CLINICAL STUDY PROTOCOL

Title: A 4-week, Phase III, multicenter, double-masked, vehicle-controlled clinical study to evaluate safety and efficacy of Oxervate® (cenegermin) 20 mcg/mL ophthalmic solution versus vehicle, in patients with severe Sjogren's dry eye disease (PROTEGO-1 study)

Study Number:	**NGF0121**
Study Name:	**PROTEGO-1**
IND:	**115892**
EUDRACT:	**2021-003346-21**
Investigational Product:	Cenegermin
Phase of the study:	III
Protocol Version - Date:	**Version No. 6.0 – 26/JAN/2022**

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List of Abbreviations and Definitions of Terms

ADR	Adverse Drug Reaction
AE	Adverse Event
BCDVA	Best Corrected Distance Visual Acuity
BID	Bis in die (twice a day)
CFR	Code of Federal Regulations
CRA	Clinical Research Associate
RC	Reading Center
CRO	Contract Research Organization
DE	Dry Eye
DED	Dry Eye Disease
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
e-CRF/CRF	Electronic/Case Report Form
EDC	Electronic Data Capture
EMA	European Medicine Agency
ETDRS	Early Treatment Diabetic Retinopathy Study
ETV	Early Termination Visit
FAS	Full Analysis Set
FDA	Food and Drug Administration
FPI	First Patient In
FPFV	First Patient First Visit
FU	Follow Up
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDEEL	Impact of Dry Eye on Everyday Life
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IUD	Intra Uterine Device
LNGFR	Low-affinity nerve growth factor receptor

LPLV	Last Patient Last Visit
MedDRA	Medical Dictionary for regulatory activities
mcg/mL	micrograms/millilitre
NEI	National Eye Institute
NIMP	Non-Investigational Medicinal Product
NGF	Nerve Growth Factor
NK	Neurotrophic Keratitis
PI	Principal Investigator
PID	Patient Identification number
PP	Per Protocol Population
PT	Preferred Term
p75NTR	p75 Neurotrophin Receptor
rhNGF	recombinant human Nerve Growth Factor
RP	Retinitis Pigmentosa
SAE	Serious Adverse Event
SAF	Safety population
SANDE	Symptom Assessment iN Dry Eye
SAP	Statistical Analysis Plan
SD	Standard Deviation
SLE	Slit-Lamp Examination
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TFBUT	Tear Film Break-Up Time
TID	Ter in die (thrice a day)
TrkA	Tropomyosin receptor kinase A
USA	United States of America
Vs	Versus
§	Section

1. STUDY SYNOPSIS

CLINICAL STUDY SYNOPSIS:	
Study Number	**NGF0121**
Title of Study	A 4-week, Phase III, multicenter, double-masked, vehicle-controlled clinical study to evaluate safety and efficacy of Oxervate® (cenegermin) 20 mcg/mL ophthalmic solution versus vehicle, in patients with severe Sjogren's dry eye disease (PROTEGO-1 study)
Study Name	PROTEGO-1
IND N°	115892
EUDRACT:	2021-003346-21
Study Centers (Country)	Approximately 10 study sites in Europe and USA
Development Phase	Phase III
Objective	The primary objective of this study is to assess the efficacy and safety of cenegermin ophthalmic solution at 20 mcg/mL concentration administered three times daily for 4 weeks in patients with severe Sjogren's dry eye disease.
Study Design and Methodology	This is a phase III, multicenter, double-masked, parallel-arm, vehicle-controlled trial.
Number of Patients	Randomization 1:1 of 90 patients to cenegermin ophthalmic solution 20 mcg/mL TID or vehicle ophthalmic solution TID for 4 weeks. Assuming a dropout rate of 10%, up to 100 patients are expected to be enrolled.
Main Inclusion criteria **1.** Male or female aged ≥ 18 years **2.** Patients with a confirmed diagnosis of Sjogren's syndrome or other autoimmune disease known to induce Sjogren's Dry Eye Disease (DED). **3.** Patients with severe Sjogren's dry eye disease characterized by the following clinical features: a. Corneal and/or conjunctival staining with fluorescein using National Eye Institute (NEI) grading system ≥3 b. SANDE questionnaire global score >25 mm c. Schirmer test I (without anaesthesia) ≥2 ≤5mm/5min **4.** The same eye (eligible eye) must fulfill all the above criteria **5.** Diagnosis of severe Sjogren's dry eye disease at least 3 months before enrollment (current use or recommended use of artificial tears for the treatment of Sjogren's related Dry Eye) **6.** Best corrected distance visual acuity (BCDVA) score of ≥ 0.1 decimal units (20/200 Snellen value) in each eye at the time of study enrolment **7.** If a female of childbearing potential, have a negative urine pregnancy test and use a highly effective method to avoid pregnancy for the duration of the trial and 30 days after the	

study treatment period. Males of reproductive potential should use effective contraception during treatment and 30 days after the study treatment period

8. Only patients who satisfy all Informed Consent requirements may be included in the study. The patient and/or his/her legal representative must read, sign and date the Informed Consent document before any study-related procedures are performed. The Informed Consent Form signed by patients and/or legal representative must have been approved by the IRB/IEC for the current study
9. Patients must have the ability and willingness to comply with study procedures.

Main exclusion criteria

1. Inability to speak and understand the local language sufficiently to understand the nature of the study, to provide written informed consent, and to allow the completion of all study assessments
2. Evidence of an active ocular infection, in either eye
3. Presence of any other ocular disorder or condition requiring topical medication during the entire duration of study in either eye
4. History of severe systemic allergy or of ocular allergy (including seasonal conjunctivitis) or chronic conjunctivitis and/or keratitis other than dry eye
5. Intraocular inflammation defined as Tyndall score >0
6. History of malignancy in the last 5 years
7. Systemic disease not stabilized within 1 month before Screening Visit (e.g. diabetes with glycemia out of range, thyroid malfunction) or judged by the investigator to be incompatible with the study (e.g. current systemic infections) or with a condition incompatible with the frequent assessment required by the study
8. Patient with a history of serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or clinically significant allergy to drugs, foods, amide local anesthetics or other materials including commercial artificial tears (in the opinion of the investigator)
9. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:
    a. are currently pregnant or,
    b. have a positive result at the urine pregnancy test (Baseline/Day 1) or,
    c. intend to become pregnant during the study treatment period or,
    d. are breast-feeding or,
    e. are not willing to use highly effective birth control measures, such as: combined (estrogen and progesterone containing) hormonal contraceptives associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, implantable, injectable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner, sexual abstinence during the entire course of and 30 days after the study treatment period

10. Any concurrent medical condition, that in the judgment of the PI, might interfere with the conduct of the study, confound the interpretation of the study results, or endanger the patient's well-being **11.** Use of topical cyclosporine, or topical ophthalmic treatments of the same class, within 14 days of screening visit (day -8). **12.** Use of topical corticosteroids, lifitegrast, autologous serum tears in either eye during the study (previous use not an exclusion criteria but must be discontinued at the screening visit) **13.** Contact lenses, TrueTear device, moisture goggles, sutureless amniotic membrane or punctum plug use during the study (previous use not an exclusion criteria but must be discontinued at the screening visit) **14.** History of drug addiction or alcohol abuse within the last year **15.** Any prior ocular surgery (including refractive, palpebral and cataract surgery) if within 90 days before the screening visit **16.** Participation in a clinical trial with a new active substance during the past 3 months prior to screening **17.** Participation in another clinical trial study at the same time as the present study	
Test/Reference Product, Dosage and Mode of Administration	Oxervate®, an ophthalmic solution containing **cenegermin 20 mcg/mL, which is a recombinant human Nerve Growth Factor (rhNGF)**; reference product is **vehicle**. Test and reference will be instilled in both eyes according to the following scheme: **Group 1:** one drop of cenegermin 20 mcg/mL will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm). **Group 2:** vehicle eye one drop will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm). The IMP will be provided in a monthly box containing 28 daily vials. Together with the IMP monthly box, patients will be provided with a sufficient number of pipettes and adaptors for the administration of the IMP.
Duration of Study	Patients will be evaluated at screening visit (day -8), baseline (day 1+2), week 2 (day 14±2), week 4 (day 28±2) or early exit, week 8 (day 56±2), week 12 (day 84±4), and week 16 (day 112±7) of follow-up. Safety evaluations will also be continued in follow-up until week 24 (day 168 ±7) **Screening Visit (day -8):** all procedures for assessment of eligibility will be performed.

	Wash-out period (from day -8 to baseline): 8 (+2) days with no further treatment except commercially available preservative free artificial tears if strictly needed, provided by the Sponsor. The use of artificial tears will be tracked in the patient's diary. **Treatment:** eligible patients will be randomized 1:1 and treated for 4 weeks with either cenegermin ophthalmic solution 20 mcg/mL or vehicle. Only the experimental IMP is allowed during the treatment period however, if strictly needed, the patient may use commercially available preservative free artificial tears provided by the Sponsor. The use of artificial tears will be tracked in the patient's diary. **Follow up:** Initially 8 weeks (from week 5 to week 12) post treatment with no further topical ocular treatment except commercially available preservative-free artificial tears provided by the Sponsor administered three times daily. Furthermore, the patient can administer additional artificial tear eye drops provided by the Sponsor only if strictly needed, and must document in the patient's diary the number of additional drops administered for each eye. Patients will then be followed up for efficacy and safety endpoints until week 16 and for safety endpoints until week 24. During the follow up period (from week 13 to week 24) other topical treatments will be allowed at discretion of the treating physician. Such treatments will be recorded by the investigators. Patients can continue any systemic treatment as required to control their autoimmune disease throughout the study; any change in systemic treatments must be recorded by the investigators. Maximum total study duration: 112±7 days for efficacy evaluations plus an additional 56±7 days for safety evaluations.
Study population	Patients with severe Sjogren's dry eye disease will be included. A total of 100 patients will be enrolled assuming a 10% drop out rate to yield 90 eligible patients.
Co- Primary Efficacy Endpoint	• Schirmer I test (without anesthesia) >10mm/5min at week 4 in the eligible eye. • Change from baseline in Symptoms questionnaire (SANDE) global score at week 12.

Secondary Efficacy Endpoints	• Change from baseline in Schirmer I test (without anesthesia) [Time frame: week 4, 8, 12 and 16]; • Change from baseline in Cornea and conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales) [Time frame: week 4, 8, 12 and 16]; • Change from baseline in Tear Film Break-Up Time (TFBUT) [Time frame: week 4, 8, 12 and 16]; • Change from baseline in Symptoms questionnaire (SANDE) scores for severity and frequency [Time frame: week 8, 12 and 16]; • Number of patients experienced worsening in symptom scores (SANDE) and/or NEI score ≥ 50% assessed at week 4; • Quality of life (IDEEL) questionnaire [Time frame: week 4, 8, 12 and 16].
Exploratory Endpoints	[redacted]
Safety Endpoint	• Incidence and frequency of Adverse Events (AEs) and Treatment-emergent adverse events (TEAEs), assessed throughout the study.

