All collected and derived ECG data including investigator's interpretation will be listed for the SAF.

10.7.2 Vital Signs

For the secondary analyses, the raw values as well as the changes from baseline of the endpoints systolic and diastolic blood pressure (mmHg), pulse rate (bpm), respiratory rate (breaths/min) and oral body temperature (°C) will be summarized by treatment for the SAF.

All collected and derived vital signs data will be listed for the SAF.

10.7.3 Laboratory Safety Data

For the secondary analyses, the raw values as well as the changes from baseline haematology and chemistry (except of FSH) will be summarized by treatment for the SAF.

All collected and derived laboratory safety data will be listed for the SAF.

10.7.4 Intraocular pressure

Intraocular Pressure is summarized for the SAF by Study Eye/Non-Study Eye and treatment group at all visits as well as changes from baseline at all post-baseline visits.

A listing will present all documented assessments of intraocular pressure as well as the respective changes from baseline for the SAF.

10.7.5 Change in BCDVA

For the SAF, the Visual Acuity Score and logMAR will be summarized per Study Eye/Non-Study Eye respectively for each treatment group at all scheduled study visits as well as changes from baseline for all Post Baseline Visits.

All raw as well as derived BCDVA data will be listed for the SAF.

10.7.6 TFBUT

For the SAF, the Average TFBUT value will be summarized per Study Eye/Non-Study Eye respectively for each treatment group at all scheduled study visits as well as changes from baseline for all Post Baseline Visits.

All documented TFBUT values including all derivations will be listed for the SAF.

10.7.7 Ocular Surface Staining

For the SAF, corneal and conjunctival staining will be summarized per Study Eye/Non-Study Eye respectively for each treatment group at all scheduled study visits as well as changes from baseline for all Post Baseline Visits.

All documented staining results including all derivations will be listed for the SAF.

10.8 Other Safety Variables

External Ocular Examination (EOE)

All evaluations of the motility of extraocular muscle Right and Up, Right, Right and Down, Left and Up, Left, Left and Down will be summarized descriptively by treatment group and study visit for the Study Eye as well as the Non-Study Eye. This analysis will be performed on the SAF. These data will also be listed for the SAF.

Incidences of proven eyelid deformity/abnormality, abnormal motor function of eyelids, corneal exposure in case of closed eyelid, proven punctal occlusion, punctal plugs, thermal or surgical occlusion or other punctal occlusion will be presented for the SAF by treatment group and study visit for the Study Eye as well as for the Non-Study.

Moreover, these data will be listed for the SAF.

Slit Lamp Evaluation

All grading assessments of different eye structure (excluding assessment for other structures) will be summarized descriptively for the SAF by treatment group and study visit for the Study Eye as well as the Non-Study Eye.

All data recorded on the eCRF page 'Slit Lamp Examination (Biomicroscopy)' will be listed for the SAF.

Fundus Ophthalmology

All data including details on detected abnormalities recorded on the eCRF will be listed for the SAF clearly indicating the mode of assessment (Dilated/Not-Dilated).

Physical Examination

All physical examination data recorded on the eCRF will be listed for the SAF.

10.9 Pharmacokinetic Evaluations

All pharmacokinetic evaluations will be presented for the PKS.
Summary descriptive statistics for both absolute and time-matched changes from baseline will be provided by treatment for rhNGF serum concentrations ELISA I as well as ELISA II at all scheduled time points including geometric mean, coefficient of variation and 95% confidence intervals for the geometric mean.

In addition, presence of rhNGF concentrations will be summarized by treatment group for ELISA I and ELISA II concentrations at each scheduled assessment time.

Serum concentration versus time profiles will be presented on linear and log/linear scale for each individual with at least one value above detection level as well as geometric mean and median values for each time-point by treatment group. For individual plots, actual sampling times will be used. For the plots of geometric mean, scheduled sampling times will be used.

All recorded PK data will be listed for the SAF, clearly indicating subjects not included in the PKS.

Further PK analysis might be performed according to an appendix of the SAP, if indicated.

10.10 Immunogenicity

All recorded ATA concentrations will be listed for the SAF, clearly indicating subjects not included in the PKS.

11 References

1	Holladay, JT (1997) Proper Method for Calculating Average Visual Acuity. Journal of Refractive Surgery, 13, pp. 388-391
2	Lemp MA. Report of the National Eye Institute/Industry Workshop on clinical trials in dry eye. CLAO J1995, 21(4):221–32.

12 Tables, Listings and Figures

In agreement with Medical Writing, numbering of the actual output will be accommodated to numbering into appendices 14 and 16.

In the column U/R it is indicated whether the item is U=Unique TLF (or first instance of a table to be repeated) or R=Repeat item.

TLF number	Title	Analysis Set	U/R
Tables			
Table 14.1-1.1	Summary of Subject Disposition	TOT	U
Table 14.1-1.2	Summary of Study Discontinuation	SAF	U
Table 14.1-2.1	Summary of Protocol Deviations	SAF	U
Table 14.1-3.1	Summary of Demographics and Other Background Information	SAF	U
Table 14.1-4.1	Summary of Selected Study Eyes	SAF	U
Table 14.1-5.1	Summary of Medical History	SAF	U
Table 14.1-6.1	Summary of Prior Medications	SAF	R
Table 14.1-6.2	Summary of Concomitant Medications	SAF	U
Table 14.1-7.1	Summary of Exposure	SAF	U
Table 14.1-7.2	Summary of Compliance	SAF	U
Table 14.2-01.1	Summary of Primary and AE Related Secondary Endpoints	SAF	U
Table 14.2-02.1	Overall Summary of Adverse Events	SAF	U
Table 14.2-03.1	Summary of TEAEs by System Organ Class and Preferred Term	SAF	U
Table 14.2-04.1	Summary of TEAEs Single Dose by System Organ Class and Preferred Term	SAF	R
Table 14.2-05.1	Summary of TEAEs Multiple Dose by System Organ Class and Preferred Term	SAF	R
Table 14.2-06.1	Summary of FUAEs by System Organ Class and Preferred Term	SAF	R
Table 14.2-07.1	Summary of Ocular TEAEs by System Organ Class and Preferred Term	SAF	R

TLF number	Title	Analysis Set	U/R
Table 14.2-08.1	Summary of Ocular TEAEs in Study Eye by System Organ Class and Preferred Term	SAF	R
Table 14.2-09.1	Summary of Ocular TEAEs in Non-Study Eye by System Organ Class and Preferred Term	SAF	R
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Table 14.4-3.2	Summary of Changes from Baseline in Haematology	SAF	U
Table 14.4-4.1	Summary of Biochemistry	SAF	U

TLF number	Title	Analysis Set	U/R
Table 14.4-4.2	Summary of Changes from Baseline in Biochemistry	SAF	U
Table 14.4-5.1	Summary of Intraocular Pressure	SAF	U
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Table 14.4-9.1	Summary of Corneal and Conjunctival Staining	SAF	U
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Table 14.5-1.1	Summary of Motility of Extraocular Muscle	SAF	U
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Table 14.6-2.2	Summary of time-matched changes from Baseline in rhNGF titres ELISA II	PKS	U
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TLF number	Title	Analysis Set	U/R
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TLF number	Title	Analysis Set	U/R
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Listing 16.2.08-5.1	Listing of Intraocular Pressure	SAF	U
Listing 16.2.08-6.1	Listing of BCDVA Assessments	SAF	U
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13 Appendices

None