Statistical Methods	The sample size of the study is calculated based on results from previous studies. Expecting a difference of 30% in Schirmer I rate of responders (defined as patients reaching a value >10mm/5min) at Week 4 and 15 points in Global SANDE score improvement at Week 12, a total sample size of 90 evaluable patients (100 pts considering 10% drop-out rate) allows to achieve an overall power of 90% to show superiority of cenegermin ophthalmic solution vs vehicle in terms of both co-primary endpoints, considering a one-sided alpha of 0.025. Summary statistics are defined for quantitative variables (number of observations, mean, standard deviation, median, minimum and maximum) and qualitative variables (number and percentage per category). If appropriate, confidence intervals around the mean or the proportions will be presented. The proportion of patients reaching a value of Schirmer I test (without anesthesia) >10mm/5min at week 4 will be analyzed by means of logistic regression. The change from baseline in SANDE global score at week 12 will be analyzed by means of an adjusted ANCOVA procedure. If the primary analysis of primary endpoint leads to rejection of the null hypotheses, key secondary endpoints will be tested in a conditional sequential manner to show superiority of cenegermin versus placebo vehicle according to the pre-defined ranking sequence. Independently of the results on primary endpoints, all secondary endpoints will be analysed at each available time point by means of descriptive statistics and by appropriate parametric tests. Data record in the questionnaires of quality of life will be presented with appropriate descriptive statistics and processed with appropriate inferential test. Change from baseline value and shift tables versus baseline may be summarized for all available post-baseline visits. CCI [redacted] will be descriptively summarized at each available time point. Any statistical testing will be descriptive in nature. Correlation between sign and symptom scores will be assessed. AEs will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA). Individual AEs will be listed in patient data listings. TEAEs will be presented in terms of the number of AEs and incidence by treatment, seriousness,

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

2. SCHEDULE OF EVALUATIONS

Study procedures	Screening (Day-8)	Visit 1 Baseline* (Day 1 + 2 days)	Visit 2 Week 2 [a] (Day 14 +/- 2 days)	Visit 3 End of treatment Week 4[a] (Day 28 +/- 2 days)	Visit 4 follow-up Week 8[a] (Day 56 +/- 2 days)	Visit 5 follow-up Week 12[b] (Day 84 +/- 4 days)	Visit 6 follow-up Week 16[c] (Day 112 +/- 7 days)	Visit 7 follow-up Week 24[c] (Day 168 +/- 7 days)
➢ Explanation to the patient of study aims, procedures and possible risks	X							
Informed Consent	X							
Inclusion/Exclusion Criteria	X	X						
Pregnancy Test	X	X		X			X	
Randomization		X						
Demographics	X							
Ocular and Systemic Medical History	X							
Previous Ocular And Systemic Medications	X	X						
SANDE	X	X	X	X	X	X	X	
IDEEL	X	X	X	X	X	X	X	
BCDVA	X	X	X	X	X	X	X	
External Ocular Examination	X	X	X	X	X	X	X	
Schirmer test I	X	X	X	X	X	X	X	
Slit Lamp Examination	X	X	X	X	X	X	X	
TFBUT	X	X	X	X	X	X	X	
Fluorescein staining (NEI scale)	X	X	X	X	X	X	X	
CCI [redacted]		X		X				
Study drug dispensation		X[d]						
Preservative free artificial teras dispensation	X	X	X	X	X			

Study procedures	**Screening (Day-8)**	**Visit 1 Baseline* (Day 1 + 2 days)**	**Visit 2 Week 2 [a] (Day 14 +/- 2 days)**	**Visit 3 End of treatment Week 4[a] (Day 28 +/- 2 days)**	**Visit 4 follow-up Week 8[a] (Day 56 +/- 2 days)**	**Visit 5 follow-up Week 12[b] (Day 84 +/- 4 days)**	**Visit 6 follow-up Week 16[c] (Day 112 +/- 7 days)**	**Visit 7 follow-up Week 24[c] (Day 168 +/- 7 days)**
Verify patient study medication dosing compliance				X				
Concomitant Ocular And Systemic Medications			X	X	X	X	X	X
Frequency of preservative free artificial tears use (n° drops/day)		X	X[e]	X[e]	X[f]	X[f]	X	X
Dispensation, check and retrieval of patient's diary	X[g]	X	X[h]	X	X[h]	X	X[h]	X[i]
Record AEs	X	X	X	X	X	X	X	X

*) visit window of +2 days; a) Visit window of ± 2 days; b) Visit window of ± 4 days; c) Visit window of ± 7 days; d) a monthly box will be given to the patients; e) During the treatment period patients can use, if strictly needed, the preservative free artificial tears provided by the Sponsor; f) During the follow up period it is allowed to use the preservative free artificial tears provided by the Sponsor; g) At screening visit only dispensation; h) During the visit 2 - week 2, visit 4 - week 8 and visit 6 – week 16, the PI or a delegate must only check if the patient has correctly completed the diary; i) At Visit 7 – week 24 only check and retival.

2.1. BACKGROUND INFORMATION

2.1.1. Nerve Growth Factor - Overview

Nerve growth factor (NGF) is a polypeptide essential for the survival and growth of sympathetic and sensory neurons, and for differentiation of neurons in the central nervous system. It binds with at least two classes of receptors: high-affinity tropomyosin receptor kinase A (TrkA), a transmembrane tyrosine kinase, and low-affinity NGF receptor (LNGFR), also known as p75 neurotrophin receptor (p75NTR).
NGF and TrkA are expressed in the anterior segment of the eye (iris, ciliary body, lens, cornea and conjunctiva), and NGF is released in the aqueous humor. Several pieces of experimental evidence suggest that NGF affects all tissues of the anterior ocular segments, playing a crucial role in the physiopathology of several anterior ocular segment diseases.

2.1.2. Chemical And Formulation Data

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

2.1.3. Rationale for cenegermin (rhNGF) therapy in patients with dry eye

Dry eye is a chronic condition of the ocular surface with severe symptoms and visual impairment, leading to worse efficiency to perform duties for an average of 184 work days and resulting in an average loss of productivity estimated in 5,000USD per year per patient[1].

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

Dry eye pathogenesis is multifactorial however, a number of common mechanisms can be identified: (i) chronic inflammation of the conjunctiva; (ii) decrease of ocular surface sensitivity; (iii) impairment of quantity of tears and/or quality of the tear film, including tear film hyperosmolarity; (iv) changes of conjunctival epithelium with squamous metaplasia and decrease of goblet cell density; (v) corneal epithelium damage[2].

Until now, treatment has been limited to the use of artificial tears to temporarily improve lubrication of the ocular surface, immunomodulation to increase tear production and decrease inflammation (Cyclosporine, Lifitegrast), or the use of topical steroids to decrease the inflammatory reaction acutely. However, chronic use of steroids is associated with several complications such as cataract development and increasing intraocular pressure[2]. Cyclosporine is topical immunomodulator indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca. Lifitegrast ophthalmic solution is a lymphocyte function-associated antigen-1 (LFA-1) antagonist

indicated for the treatment of the signs and symptoms of dry eye disease. On the other hand, experimental and clinical evidence suggests that NGF may affect all the pathogenic mechanisms of dry eye, potentially restoring ocular surface homeostasis[3]. Human corneal epithelium, keratocytes, and endothelium produce NGF, and express its high affinity tyrosine kinase receptor A (TrkA), and experimental studies demonstrated that the activation of TrkA receptors by NGF plays a key role in corneal sensory nerve survival[4].

Indeed, several studies have shown that NGF is involved in the regulation of tear film production. In fact, NGF, TrkA and p75, as well as other neurotrophins (NTs) and related receptors, are expressed by the rat lacrimal gland tissue; moreover, NGF has been quantified in human tears, indicating that NGF is basally released by the lacrimal gland[5–7]. These data suggest that NGF may play a role in the maintenance of the tear film and in its alterations in drying ocular surface diseases. Specifically, considering that NGF potentially affects all the components of the ocular surface (cornea, conjunctiva, lacrimal gland, and sensory innervation), it might play an important role during dry eye disease. In line with this hypothesis: NGF topical administration in a dog experimental model of dry eye has been shown to increase tear production, conjunctival goblet cell density and corneal transparency[2,8]; an increased tear concentration of NGF has been reported in patients affected by keratoconjuctivitis sicca[9,10]; NGF stimulates glycoconjugate secretion by conjunctival goblet cells, without affecting cell proliferation[11].

In several systemic autoimmune conditions, including Sjogren syndrome, the lacrimal gland can be attacked by the immune system resulting in an ocular manifestation of aqueous deficient DE (ADDE)[12]. In fact, the leading cause of ADDE in dogs is immune-mediated lacrimal gland dysfunction comparable to Sjogren syndrome in humans[13]. In addition, NGF was found to increase secretory mucin MUC5AC secretion by conjunctival goblet cells, without affecting cell proliferation[11]. The major secretory gel forming mucin MUC5AC plays a crucial role in the maintenance of a wet-surfaced ocular surface phenotype, in the clearance of debris from the ocular surface epithelia (including pathogens and allergens), and in the stability of the tear film, contributing to preventing its premature evaporation[14,15]. The underlying tear film dysfunction discussed thus far encompasses alterations in one, or all, of its layers (i.e. the aqueous, mucin, and lipid layer). Tear production is controlled by the lacrimal functional unit (LFU) consisting of the cornea, conjunctiva, lacrimal glands, meibomian glands, and the neuronal network that connects them[14]. When corneal nerves are stimulated, afferent signals are triggered through the ophthalmic branch of the trigeminal nerve (V). In turn, efferent secretomotor nerve endings stimulate lacrimal glands, meibomian glands and conjunctival goblet cells to secrete aqueous, lipids and mucin contents of the tear film, respectively[14–16]. Likewise, when corneal sensory nerves are stimulated, they release neuromediators that provide trophic support for the corneal epithelium and keratocytes. These corneal epithelial cells and keratocytes release neuropeptides, neurotrophins and growth factors (e.g. NGF) that influence survival, differentiation, and maturation of nerve fibers[17]. Thus, neurosensory abnormalities play a key etiological role in DE that remains largely unaddressed by current approved therapeutics.

Specifically, considering that NGF potentially affects all the components of the ocular surface (cornea, conjunctiva and goblet cells, lacrimal gland, and sensory innervation), it may play an important role in Sjogren's DE disease. In addition, considering the mechanism of action of NGF on improving ocular surface sensory nerve function, which will induce in turn an improvement in reflex tear secretion and blink rate, it is expected that the effects on both signs and symptoms of DE may be long-lasting.

2.2. A SUMMARY OF CLINICAL DATA

Cenegermin has been already studied in healthy volunteers and in different subject populations.
In the completed Phase I study in healthy volunteers (CCI), a total of 58 subjects (out of 74 enrolled) were treated with single and multiple doses of different concentrations of cenegermin ophthalmic solution showing a good safety profile.
Furthermore, a Phase I/II multicenter, double-masked, vehicle-controlled study evaluating the safety and efficacy of cenegermin at 10 and 20 mcg/mL six times a day in 174 patients with stage 2 and 3 of Neurotrophic Keratitis (study CCI) was completed in 2015; the study demonstrated that cenegermin was very well tolerated and effective in patients with NK. A successful confirmatory study (CCI) was completed in 2016 and marketing authorization for cenegermin 20 mcg/mL ophthalmic solution was granted by the EMA in July 2017 for treatment of moderate to severe NK. While in the US cenegermin was approved by FDA in 2018 for all stages of Neurotrophic Keratitis. Cenegermin is distributed under the trade name Oxervate® 20 mcg/mL ophthalmic solution.
In addition to NK, cenegermin ophthalmic solution has been evaluated in a Phase I/II study in retinitis pigmentosa (RP) patients at 60 and 180 mcg/mL dosages CCI), and in a phase I/II study in glaucoma at a dose of 60 mcg/mL CCI). An open label, uncontrolled study showed that 4 weeks treatment with cenegermin ophthalmic solution at 20 mcg/mL and 4 mcg/mL concentrations was safe and effective in improving symptoms, corneal staining and tear production in patients with dry eye disease CCI). These results prompted two additional phase II RCTs in patients with dry eye disease (CCI) and in those with ocular discomfort symptoms following refractive surgery CCI); these also confirmed the favourable tolerability profile of cenegermin ophthalmic solution at a concentration of 20 mcg/mL when used up to 6 times daily for 8 weeks.
In the double-masked vehicle-controlled Phase II study CCI no major differences were observed in efficacy when comparing the two administration regimens (6 vs. 2 times/day) and treatment durations (8 weeks vs. 4 weeks) in a subset of patients with similar characteristics (hyposecretive dry eye with Schirmer test <10mm/5min).

An additional phase II study (CCI) was specifically designed to further prove this hypothesis. It was a 4 week, Phase 2, multicenter, randomized, double-masked, vehicle-controlled, parallel group study with 12 weeks of follow-up to evaluate safety and efficacy of cenegermin ophthalmic solution versus vehicle, in patients with moderate to severe dry eye, started in 2018 in patients with hyposecretive dry eye (with or without an evaporative component caused by the reduced tear film), including patients diagnosed with primary Sjogren syndrome.

In this phase II trial a total of 261 patients were randomized 1:1:1 to receive either cenegermin TID (i.e. 3 times/day), or cenegermin BID (i.e. 2 times/day), or vehicle.

The primary endpoint of the study was the change from baseline in Schirmer I test (without anaesthesia), while key secondary endpoints included change from baseline in SANDE scores for severity and frequency, cornea and conjunctiva vital staining, TFBUT and quality of life. Despite the primary endpoint at $p<0.025$ was not reached in the overall population, the study has clearly shown a superior efficacy of both cenegermin arms versus placebo, especially considering that due to the Covid-19 emergency recruitment was interrupted when only 261 (the minimum number required) out of the 300 patients initially planned were randomized. However, when analyzing the percentage of patients achieving more than 10 mm in Schirmer I test scores at the end of

treatment, as pre-specified in the statistical analysis plan (SAP), the difference between both cenegermin dose regimens vs. placebo was statistically significant for both study groups (p=0.028 for cenegermin TID and p=0.007 for cenegermin BID [P-value versus Vehicle TID based on Fisher's exact test]). It is notewhorty that, while no clear dose ranging effect was observed on the tear function endpoints, symptoms recorded by the DE-specific and validated SANDE questionnaire showed a significant persistence of the beneficial effect of the higher dose-regimen (TID) over time and up to the full 16 weeks after the end of the 4 weeks treatment cycle. In fact, statistical significance vs. vehicle was reached at all follow-up timepoints for the TID with best result at week 12 (p=0.002 based on t-test; missing data imputed by LOCF). In addition, no difference was observed in the safety and tolerability profile of the experimental treatment. Specifically, up to 3 instillations per day of cenegermin ophthalmic solution in both eyes confirmed to be safe and well tolerated. The two cenegermin study groups have shown a similar tolerability. No serious adverse events were reported in the study. Adverse reactions were mild and transient, and occurred only during the 4-weeks treatment period of the 16-weeks cycle.

The results of these three randomized controlled studies in dry eye disease patients, as well as the results on reflex tear secretion obtained in the neurotrophic keratitis trials CCI and CCI, suggests that cenegermin may be a safe and effective treatment option for patients with hyposecretive/aqueous tear-deficient dry eye disease. In all studies rhNGF was well tolerated and the reported ocular AEs were generally transient and mostly mild and moderate in intensity during the controlled treatment period and the follow-up period.

In the Phase I, randomized, double-masked, placebo-controlled study to evaluate the safety, tolerability and pharmacokinetics of rhNGF eye drops in healthy volunteers of Japanese ethnicity (CCI), a total of 30 subjects were enrolled. Twenty subjects were treated with a single dose of cenegermin ophthalmic solution at a 20 mcg/mL concentration and a total of 10 subjects were treated with vehicle. On day 1 patients received only one dose of cenegermin, then the remainder of the trial they received one drop six times a day. Both the single and multiple doses of cenegermin delivered topically as ophthalmic solution were safe and well tolerated by healthy subjects of Japanese ethnicity. No clinically important changes in ECG were observed after dosing with cenegermin. There was no apparent relationship between treatment and plasma cenegermin levels, and this study did not give any indication for an immunogenic potential of cenegermin in humans after ophthalmic treatment.

2.3. STUDY RATIONALE

The data reported above, together with the evidence of cenegermin ophthalmic solution effectiveness in the treatment of patients affected by corneal epithelial defects and ulcers, and moderate to severe dry eye make cenegermin a strong candidate for the treatment of severe Sjogren's dry eye disease[4,10,18].

As part of the development plan, the present study was designed in order to evaluate the safety and efficacy of Oxervate® (cenegermin ophthalmic solution, rhNGF) versus vehicle in patients with severe Sjogren's dry eye disease. For additional information regarding the development of rhNGF, please consult the current IB.

2.3.1. Risk assessment/benefit evaluation

This study is considered a low-risk interventional trial. It is conducted with a previously tested dose of medicinal product already authorized and commercialized for the treatment of NK in Europe, US and China.
The proposed study is conducted in patients with severe Sjogren's dry eye disease, an indication for which Oxervate® (cenegermin) ophthalmic solution is not yet approved, however two preliminary clinical studies were already conducted in patients with dry eye demonstrating that cenegermin is well tolerated in this condition.
The risk of this study is comparable to the risk of the standard of care, in fact the dose proposed in this protocol is cenegermin 20 mcg/mL three drops per day instilled in each eye, which is well below the dose approved for the treatment of NK (cenegermin 20 mcg/mL six drops/eye per day).
Considering what is discussed above, no particular safety risks are foreseen with respect to the safety profile of the marketed product Oxervate® (cenegermin 20 mcg/mL ophthalmic solution).

The patients with severe Sjogren's dry eye disease participating in this study may potentially benefit from the application of cenegermin for 28 days (4 weeks).

Any possible risk derived from the administration of cenegermin in the specific population involved in this study will be minimized by integrated monitoring which includes clinical observations, Ocular examination, laboratory tests.

2.3.2. Description of the Investigational Product

The investigational medicinal product (IMP) consists of a sterile isotonic solution for ophthalmic administration, containing cenegermin 20 mcg/mL as drug substance.

The matching placebo vehicle consists of a sterile isotonic solution.

Further information is given in section 5.

3. OVERALL STUDY DESIGN AND INVESTIGATIONAL PLAN

3.1. STUDY OBJECTIVES

The study objective is to assess the efficacy and safety of cenegermin (rhNGF) ophthalmic solution at 20 mcg/mL concentration administered three times daily for 4 weeks in patients with severe Sjogren's dry eye disease.

3.2. STUDY ADMINISTRATIVE STRUCTURE

This study will be performed at approximately 10 study centers located in the USA and Europe. At each study center, the Principal Investigator (PI) will be responsible for ensuring that the investigation is conducted according to the signed Investigator agreement, the protocol, GCP guidelines, and local regulations.

The PI at each study center will be responsible for the management of the study, which will consist of maintaining the study file and the patient records, corresponding with the IRB, and completing the case report forms (eCRFs) and reporting SAEs within 24 hours of initial awareness.
The PI is responsible for supervising any individual or party to whom the investigator delegates trial related duties and functions conducted at the trial site.
If the investigator/institution retains the services of any individual or party to perform trial-related duties and functions, the investigator/institution should ensure this individual or party is qualified to perform those trial-related duties and functions and should implement procedures to ensure the integrity of the trial-related duties and functions performed and any data generated.

3.3. OVERALL STUDY DESIGN

This is a 4 week phase III, multicenter, double-masked, vehicle-controlled study to evaluate safety and efficacy of cenegermin ophthalmic solution at 20 mcg/mL solution versus vehicle, in patients with severe Sjogren's dry eye disease
Patients will be evaluated as per Section 2 "SCHEDULE OF EVALUATIONS".

During the screening (day-8) all procedures for inclusion will be performed. From the day of screening the patients will stop any kind of further treatment, except commercially available preservative free artificial tears provided by Sponsor for a period of 8 days and 10 days as maximum: day -8 until the baseline visit (day 1+2), Figure 1. At the end of the wash out period (day -8 to baseline), patients meeting the entry criteria for this study will be randomized 1:1 and treated for 4 weeks with either cenegermin ophthalmic solution 20 mcg/mL TID or vehicle TID.
During the 4 weeks of masked treatment only the administration of IMP is allowed. Nevertheless, if strictly needed, the patient can take preservative free artificial tears (provided by the Sponsor). The use (n° drops/day) of preservative free artificial tears will be clearly documented in a patient's diary and in the eCRF.

Eight weeks post treatment with no further topical ocular treatment except commercially available preservative-free artificial tears provided by the Sponsor three times daily will follow the completion of the double-blind treatment. During this follow up period, the patient can administer additional artificial tear eye drops, provided by Sponsor, only if strictly needed, and must document in the patient's diary the number of additional drops administered for each eye.
Patients will then be followed up for efficacy and safety endpoints until week 16 and for safety endpoints until week 24. During the follow up period after week 12 until week 24 other topical treatments will be allowed at discretion of the treating physician. Such treatments will be recorded by the investigators.
Throughout the study, the patient can continue any systemic treatment as required to control their autoimmune disease, and any change in systemic treatments must be recorded by the investigators.
The total duration of the study is 25 weeks including 1 week of screening.

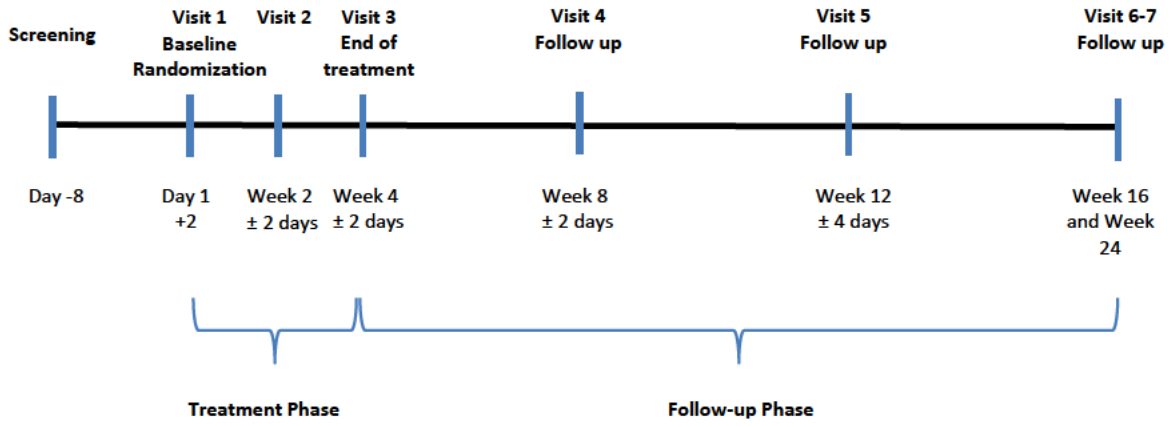

Figure 1. Study duration

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

The dose proposed in this study (cenegermin 20 mcg/mL) has already been tested both in heathy volunteers for five consecutive days ([redacted] and [redacted]) and in patients affected by several ocular surface diseases, including moderate to severe dry eye ([redacted]).

Cenegermin 20 mcg/mL (one drop in each eye six times daily for 8 weeks), was also tested in the Phase I/II of the NK studies ([redacted]), dry eye ([redacted]) and in patients after cataract and refractive surgery ([redacted]).

Cenegermin 20 mcg/mL (one drop in each eye three times daily for 8 weeks) was also tested in the Phase II ([redacted]) in moderate to severe dry eye.

Cenegermin 20 mcg/mL administered two times daily, demonstrated to be safe and well tolerated CCI will also ensure the lubrification of the ocular surface in the present study where the use of artificial tears will be not allowed during the treatment period.

Higher doses of rhNGF (up to 180 mcg/mL) were administrated to patients with diseases of the back of the eye, such as CCI (Study CCI) for up to 168 days.

The 20 mcg/mL concentration of cenegermin is well sustained by the current manufacturing process and is used in a commercial formulation.

A dose regimen of 3 drops per day of cenegermin ophthalmic solution and a treatment duration of 4 weeks has been selected based on the results of the previous clinical trials described above.

The administration of a total of 3 drops per day for all the study arms has been chosen to guarantee to patients randomized to the vehicle arm to have the minimum amount of lubrication that is compatible with symptoms' relief. A double-blind study design was adopted to minimize systematic bias. Randomization is expected to minimize patient selection bias and increase baseline comparability between treatment groups. The use of placebo control is critical to the study design for providing an accurate estimate of the additive benefit of pharmacotherapy.

The use of the drug's vehicle as placebo helps in making the latter as indistinguishable from the cenegermin solution.

Patients with insufficient therapeutic response, tolerability issues, or worsening of symptoms may be discontinued at any time during the study.

4. SELECTION OF STUDY POPULATION

Patients ≥ 18 years with severe Sjögren's dry eye will be included. A total of 100 patients will be enrolled. The safety and efficacy of cenegermin ophthalmic solution will be investigated in these patients.

4.1. INCLUSION CRITERIA

To be eligible for inclusion into this study, each patient must fulfil the following inclusion criteria:

1. Male or female aged ≥ 18 years
2. Patients with a confirmed diagnosis of Sjögren's syndrome or other autoimmune disease known to induce Sjögren's Dry Eye Disease (DED).
3. Patients with severe Sjögren's dry eye disease characterized by the following clinical feautures:
    a. Corneal and/or conjunctival staining with fluorescein using National Eye Institute (NEI) grading system ≥3
    b. SANDE questionnaire >25 mm
    c. Schirmer test I (without anaesthesia) ≥2 ≤5mm/5min
4. The same eye (eligible eye) must fulfill all the above criteria
5. Patients diagnosed with severe Sjögren's dry eye disease at least 3 months before enrolment (current use or recommended use of artificial tears for the treatment of Sjogren's related Dry Eye)
6. Best corrected distance visual acuity (BCDVA) score of ≥ 0.1 decimal units (20/200 Snellen value) in each eye at the time of study enrolment
7. If a female of childbearing potential, have a negative urine pregnancy test and use a highly effective method to avoid pregnancy for the duration of the trial and 30 days after the study treatment period . Males of reproductive potential should use effective contraception during treatment and 30 days after the study treatment period.
8. Only patients who satisfy all Informed Consent requirements may be included in the study. The patient and/or his/her legal representative must read, sign and date the Informed Consent document before any study-related procedures are performed. The Informed Consent form signed by patients and/or legal representative must have been approved by the IRB/IEC for the current study
9. Patients must have the ability and willingness to comply with study procedures

EXCLUSION CRITERIA

1. Inability to speak and understand the local language sufficiently to understand the nature of the study, to provide written informed consent, and to allow the completion of all study assessments
2. Evidence of an active ocular infection, in either eye
3. Presence of any other ocular disorder or condition requiring topical medication during the entire duration of study in either eye

4. History of severe systemic allergy or of ocular allergy (including seasonal conjunctivitis) or chronic conjunctivitis and/or keratitis other than dry eye
5. Intraocular inflammation defined as Tyndall score >0
6. History of malignancy in the last 5 years
7. Systemic disease not stabilized within 1 month before Screening Visit (e.g. diabetes with glycemia out of range, thyroid malfunction) or judged by the investigator to be incompatible with the study (e.g. current systemic infections) or with a condition incompatible with the frequent assessment required by the study
8. Patient had a serious adverse reaction or significant hypersensitivity to any drug or chemically related compounds or had a clinically significant allergy to drugs, foods, amide local anesthetics or other materials including commercial artificial tears (in the opinion of the investigator)
9. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) are excluded from participation in the study if they meet any one of the following conditions:
    a. are currently pregnant or,
    b. have a positive result at the urine pregnancy test (Baseline/Day 1) or,
    c. intend to become pregnant during the study treatment period or,
    d. are breast-feeding or,
    e. are not willing to use highly effective birth control measures, such as: combined (estrogen and progesterone containing) hormonal contraceptives associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, implantable, injectable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner, sexual abstinence during the entire course of and 30 days after the study treatment period.
10. Any concurrent medical condition, that in the judgment of the PI, might interfere with the conduct of the study, confound the interpretation of the study results, or endanger the patient's well-being
11. Use of topical cyclosporine, , or topical ophthalmic treatments of the same class, within 14 days of screening visit (day -8)
12. Use of topical corticosteroids, lifitegrast, autologous serum tears in either eye during the study (previous use not an exclusion criteria but must be discontinued at the screening visit)
13. Contact lenses, True Tear device, moisture goggles, sutureless amniotic membrane or punctum plug use during the study (previous use not an exclusion criteria but must be discontinued at the screening visit)
14. History of drug addiction or alcohol abuse in the last 2 years
15. Any prior ocular surgery (including refractive, palpebral and cataract surgery) if within 90 days before the screening visit

16. Participation in a clinical trial with a new active substance during the past 3 months
17. Participation in another clinical trial study at the same time as the present study.

4.2. ELIGIBLE EYE

Assuming that all the inclusion/exclusion criteria are met in both eyes, the worse eye (eligible eye) will be determined **at the baseline visit using** a stepladder approach, as follows:

1. Schirmer's Test (since this is the primary endpoint)-Worse eye determined as eye with lower Schirmer I score.
2. NEI score (cornea+conjunctival staining) - If Schirmer I score is the same in both eyes, worse eye will be determined by NEI score (cornea+conjunctival staining).

If all of the above are identical, determination of the worse eye will be based on the Investigator's judgement, otherwise **simply use the right eye** as the eligible eye.

4.3. ASSIGNMENT OF PATIENT NUMBER

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

4.4. RANDOMIZATION

Eligible patients will be randomized in a 1:1 ratio to either cenegermin ophthalmic solution 20 mcg/mL TID (~50 patients) or vehicle ophthalmic solution TID (~50patients).

Each randomized patient will be allocated with randomization number, according to the stratified randomization list. Drop outs after randomization will not be replaced.

Randomization will be stratified by site to ensure balanced assignment across treatment groups. The stratified permuted block randomization list will be generated with a computer procedure by an independent statistician not involved in the conduct of the study.

Randomization will be performed through IRS. Each Patient Kit number will be randomly associated with a treatment group. The randomization list will be provided to the facility responsible for IMP packaging/labelling for the purpose of IMP preparation. Each randomized patient will be allocated with randomization number according to the stratified randomization list. Dropouts after randomization will not be replaced

The enrollment of patients will be scheduled in order to assure an inclusion of approximately 100 patients to ensure at least 90 patients assuming up to 10% dropout rate.

4.5. MASKING

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

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

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

The investigator will be provided with a protected access to the randomization list so only in case of a medical emergency the Investigator can open the treatment allocation for a specific patient.
Also Dompé's Pharmacovigilance contact person will be provided with a protected access to randomization list , so if required by the Pharmacovigilance activities the Pharmacovigilance contact person can open the treatment allocation for a specific Patient.

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

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

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

5. STUDY MEDICATION

5.1. DESCRIPTION OF PRODUCT

5.1.1. Presentation of Non Investigational Medicinal Product (NIMP)

TEST PRODUCT NIMP	Blink® Tears or equivalent preservative-free artificial tears
Pharmaceutical form	Lubricating Eye Drops
Administration	One drop of Blink® Tears or equivalent will be instilled in both eyes during the screening week, only if strictly needed by the patient. The patient must document in the patient's diary the n° of additional drops administered for each eye. One drop of Blink® Tears or equivalent will be instilled in both eyes during the 4 weeks of masked treatment, only if strictly needed by the patient. The patient must document in the patient's diary the n° of additional drops administered for each eye. One drop of Blink® Tears or equivalent will be instilled in both eye TID (morning, afternoon and evening) during the initially 8 weeks of follow up. The patient, only if strictly needed, can administer additional drops and must document in the patient's diary the n° of additional drops administered for each eye.
Administration route	Ocular

5.1.2. Presentation of Investigational Medicinal Product

TEST PRODUCT IMP	Cenegermin (rhNGF 20 mcg/mL) and/or Placebo vehicle (Vehicle vials).
Manufacturer active substance	Dompé Farmaceutici S.p.A., Italy

Manufacturer finished product	Bulk drug product is manufactured by PPD Secondary packaging and labelling is performed by PPD
Pharmaceutical form	Ophthalmic sterile solution
Dose	Test and reference will be instilled in both eyes according to the following scheme: **Group 1**: one drop of cenegermin 20 mcg/mL will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm). **Group 2**: vehicle : one drop of vehicle ophthalmic solution will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm).
Administration route	Ocular

5.2. FORMULATION AND PACKAGING

The Investigator will be provided with a subject monthly box containing 4 weekly boxes of frozen IMP solutions (-20 ± 5°C) that, in-turn, contain 7 daily vials to be stored in refrigerator at 2-8 °C (36° F to 46° F) or at room temperature not exceeding 12 hours. Each vial contains:

- cenegermin, at concentrations of 20 mcg/mL, and/or
- vehicle (placebo).

Together with the IMP monthly box, the patients will be provided with a sufficient number of pipettes and adapters and wipes to be used for the administration of the IMP.
Pipettes, and adapters and wipes will be provided separately in single sterile packages and may be kept at room temperature.
The vial adapter is a device for safe transfer of drug between vial and pipette The pipette is used with an adapter consisting of a connecting device with dual connections: one end for the pipette and one end for the vial. The patient will need to:

1. Put the adapter on the top of the vial (after removing the plastic seal) by piercing the septum

2. Put the pipette on adapter inlet
3. Draw the solution contained in the vial with the pipette until this reaches its capacity
4. Remove the pipette and use it as a dropper to administer one drop of IMP into each eye.

5.3. STORAGE AND HANDLING OF IMP

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

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

A temperature probe and data logger will accompany the drug on shipment. It is essential that the investigational sites will verify the temperature excursion during shipment vs. the acceptable storage conditions, in order to identify potential stability concerns during shipment. These must be immediately communicated to the Sponsor that will decide upon appropriate actions to be taken. The IMP will be stored in a locked place, sheltered from light. The vials will be not shaken since agitation of vials may cause foaming and/or particle formation.

On Day 1 (baseline visit), the study personnel will give to the patient the monthly boxes containing the study medications

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

The weekly box must be kept at 2-8°C for 7 days protected from light; the daily vial can be kept at room temperature, before the patient will use the single vial for each instillation (both eyes) as long as 12 hours are not exceeded. Agitation of vials may cause foaming and/or particle formation. Drug preparation and administration instructions will be provided separately to the site and to the patients.

Together with the IMP monthly box, the patient will also receive a separate administration kit of vial adapters (one per vial), pipettes (3 per vial) and disinfectant wipes (3 per vial); some spare samples could be provided to ensure product administration.

IMP ophthalmic solution instruction will be provided to patients.

Patients will use one vial to instill one drop in both eyes (**group 1**: cenegermin TID; **group 2**: vehicle TID) three times a day (approximately every 6 hours), starting in the morning when they wake up, for a total duration of 4 weeks"..

The contents of each vial are for the daily administration to both eyes only. After the last administration the used vial should be returned to the original medication box and is not to be reused.

Any deviations from the recommended storage conditions should be immediately reported by the pharmacist to the Sponsor and Investigator, and the use of the drug should be suspended until they have given authorization for its continued use. The IMP supplies are to be used only in accordance with this protocol. The Investigator will not use any drug samples for other purposes (e.g., treating patients or deviating from the protocol with regard to dose regimen, duration of treatment, etc.). Under no circumstances will the Investigator give any drug samples to a third party.

5.4. DOSE, ROUTE AND SCHEDULE OF IMP ADMINISTRATION

5.4.1. Administration route

The Administration route is topical ophthalmic application.

5.4.2. Dose regimen

In all patients both eyes will be treated for a period of 4 weeks.

The dosing scheme of the different study groups is summarized below:

Group 1: one drop of cenegermin 20 mcg/mL will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm).

Group 2: one vehicle drop will be instilled in both eyes three times daily (every 6 hours, e.g. 7:00 am, 01:00 pm; 07:00 pm).

During the 4 weeks of masked treatment only the administration of IMP is allowed. Nevertheless, if strictly needed, the patient can take a preservative free artificial tears (provided by the Sponsor). The use (n° drops/day) of preservative free artificial tears will be clearly documented in a patient's diary and in the eCRF.

Both the patients and the investigator will be masked to the study treatment.
Information about the study drug administration, and comments, will be recorded on the appropriate page of the eCRF.

5.5. ACCOUNTABILITY OF THE IMP

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

At the week 4 (end of treatment visit) the patient will return the used or unused study boxes/vials to the Investigator.

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

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

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

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

During the course of the study and until its conclusion, the Investigator will complete the drug accountability forms. Partially used or unused study drug boxes will be verified by the Investigator and within one month after completion of the trial the partially used and unused study medication will be shipped to the Sponsor or will be destroyed after authorization by the Sponsor or by an authorized company according to GCP regulations.

5.6. CONCOMITANT MEDICATION

As a general rule, no ophthalmic medication other than study drug will be given to the patient from the screening day until all of the final study evaluations have been completed, except for preservative free artificial tears provided by the Sponsor.

The preservative free artificial tears will be provided by the Sponsor and can be used according to the following scheme:

1) During the 4 weeks of masked treatment, only if strictly needed, the patient will instill one drop in both eyes.
2) During the 8 weeks of Follow-up period post-treatment one drop will be instilled in both eyes TID (morning, afternoon and evening).

The use (n° drops/day) of preservative free artificial tears will be clearly documented in the patient's diary and eCRF.

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 will be clearly documented on the Concomitant Medications eCRF page.
Medication entries should be specific to product name (if a combination drug product) and spelled correctly. The dose, unit, frequency, route of administration, start date, discontinuation date, and indication should also be recorded. For medications administered only one time, the frequency column may reflect "once."

6. STUDY PROCEDURE AND ASSESSMENTS

The first patient first visit (FPFV) is defined as the 1st visit performed at one of the clinical centers by the 1st screened patient. The last patient, last visit (LPLV) is defined as the last visit performed at one of the clinical centers by the last patient (i.e., the last visit foreseen by the study protocol), independently of whether the patient completed or withdrew from the study. The First Patient In (FPI) is defined as the first randomized patient at one of the clinical centers.

Patients will be evaluated according to the following scheme:

Interventional phase

- Screening visit (day -8),
- Baseline (day 1+2),
- Week 2 (day 14±2),
- Week 4 (day 28±2) or early exit

Follow up visits

- Week 8 (day 56±2)
- Week 12 (day 84±4)
- Week 16 (day 112±7)
- Week 24 (day 168±7)

The schedule of study procedures and assessments is tabulated by visit in the Schedule of Evaluations in § 2.0.

The descriptions of the procedures to be performed at each visit are provided below.

6.1. SCREENING AND RANDOMIZATION VISITS

During the screening visit (day -8) all procedures for will be performed. From the day of screening the patients will stop any kind of further ophthalmic treatment, until all of the final study evaluations have been completed, except preservative free artificial tears (wash out period from day -8 to baseline, Day 1 +2). The wash out period should not be less than 8 days and more than 10 days.
At the end of the screening period, patients meeting the entry criteria for this study will be randomized 1:1 and treated for 4 weeks with either cenegermin ophthalmic solution 20 mcg/mL TID or vehicle TID.

Slit lamp examination and the fluorescein staining (Corneal and/or Conjunctival staining with Fluorescein) will be performed at different time-points, as specified in the section 2 of "SCHEDULE OF EVALUATIONS" and procedure will be detailed on the informed consent form.

An independent Blinded Reading Center (RC) will also be used to assess the images collected at protocol-specified time points and sent to the RC as specified in the RC procedure manual.

The RC will provide instructions for the slit lamp photography and the fluorescein staining procedures.

	Day	Procedures/Assessments
Screening visit	*Day -8*	The following procedures will be performed (order below is mandatory): ➢ Explanation to the patient of study aims, procedures and possible risks ➢ Informed consent signature ➢ Screening number allocation ➢ Patient eligibility: Inclusion/exclusion criteria evaluation ➢ Pregnancy test for female patients of childbirth potential ➢ Demographic data ➢ Ocular and systemic medical history ➢ Previous ocular and systemic medications (prior to start the study) ➢ Ocular examination of both eyes, to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the eyelid (Meibomian glands), eyelid (erythema), eyelid (edema), lashes, conjunctiva erythema, lens, iris, anterior chamber, corneal horizontal diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluorescein staining) • ➢ AE collection ➢ Preservative free artificial tears dispensation The Investigator will dispense to the patients the preservative free artificial tears (Blink® Tears or equivalent), provided by Sponsor, to be used only if strictly needed by the patient (the patient must follow the instruction in the product leaflet). The patients will be instructed to enter information of self-administration of Blink® Tears or equivalent (if the administration has been occur per eye) and AE occurrence into the diary. The diary will be given from the PI or delegate during this visit.
At home	*Day -8 – Day 1+2 (baseline)*	➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Artificial tears use during the wash-out period (diary) Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments

Visit 1 Baseline	*Day 1 +2*	The following procedures will be performed (order below is mandatory): ➢ Pregnancy test for female patients of childbirth potential ➢ Previous ocular and systemic medications (prior to start the treatment) ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the eyelid (Meibomian glands), eyelid (erythema), eyelid (edema), lashes, conjunctiva erythema, lens, iris, anterior chamber, corneal horizontal diameter • TFBUT • Ocular surface staining (NEI score – corneal and conjunctival fluorescein staining) • Schirmer test II (with Anesthesia) ➢ Patient eligibility: Inclusion/exclusion criteria evaluation ➢ Randomization ➢ Study drug dispensation ➢ Preservative free artificial tears dispensation ➢ AE collection During visit 1, Day 1+2 or baseline, PI or delegate must check and collect the patient's diary concerning the screening period and provide a new diary for the treatment period. The PI or a delegate must check if patient has correctly completed the diary. If not, the site staff must retrain the patient. The Investigator will dispense to the patients their monthly box containing the study drug for the following 4 weeks together with an adequate number of adapters and pipettes. The Investigator will dispense to the patients the preservative free artificial tears (Blink® Tears or equivalent), provided by Sponsor, to be used only if strictly needed by the patient (the patient must follow the instruction in the product leaflet). After completing baseline evaluation patients will be administered, by PI, with the study treatment as per instructions, and will self-administer at home the subsequent doses. Patients will return to the clinical site on Day 14 (±2) (Week 2, visit 2).

6.2. STUDY VISITS AND FOLLOW-UP ASSESSMENTS

After the first two weeks of treatment the patient will undergo a medical assessment during the visit 2 (week 2). Following the completion of the double-blind treatment (visit 3, week 4), patients will be followed up for efficacy until visit 6 (week 16) and safety assessments until vist 7 (week 24). During the follow-up period until

week 12 patients will not use further ophthalmic treatment except preservative free artificial tears, provided by Sponsor, one drop instilled in both eyes three times daily (morning, afternoon and evening). The patient, only if strictly needed, can administer additional drops and must document in the patient's diary the n° of additional drops administered for each eye. After week 12 (visit 5), patients will be followed-up for additional 12 weeks until the end of the study (visit 7, week 24) during which patients can use others artificial tears different from the artificial tears provided by the Sponsor.

Slit lamp examination and the fluorescein staining (Corneal and/or Conjunctival staining with Fluorescein) will be performed at different time-points, as specified in the section 2 of "SCHEDULE OF EVALUATIONS" and procedure will be detailed on the informed consent form.

An independent Blinded Reading Center will also be used to assess the images collected, as described in the previous paragraph.

	Day	**Procedures/Assessments**
At home	*Days 1-14* ±*2*	➢ Self-administration at home of the IMP, three times daily every 6 h for both eyes (diary) ➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Recording possible use of preservative free artificial tears (diary). Data will be recorded by the patient in the patient's diary.

	Day	Procedures/Assessments
Visiti 2 Week 2	*Day 14±2*	The following procedures will be performed (order below is mandatory): ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal Horizontal Diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluorescein staining) • ➢ Concomitant ocular and systemic medications ➢ Frequency of patient's artificial tear use during first 2 weeks of treatment (to be reported in eCRF) ➢ AE monitoring During the visit 2, week 2, the patient must bring the diary assigned during the previous visit. The PI or a delegate must check if patient has correctly completed the diary. If not, the site staff must retrain the patient. At completion of the assessment the patient will be discharged and will be asked to return for the end of treatment visit on day 28±2 (Visit 3 week 4). The Investigator will dispense to the patients the preservative free artificial tears (Blink® Tears or equivalent), provided by Sponsor, to be used only if strictly needed by the patient (the patient must follow the instruction in the product leaflet).
At home	*Days 14 ±2–28 ± 2*	➢ Self-administration at home of the IMP, three times daily every 6 h for both eyes (diary) ➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Recording possible use of preservative free artificial tears (diary). Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments
Visit 3 week 4	*Day 28*±2	The following procedures will be performed (the below order is mandatory): ➢ Pregnancy test for female patients of childbirth potential ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal Horizontal Diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluorescein staining) • Schirmer Test II (with Anesthesia) ➢ Assessment of compliance to treatment (from patient diary and IMP reconciliation from returned weekly boxes) ➢ Concomitant ocular and systemic medications ➢ Frequency of patient's artificial tear use during the last 2 weeks of treatment (to be reported in eCRF). ➢ AE monitoring ➢ Verify patient study medication dosing compliance During visit 3, week 4, PI or delegate must check and collect the patient's diary concerning the treatment period and provide a new diary for the follow up period. At completion of the assessment the patient will be discharged and will be asked to return for the follow up visit on day 56±2 (Visit 4 week 8). The Investigator will dispense to the patients the preservative free artificial tears (Blink® Tears or equivalent), provided by Sponsor, to be self-administered three times daily, one drop in both eyes during the first 4 weeks of Follow up. The PI or delegate has to explain to the patients that, only if strictly needed, they can administer an additional numbers of drops of Blink® Tears or equivalent, by documenting all information in the patient's diary.
At home	*Days 28* ±2–*56* ± *2*	➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Artificial tears use during 4 weeks of FU (diary) Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments
Visit 4 Follow up week 8	*Day 56± 2*	The following procedures will be performed (the below order is mandatory): ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal Horizontal Diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluorescein staining) ➢ Concomitant ocular and systemic medications ➢ Frequency of patient's artificial tear use during 4 weeks of FU (to be reported in eCRF). ➢ AE monitoring During visit 4, week 8, PI or delegate must check the patient's diary concerning the follow up period. The Investigator will dispense to the patient the preservative free artificial tears (Blink® Tears or equivalent), provided by Sponsor, to be self-administered three times daily, one drop in both eyes during the second 4 weeks of Follow up. The PI or delegate has to explain to the patients that, only if strictly needed, they can administer an additional numbers of drops of Blink® Tears or equivalent, by documenting all information in the patient's diary.
At home	*Days 56 ±2–84 ± 4*	➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Artificial tears use during 4 weeks of FU (diary) Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments
Visit 5 Follow up week 12	*Day 84 ± 4*	The following procedures will be performed (the below order is mandatory): ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal Horizontal Diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluoresce instaining) ➢ Concomitant ocular and systemic medications ➢ Frequency of patient's artificial tear use during 4 weeks of FU (to be reported in eCRF). ➢ AE monitoring During visit 5, week 12, PI or delegate must check and collect the patient's diary concerning the follow up period and provide a new diary for the second follow up period. The Investigator will instruct the patient that during the follow up period (from week 13 to week 24) other topical treatments will be allowed at discretion of the treating physician. Such treatments will be recorded by the investigators. The patient has to register the use other preservative free artificial tears in the patient's diary.
At home	*Days 84 ± 4–168 ± 7*	➢ Recording any new or changes in concomitant medications (diary) ➢ Recording any unusual medical conditions - AE monitoring (diary) ➢ Artificial tears use during 4 weeks of FU (diary) Data will be recorded by the patient on the patient's diary.

	Day	Procedures/Assessments
Visit 6 Follow up week 16	*Days 112* ± 7	The following procedures will be performed (the below order is mandatory): ➢ Pregnancy test for female patients of childbirth potential ➢ Ocular examination of both eyes to be performed by the physician investigator: • Assessment by SANDE questionnaire • Assessment by IDEEL questionnaire • Assessment of best corrected distance visual acuity (BCDVA) • External Ocular Examination • Schirmer test I (without anesthesia) • Slit-lamp examination (SLE) to assess the Eyelid - Meibomian glands, Eyelid – Erythema, Eyelid - Edema Lashes, Conjunctiva Erythema, Lens, Iris, Anterior Chamber, Corneal Horizontal Diameter • TFBUT • Ocular surface staining (NEI score - corneal and conjunctival fluorescein staining) • ➢ Concomitant ocular and systemic medication ➢ Frequency of patient's artificial tear use during 4 weeks of FU (to be reported in eCRF) ➢ AE monitoring PI or delegate must check the patient's diary concerning the previous follow up period.

Visit 7 Follow up week 24 **Final visit or early termination visit (ETV)**	*Days 168 ± 7*	The End of Study (EoS) visit is defined as the last follow-up at week 24. In case of premature study discontinuation, patients will undergo an Early Termination Visit (ETV). ➢ Concomitant ocular and systemic medication ➢ Frequency of patient's artificial tear use during 8 weeks of FU (to be reported in eCRF) ➢ AE monitoring PI or delegate must check and collect the patient's diary concerning the last follow up period.

6.3. EARLY WITHDRAWAL FROM THERAPY OR ASSESSMENT

6.3.1. Primary Reason For Discontinuation From The Study

A premature discontinuation will occur when a patient who signed the ICF ceases participation in the study, regardless of circumstances, before the completion of the study protocol procedures. Patients can be prematurely discontinued from the study for one of the following reasons:

- **Adverse event** (AE): Any significant AE that, in the opinion of the Investigator or concerned patient, is not compatible with study continuation.
- **Disease progression or worsening,** that according to the opinion of the Investigator is not compatible with study continuation..
- **Death**.
- **Lost to follow-up** (Every effort must be made to contact the patient; a registered letter must be sent).
- **Non-compliance with study drug**: an indication that a patient has not agreed with or followed the instructions related to the study medication.
- **Severe Protocol violation**: an event or decision that stands in contrast to the guidelines set out by the protocol.

- **Study terminated by the Sponsor**: an indication that a clinical study was stopped by Sponsor.
- **Withdrawal of consent**: study discontinuation requested by a patient for whatever reason.
- **Other reasons**, such as administrative reasons or pregnancy.

Before removal, each case should first be discussed with Dompé farmaceutici S.p.A.

The reasons for premature discontinuation from the study will be reflected on the Study Termination Record of the eCRF. Unless the patient has withdrawn consent, the follow up visit assessments should be performed as detailed in § 6.

The investigator should advise patients that prematurely discontinue on any therapies or treatments for their condition and refer them for further treatment as appropriate

6.3.2. Discontinuation procedures

Treatment discontinuation
Patients who discontinue the treatment will not be withdrawn from the study by default, but will be asked to complete safety and efficacy observations as per the protocol, unless otherwise they withdraw their consent.

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

- ask the patient to undergo, as far as possible, a final medical visit (Early Termination Visit - ETV) to examine the patient's health conditions. This examination will verify that all values tested at screening have remained within a clinically acceptable range (i.e., not clinically significant changes compared to screening).
- arrange for alternative medical care of the withdrawn patient, if necessary.
- report in the eCRF date and time of the last dose administration, and date and primary reason of study discontinuation.
- record in the eCRF any follow-up if the patient is withdrawn for an AE. AE's should be followed until resolution.

6.3.3. Replacement procedure

Patients in this study who prematurely discontinue treatment will not be replaced.

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

6.4. END OF STUDY

Patients completing the double-blind treatment and follow-up (Visits 1 Baseline to Visit 7 Follow up) will be considered completers.

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

7. ENDPOINTS

7.1. STUDY ENDPOINTS

The study objective is to assess the efficacy and safety of cenegermin when administered as ophthalmic solution to patients with moderate to severe dry eye.

Evaluation of the clinical efficacy and safety during and at the end of treatment with cenegermin, will be performed on the basis of the following assessments at each time point.

Evaluations will be performed on day -8 (screening), day 1+2 (visit baseline), day 14±2 (week 2), day 28±2 (week 4 End of treatment), day 56±2 (week 8 Visit 4 FU), day 84±4 (week 12 Visit 5 FU), day 112±7 (week 16 Visit 6 FU) and day 168±7 (Week 24 Visit 7 FU), according to the schedule of evaluation (§ 2)

7.1.1. Co-Primary endpoints

- Schirmer I test (without anesthesia) >10mm/5min at week 4.
- Change from baseline in Symptoms questionnaire (SANDE) global score at week 12

7.1.2. Secondary endpoints

- Change from baseline in Schirmer I test (without anesthesia) [Time frame: week 4, 8, 12 and 16];
- Change from baseline in Cornea and conjunctiva vital staining with fluorescein (National Eye Institute [NEI] scales) [Time frame: week 4, 8, 12 and 16];
- Change from baseline in Tear Film Break-Up Time (TFBUT) [Time frame: week 4, 8, 12 and 16];
- Change from baseline in Symptoms questionnaire (SANDE) scores for severity and frequency [Time frame: week 8, 12 and 16];
- Number of patients experienced a worsening in symptom scores (SANDE) and/or NEI score ≥ 50% assessed at week 4;
- Quality of life (IDEEL) questionnaire [Time frame: week 4, 8, 12 and 16].

7.1.3. Exploratory endpoint

- CCI [redacted]

assessed at week 2;

- CCI [redacted]

7.1.4. Safety endpoint

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

8. EVALUATION OF ADVERSE EVENTS AND SAFETY INFORMATION

8.1. DEFINITIONS

Adverse Event

An **Adverse Event** (**AE**) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Adverse Drug Reaction

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

The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product. For the purposes of regulatory safety reporting, "reasonable possibility" means there are facts (evidence) or arguments to suggest a causal relationship between the drug and the adverse event. Adverse events are to be considered unrelated if the relationship to the study drug, as described in the table in § 8.3.1, is none or unlikely; whereas any AE reported in the study having a possible, probable or highly probable relationship to study drug will be considered as an ADR.

Serious Adverse Event

A **Serious Adverse Event** (**SAE**) is defined as any adverse experience that, in the view of either the Investigator or Sponsor, meets any of the following criteria:

- results in death,
- is life-threatening (i.e. the patient was at immediate risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization.

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

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

- is a congenital anomaly/birth defect,
- is an important medical event.

NOTE: An important medical event is an event that may not result in death, be life threatening, or require hospitalization, but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the patient's wellbeing and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization or the development of drug dependency or drug abuse

Pre-planned hospitalization or hospitalization for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition are not considered to be SAEs (see Par. 8.4.2). These events must be recorded in the AE page of the eCRF where a variable will be ticked to indicate that they are not SAEs. Death shall always be reported as SAE and cause of death shall always be specified when known.

Unexpected Adverse Event/Reaction

An AE or ADR is considered unexpected if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed and listed in the Investigator Brochure. Events that are mentioned in the Investigator Brochure (section Reference Safety Information) as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation are considered unexpected. The determination of expectedness is made by the Sponsor on the basis of the IB Reference Safety Information (RSI) section.

Suspected serious unexpected adverse reaction

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

Adverse Events (AEs) of special Interest (Sight-threatening Events)

The following adverse events are considered to be of special interest and by default shall be reported as SAEs (medically important criteria):

- AEs that caused a decrease in visual acuity of >30 ETDRS letters or > +0.6 LogMAR (compared with the last assessment of visual acuity at the last visit) lasting >1 hour
- AEs that caused a decrease in visual acuity to the level of Light Perception or worse lasting >1 hour
- AEs that required surgical intervention (e.g., conventional surgery, vitreous tap or biopsy with intravitreal injection of anti-infectives, or laser or retinal cryopexy with gas) to prevent permanent loss of sight
- AEs associated with severe intraocular inflammation (i.e., 4+ anterior chamber cell/flare or 4+ vitritis)
- AEs that, in the opinion of the Investigator, may require medical intervention to prevent permanent loss of sight.

8.2. ADVERSE EVENT (AE) MONITORING

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

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

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

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

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

8.3. RECORDING

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

Adverse Events:

All AEs (non-serious and serious) that occur during the course of the study will be recorded in the eCRF. Any pre-existing medical conditions or signs/symptoms present in a patient prior to the start of the study (i.e., before informed consent is signed) should be specified in the dedicated eCRF sections. Subsequent to signing an informed consent form, all untoward medical occurrences that occur during the course of the study must be documented on eCRF. AEs will be collected till last Follow Up visit (week 24).

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

Serious Adverse Events:

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

8.3.1. Relationship of AEs to the Investigational Product

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

Relationship of the Adverse Event to the IMP

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

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

8.3.2. Severity of AEs

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

Severity of the Adverse Event

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

8.4. SERIOUS ADVERSE EVENT REPORTING

8.4.1. Reporting Procedure for Investigators to Dompé and CRO

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

PPD **Safety reporting**
Safety.PPD ;
Dompé Contact information
Dompé Drug Safety
PPD

Dompé Medical Expert
PPD

Dompé Clinical Operations
PPD

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

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

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

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

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

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

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

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

8.4.2. Conditions that should not be reported as serious adverse events

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

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

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

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

8.4.3. Reporting Procedure to EC/IRB and to Regulatory Authorities

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

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

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

The Investigators in turn shall notify their IRB.

According to US regulations, the Investigators will be informed in an unblinded fashion: Dompé will open the blind and report only SUSAR referred to Oxervate® (cenegermin) ophthalmic solution.

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

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

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

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

Dompé shall report SUSAR to the Regulatory Authorities in the EU and to ECs, as per applicable requirements, within 7/15 calendar days.

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

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

8.4.4. Periodical Reporting to Regulatory Authorities

Dompé shall be responsible to prepare and submit annual safety reports (Development Safety Update Report – DSUR) to relevant Regulatory Authorities.

8.5. UNMASKING OF THE STUDY TREATMENT

Masked information on the identity of the assigned investigational product will be provided for each patient. Unblinding can only occur in case of emergency, when knowledge of the treatment identity is essential for treating the subject. If the treatment code needs to be broken in the interest of patient safety for a medical emergency, the Investigator is allowed to break the treatment code for the specific patient, even before informing the Sponsor. The Investigator must always notify the Sponsor, so that the reason for any premature unmasking can be documented, by means of a communication to CRO/Dompé Drug Safety to the contact details in the section "Contact Information" and to Dompé Medical Expert. The Investigator will inform the Dompé representative (Dompé Medical Expert) if an emergency unmasking was performed without revealing the treatment identity, in order to avoid a dissemination of unmasked information..

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

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

8.6. FOLLOW-UP OF PATIENTS WITH ADVERSE EVENTS (AES)

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

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

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

8.7. PREGNANCY IN THE CLINICAL TRIAL

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

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

The Investigator must report every pregnancy on a pregnancy report form as soon as possible (within 24 hours of learning of the pregnancy) to PPD /Dompé Drug Safety contacts reported at Paragraph 8.4.1, even if no AE has occurred, and follow it to term.

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

If the pregnancy is associated with a SAE (e.g. if the mother is hospitalized for dehydration), in addition to the pregnancy report form, a separate SAE report form must be filed as described in § 8.4 with the appropriate serious criterion (e.g. hospitalization) indicated on the SAE report form. Miscarriage, stillbirth and any malformation/disease must be reported as a SAE. Any pregnancy leads to the immediate cessation of the study treatment.

8.8. ADVERSE EVENTS CAUSING TREATMENT DISCONTINUATION

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

8.9. OVERDOSE

Overdose (accidental or intentional) which may or may not result in serious adverse reactions, shall be reported to Sponsor Drug Safety/PPD by email or fax, following the same procedure for SAE, within 24 hours from the Investigator's knowledge of its occurrence. This includes reports related to drug intake through different routes (e.g. ingestion) or with suicidal intentions and consequent drug overdose.
An overdose of Oxervate®20 mcg/mL of cenegermin ophthalmic solution is defined as the administration of 50% or more additional drops on any given treatment day.

The Investigator shall provide in the SAE form information about symptoms, corrective treatment and outcome of overdose. The Medical Expert should be contacted to discuss corrective treatment, if necessary.

9. STATISTICS

9.1. SAMPLE SIZE

The sample size of the study is calculated based on results from previous studies.

Considering a randomization ratio 1:1 and a one-sided alpha of 0.025, a total of 90 evaluable patients will allow to achieve an overall power of 90% to show superiority of cenegermin 20 mcg/mL ophthalmic solution vs vehicle in terms of both co-primary endpoints, expecting a difference of 30% in Schirmer I rate of responders in favour of cenegermin 20 mcg/mL ophthalmic solution treatment at Week 4 and a 15 points Global SANDE score improvement at Week 12 (with a SD of 20 points).

Since both endpoints must be met in order to claim superiority, no multiplicity correction of alpha is required Assuming that 10% of subjects will not be evaluable for primary analysis, a total of approximately 100 subjects is expected to be enrolled.

9.2. ANALYSIS POPULATION

The following population will be defined:

- The Safety (SAF) population will consist of all randomized patients who received at least one dose of the investigational product. Safety population will be analyzed according to the actual treatment received. The SAF population will be used to present results on safety data.
- The Full Analysis Set (FAS) population will consist of all randomized patients who received at least one dose of the investigational product. FAS population will be analyzed according to ITT principle, i.e. by treatment allocation. The FAS population will be used for the primary analyses of the study and to present results on efficacy data.
- The Per Protocol (PP) population will consist of all randomized patients who received at least one dose of the investigational product and do not have Major Protocol Deviations. The PP population will be used for sensitivity analyses.

9.3. OVERVIEW OF PLANNED STATISTICAL ANALYSES

The study plans for the following statistical analyses:

- Final analysis: this analysis will be conducted when all enrolled subjects have completed the treatment at week 4 and FU at week 12. After data up to week 12 have been locked, results will be provided in an aggregate way without unblinding the investigators and study personnel about treatment codes.
- Addendum to final analysis: this additional analysis will be conducted when all enrolled subjects have completed the FU at week 24, and the study database has been locked and unblinded. A dedicated addendum to the CSR will be created.

9.4. STATISTICAL METHODOLOGY

9.4.1. General Considerations

Appropriate descriptive statistics will be produced by treatment arms according to the nature of the variable. For continuous data, number of observations, mean, standard deviation, median and range (minimum and maximum) will be presented. For qualitative data, frequency distributions and percentages per category will be presented. If appropriate, confidence intervals around the mean or the proportions will be presented. The number of subjects with missing data will be presented under the "Missing" category. Missing values will not be included in the denominator count when computing percentages. When continuous data will be summarized, only the non-missing values will be evaluated for computing summary statistics. Any exception will be declared.

For the purpose of efficacy analysis in this trial, the statistical analysis (both descriptive and inferential) of some endpoints will be done on the eligible eye when applicable. Details will be provided in the statistical analysis outputs as notes.

Unless otherwise specified, the significance level used for statistical testing will be 0.05 and two-sided tests will be used. All patient data collected on the CRF will be listed by patient and centre.

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

9.4.2. Primary Estimand

A Treatment Policy Estimand is defined by the following:

- Population: Subjects in the FAS population (section 9.2);
- Variable: both signs (Schirmer I test) and symptoms (SANDE global score) endpoints at week 4 and 12, respectively;
- Intercurrent event: The occurrence of an intercurrent event is irrelevant. All observed values will be used regardless of occurrence of an intercurrent event. Retrieved drop-outs will be used for data imputation of missing data;
- Population-level summary: proportion of patients reaching a value of Schirmer I test (without anesthesia) >10mm/5min at week 4, and change from baseline in the global SANDE score at week 12.

9.4.3. Analysis of efficacy variables

9.4.3.1. Primary analyses

The following null hypothesis is defined on the first primary endpoint: the proportion of patients reaching a value of Schirmer I test (without anesthesia) >10mm/5min at week 4 in cenegermin (rhNGF) is lower or equal than control:

H_{01}: $T_{rhNGF} \leq T_{CONTROL}$

H_{11}: $T_{rhNGF} > T_{CONTROL}$

where T_{rhNGF} and $T_{CONTROL}$ are the proportion of patients reaching a value of Schirmer I test (without anesthesia) >10mm/5min at week 4 for cenegermin and control groups, respectively. The null hypothesis H_{01} will be rejected if the associated primary analysis p-value will be lower than 0.025.

This primary endpoint will be analyzed by means of a logistic regression adjusting by pre-defined baseline factors (site, gender, age class, baseline Schirmer I test value) and a one-sided test will be used to test for differences between treatment groups.

Similarly, the following null hypothesis is defined on the co-primary endpoint: the change from baseline in the global SANDE score at week 12 in cenegermin is lower or equal than control:

H_{02}: $\mu_{rhNGF} \leq \mu_{CONTROL}$

H_{12}: $\mu_{rhNGF} > \mu_{CONTROL}$

where μ_{rhNGF} and $\mu_{CONTROL}$ are the change from baseline in the global SANDE score at week 12 for cenegermin and control groups, respectively. The null hypothesis H_{02} will be rejected if the associated primary analysis p-value will be lower than 0.025.

This coprimary endpoint will be analyzed by means of an ANCOVA model adjusting by pre-defined baseline factors (site, gender, age class, baseline global SANDE score) and a one-sided test will be used to test for differences between treatment groups.

Both null hypotheses H_{01} and H_{02} must be rejected in order to claim superiority of cenegermin over control. Consequently, no multiplicity correction of type I error will be applied on primary endpoints analysis.

Since patients who discontinue the treatment with the IMP will not be withdrawn from the study but will be asked to complete safety and efficacy assessments as per the protocol, missing data under treatment policy strategy will be addressed by modeling patients with missing data after retrieved drop-outs (based on assumption of missing data would have been like retrieved drop-outs if they were assessed). Retrieved drop-out patients are defined as patients who discontinue study treatment and decide to remain in the study by following the schedule of assessments and continuing to adhere to protocol requirements.

If enough retrieved drop-out data are available to allow convergence of a regression model, a Multiple Imputation model based on retrieved drop-outs (MI-RD) will be used for analysis. Specifically, MI will be performed based on the subjects' allocated treatment arm and observed values (baseline and intermediate) as covariates in a regression model using data from subjects that discontinued the treatment but have the primary endpoint measurement with gender, and age class as covariates. For each co-primary endpoint, MI-RD will be implemented into different steps:

- Intermittent missing data up to primary time point (Week 4 for Schirmer I test, and Week 12 for SANDE) will be imputed using MCMC methods assuming missing at random (MAR) data;
- The remaining missing values with a monotone missing data pattern will be imputed based on observed data of retrieve dropouts;

- the fully imputed datasets will be analyzed and results will be combined using Rubin's to draw inference.

If not enough data was retrieved after study treatment discontinuation for assure the convergence of the MI-RD regression model (the final decision will be done at the time of the analysis and reported in the CSR; different approaches might be used for the two coprimary endpoint), the same model will be fit using data from subjects of control group, washing-out the effect of treatment; this approach does not assume benefits for cenegermin in case of discontinuation and limits a post-discontinuation clinical effect to that of vehicle. Specifically, wash-out approach will be implemented in this way:

- Intermittent missing data up to primary time point (Week 4 for Schirmer I test, and Week 12 for SANDE) will be imputed using MCMC methods assuming MAR;
- The remaining missing values in the vehicle group with a monotone missing data pattern will be imputed based on observed data of the control patients with available endpoint, while the remaining missing values in the treatment group with a monotone missing data pattern will be imputed based on observed and imputed data of the control patients;
- All imputed data will be pooled and analyzed, and results will be combined using Rubin's to draw inference.

Whether for MI-RD or wash-out approach, the adjusted estimated treatment differences between cenegermin and vehicle (difference in proportions for Schirmer's test and differences in means for SANDE) will be displayed together with the corresponding two-side 95% confidence intervals and p-values.

9.4.3.2. Sensitivity analyses

The following sensitivity analyses are defined to assess the robustness of results on each primary endpoint versus assumptions used in the statistical model for the main estimators :

- The comparison between treatment and control will be performed in the FAS population by means of MI under MAR assumption instead of missing not at random (MNAR). MI will be implemented in two steps:
    - Intermittent missing data up to primary time point (Week 4 for Schirmer I test, and Week 12 for SANDE) will be imputed using MCMC methods assuming MAR. A separate imputation model will be used for each treatment arm. The imputation models will include the observed values (baseline and intermediate), gender, and age class as covariates. In case of non-convergence or non-estimability issues, a single model will be considered with treatment arm added as explanatory variable to the model;
    - The remaining missing values with a monotone missing data pattern will be imputed based on data of corresponding treatment group.

  Each imputed dataset will be analyzed using observed and imputed as described in section 9.4.3.1. Rubin's rule will be used for combining results to draw inference. The adjusted estimated treatment differences between cenegermin and vehicle (difference in proportions for Schirmer's test and

differences in means for SANDE) will be displayed together with the corresponding two-side 95% confidence intervals and p-values.

- A tipping point strategy will be used as a sensitivity analysis for missing data for assessment of superiority (if shown) of cenegermin. For each coprimary endpoint, tipping point, will assess how departures from MI under MNAR assumptions must be in order to overturn conclusions from the primary superiority analysis. Tipping point will be based on iterative application of MI-RD (or MI under wash-out approach), where the imputed values for the cenegermin arm are shifted at a constant Δ to represent a worse effect in each iteration. The tipping points are the smallest Δs at which no statistical significance is shown ($p \geq 0.025$).

In addition, for each primary endpoint, the regression models detailed in section 9.4.3.1 will be performed for supportive purposes:

- by considering complete cases only (i.e. without considering patients with missing primary endpoint);
- on the PP set instead of FAS.

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

9.4.3.3. Secondary analyses

In case analysis of the primary endpoints leads to rejection of null hypotheses, the following key secondary endpoints will be tested in a conditional sequential manner to show superiority of cenegermin versus control (at alpha one-sided 0.025) according to the following ranking:

1. Change from baseline in Schirmer I test (without anesthesia) at week 8;
2. Change from baseline in SANDE scores for severity at week 12;
3. Change from baseline in SANDE scores for frequency at week 12;
4. IDEEL modules (Quality of Life [27 questions], Dry eye Treatment satisfaction & Bother [10 questions], and Dry eye Symptom bother [20 questions]) at week 12 and at week 4 (in this order);
5. Change from baseline in Cornea and conjunctiva vital staining with fluorescein NEI scales at week 4, week 8 and at week 12 (in this order);
6. Change from baseline in TFBUT at week 4, week 8 and at week 12 (in this order).

This hierarchical test strategy protects the family-wise false positive error rate at the overall one-sided 0.025 level.

Key secondary endpoints 1., 2., and 3. will be analyzed as detailed in section 9.4.3.1.

Changes from baseline in each IDEEL modules scale score will be analyzed using an mixed model for repeated measures (MMRM). The analyses will include the fixed, categorical effects of treatment, visit (4 levels: Weeks

4, 8, 12, 16) and treatment by visit interaction. Subject will be considered as a random effect. The covariance matrix used will be "unstructured". Comparisons versus vehicle TID will be provided using least square means at each visit and overall. Missing data will be imputed according to the questionnaire manuals.

Changes from baseline in TFBUT and Fluorescein staining (NEI scale) will be analyzed by means of an ANCOVA model adjusting by pre-defined baseline factors (site, gender, age class, corresponding baseline value). Missing data will be imputed according to MI-RD (or wash-out approach, see section 9.4.3.1).

In case of not rejection of null hypotheses, the above test strategy will not be performed. Instead, independently of results on primary endpoints, descriptive in nature analyses will be performed on all secondary endpoints at each available timepoints by means of descriptive statistics and by appropriate parametric tests depending on the nature of the variable and its distribution. Data transformation might be used in order to satisfy the assumption of normality requested by parametric statistical tests. In case such assumptions are not met, non-parametric counterpart tests will be used. Change from baseline value (for continuous variables) and shift tables versus baseline (for categorical variables) will also be summarized for all post-baseline visits.

Further details will be provided in the SAP.

9.4.4. Analysis of CCI variables

In addition to descriptive statistics at each available time point, CCI variables will be analyzed by means of inferential tests depending on their nature and distribution (all confidence intervals and statistical tests on CCI endpoints are of descriptive nature). Change from baseline value (for continuous variables) and shift tables versus baseline (for categorical variables) might be reported for all post-baseline visits.

Correlation between sign and symptom scores at different time points will be assessed.

9.4.5. Analysis of safety variables

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

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

Individual AEs will be listed in patient data listings.

9.4.6. Subgroup analysis

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

- Age class (≤Median, >Median),
- Race,
- Ethnicity.

Statistical tests for interaction (between subgroup variable and treatment arm) will be performed before investigate further subgroups: analyses will be performed if interaction tests between treatment and variable is statistically significant at 15% nominal level. Variables that may be evaluated after test for interaction are:

- Region (EU, US),
- Sjögren's syndrome (primary, secondary),
- Medical history,
- Concomitant medication.

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

9.4.7. Missing data

All reasonable efforts will be made to reduce the rate of missing data. Investigators will be trained about the importance of patient retention and full data capture. Also, any reasonable attempts should be made by the Investigators to emphasize continued subject's participation for the full duration of the trial. However, in order to minimize missing data, if a patient cannot refer to the site for a planned follow-up visit, the Investigator will try to obtain any relevant information from the patients, including documents/laboratory results available from local medical care.

10. ETHICAL CONSIDERATIONS

10.1. INSTITUTIONAL REVIEW BOARD AND INDEPENDENT ETHICS COMMITTEE

United States

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

Europe

This study will be carried out in full compliance with the guidelines of ethics committee (EC) and government agencies of each respective country as well as the European Union (EU) Clinical Trial Directive (Directive 2001/20/EC), where applicable. Before the study begins, the Sponsor will require approval from an EC and Regulatory agency. During the course of the study, the Sponsor or authorized contract research organization (CRO) representative will provide timely and accurate reports to the EC on the progress of the study, at intervals not exceeding 1 year (or as appropriate), and will notify the EC of SAEs or other significant safety findings. The study protocol, ICF, information sheet advertisements, and amendments (if any) will be approved by the EC at the study centers in conformance with the EU Clinical trial directive (Directive 2001/20/EC), and local regulations.

10.2. ETHICAL CONDUCT OF THE STUDY

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

10.3. DATA MONITORING COMMITTEE

A Data Monitoring Committee is not required for this trial considering the following point:

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

10.4. PATIENT INFORMATION AND CONSENT

Eligible patients can take part in the study only after providing the written informed consent approved by the EC/IRB. Informed consent must be obtained before starting any procedure pertaining to the study (i.e. all the procedures described in the protocol). A Patient Information Sheet and Informed Consent Form, which meet regulatory requirements and are appropriate for this study, will be provided to the patient.

Each patient will read or be read (if he or she cannot read or write), assent understanding of, and sign or thumbprint an instrument of informed consent and after having had an opportunity to discuss them with the PI before signing; each patient will be made aware that he or she may withdraw from the study at any time.
The informed consent statement contains all the elements of informed consent and contains all the core elements and mandatory statements as defined in the CFR. Signed copies of the ICF and Patient Information Sheet will be given to the patient, and both documents will be placed in the Investigator's site files. A unique patient identification (PID) number will be assigned according to § 4.3 of the protocol at the time the patient signs the ICF.

10.5. CONFIDENTIALITY

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

On the CRFs, or Diary,patients will be identified ONLY by the assigned patient number. If patient names are included on copies of documents submitted to Dompé farmaceutici s.p.a. or CRO, the names will be obliterated or masked and the assigned patient number added to the document.

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

10.6. COMPENSATION FOR MEDICINE-INDUCED INJURY AND INDEMNIFICATION

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

11. DATA HANDLING AND RECORD KEEPING

11.1. CASE REPORT FORMS

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

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

The eCRF must be available for review to designated Dompé's representatives at each scheduled monitoring/audit visit.The PI is responsible for verifying that all data entries in the eCRFs are accurate and correct. The PI must sign the completed eCRF before database lock and its submission to the Sponsor.

Patient's diary

The patient will report in the Diary the details of self-administration at home of the IMP, as well as any new or changes in concomitant medications, any unusual medical conditions and the use of artificial tears. It is responsibility of the Investigator to explain to each patient how to enter the data in the Diary and to check the data inserted in the Diary to ensure correct completion as well as correct administration of the IMP.

The information collected in the Diary will be part of the patient's data collected.

Data reported on Diary is the sole property of Dompé and should not be made available in any form to third parties, except for authorized Dompé designee or representatives of appropriate Health/Regulatory Authorities, without written permission from Dompé.

11.2. DATA MANAGEMENT

Main Data Management activities and procedures will be accurately described in the DMP, created by PPD and approved by Dompè.

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

or her patients' data, will be collected. . Reconciliation of study data and SAEs between Clinical and Drug Safety database will be performed by PPD in ongoing basis and the before database lock. Procedure will be detailed in the DMP.

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

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

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

11.3. DOCUMENTATION REQUIRED PRIOR TO INITIATION OF AND DURING THE STUDY

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

- Current, signed and dated Curriculum Vitae of Principal Investigator and any Sub-Investigators/co-workers. Updates should be provided at least every two years.
- A signed page of the final protocol and any amendments.
- A signed copy of the study Financial Agreement/Clinical Study Agreement with the CRO, including all study specific costs.
- List and any updates of delegated responsibility (Study Team Signature List / Delegation of Responsibilities form).
- A financial disclosure agreement completed and signed by the PI and all Sub-Investigators listed on Form FDA 1572. If applicable, the PI will provide an updated financial disclosure agreement to the Sponsor 1 year after the completion of the study.

11.4. ESSENTIAL DOCUMENT RETENTION

The Investigator will retain copies of all the essential documents (as defined by ICH-GCP E6 R2) until at least 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the Investigational Product. These documents should be retained for a longer period however if required by the applicable regulatory requirements. The Investigator should take measures to prevent accidental or premature destruction of these documents.
The essential documents include at least: the signed protocol, copies of the completed eCRFs, signed Patient Informed Consent Forms from all patients who consented, hospital records and other source documents, and all other documentation included in the Investigator Site File and Pharmacy/Dispensing File.

The Investigator will inform the CRO of the storage location of these essential documents and must contact the Sponsor before disposing of any. If the Investigator wishes to assign the files to someone else or to remove them to another location, he/she should consult with the Sponsor. about this change.
Dompé farmaceutici S.p.A. will inform the Investigator in writing when these documents no longer need to be retained.

12. STUDY MANAGEMENT

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

12.1. REGULATORY BODY APPROVAL

Dompé or the CRO or other consultant appointed by Dompé will obtain the necessary approval from the Competent Authorities, as needed, prior to initiation of the study. The study will not be started until written approval from the relevant Competent Authorities (or no objection within the timeframe set by the local regulation, as applicable) has been received by Dompé.
Where appropriate, any amendments to the Protocol will be sent to the Competent Authority/FDA.

12.2. MONITORING

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

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

Monitoring visits or remote monitoring will be conducted to confirm that e.g.:
• The investigational team is adhering to the study protocol
• Informed consent has been adequately obtained from all participants prior to study specific procedures being performed
• AEs have been reported and followed up as required and any SAEs have been reported according to the requirements
• Data are being accurately recorded in the eCRFs in a timely manner

• The Investigator's Site File is being adequately maintained
• Facilities and staffing resources are, and remain, acceptable and sufficient throughout the study
• The Investigator and the site are receiving sufficient information and support throughout the study

Moreover, during on-site monitoring visits the data recorded in the eCRFs, source documents and other study-related records will be compared against each other in order to ensure accurate data that reflect the actual existence of the subject in the study i.e. source data verification.
Monitoring procedures are described in the concerned Standard Procedural Documents of the CRO in charge and in the Monitoring Plan, which also encompasses situational changes, e.g. intermediate switch to remote monitoring only if site visits are not possible; details concerning the nature and extent of Source Data Verification are also provided in the Monitoring Plan.

12.3. ACCESS TO RECORDS

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

12.4. AUDIT AND INSPECTION

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

12.5. PROTOCOL AMENDMENTS

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

12.6. DISCONTINUATION OF THE STUDY

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

12.7. PUBLICATIONS

All data generated in this study will be the property of Dompé farmaceutici S.p.A. Publication of the results by the PI will be subject to mutual agreement between the PI and Dompé farmaceutici S.p.A.
The Sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the Investigator publishing in peer reviewed journals; presenting results at scientific congresses; and posting information and results on internet-based public registers and databases.

In any case, study results will be communicated in full to the related competent authorithies for the countries involved by the submission of a complete Clinical Study Report.

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

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

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

On an exceptional basis, the Sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures etc.) to seek necessary intellectual property protection. This is because early disclosure of such a data could, in some circumstances, prevent or negatively impact patentability.

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14 APPENDICES

14.1 APPENDIX 1-SPONSOR APPROVAL PAGE

NGF0121 (PROTEGO-1 study): A 4-week, Phase III, multicenter, double-masked, vehicle-controlled clinical study to evaluate safety and efficacy of Oxervate® (cenegermin) 20 mcg/mL ophthalmic solution versus vehicle, in patients with severe Sjogren's dry eye disease

Sponsor Medical Expert: PPD PPD _____Date: ____/_____/_____

PPD

Sponsor Chief Medical Officer PPD PPD _____Date: _____/____/_____

Flavio Mantelli, Chief Medical Officer

Sponsor Clinical Trial Manager: PPD PPD _____Date: _____/____/____

PPD

14.2 APPENDIX 2-INVESTIGATOR'S SIGNATURE PAGE

Investigator's Statement

I have read study protocol NGF0121 *(PROTEGO-1)* from the title *"A 4-week, Phase III, multicenter, double-masked, vehicle-controlled clinical study to evaluate safety and efficacy of Oxervate® (cenegermin) 20 mcg/mL ophthalmic solution versus vehicle, in patients with severe Sjogren's dry eye disease (PROTEGO-1 study)*" and agree to conduct the study as outlined in the protocol, and in accordance with the Declaration of Helsinki, ICH-GCP E6 (R2) and any local regulations, being responsible for personally supervise the study conduct and ensure study staff complies with protocol requirement.

Name of Principal Investigator (block letters):_____

Signature: _____ Date: _____

14.3 APPENDIX 3 AMERICAN-EUROPEAN CONSENSUS CRITERIA FOR SJÖGREN'S SYNDROME

Reference: Vitali C, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002; 61:554-558[19].

In order to make a diagnosis of Sjögren's syndrome, the following criteria must be met:

I. Ocular Symptoms (at least one)

- Symptoms of dry eyes for at least 3 months
- A foreign body sensation in the eyes
- Use of artificial tears 3 or more times per day

II. Oral Symptoms (at least one)

- Symptoms of dry mouth for at least 3 months
- Recurrent or persistently swollen salivary glands
- Need for liquids to swallow dry foods

III. Ocular Signs (at least one)

- Abnormal Schirmer's test, (without anesthesia; ≤5 mm/5 minutes)
- Positive vital dye staining of the eye surface

IV. Histopathology

- Lip biopsy showing focal lymphocytic sialoadenitis (focus score ≥1 per 4 mm2)

V. Oral Signs (at least one)

- Unstimulated whole salivary flow (≤1.5 mL in 15 minutes)
- Abnormal parotid sialography
- Abnormal salivary scintigraphy

VI. Autoantibodies (at least one)

- Anti-SSA (Ro) or Anti-SSB (La), or both

For a primary Sjögren's syndrome diagnosis:

- Any 4 of the 6 criteria, must include either item IV (Histopathology) or VI (Autoantibodies)
- Any 3 of the 4 objective criteria (III, IV, V, VI)

For a secondary Sjögren's syndrome diagnosis:

- In patients with another well-defined major connective tissue disease, the presence of one symptom (I or II) plus 2 of the 3 objective criteria (III, IV and V) is indicative of secondary SS.

Exclusion Criteria

- Past head and neck radiation treatment
- Hepatitis C infection
- Acquired immunodeficiency syndrome (AIDS)
- Pre-existing lymphoma
- Sarcoidosis
- Graft versus host disease
- Current use of anticholinergic drugs