18 May 2017
EMA/351805/2017
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

**OXERVATE**

International non-proprietary name: cenegermin

Procedure No. EMEA/H/C/004209/0000

**Note**

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.
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<tbody>
<tr>
<td>A280</td>
<td>Absorbance at 280 nm</td>
</tr>
<tr>
<td>AC</td>
<td>Acceptance criteria</td>
</tr>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AEX</td>
<td>Anion exchange chromatography</td>
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<tr>
<td>AQL</td>
<td>Acceptable Quality Level</td>
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<tr>
<td>Arg</td>
<td>Arginine</td>
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<tr>
<td>AS</td>
<td>Active substance</td>
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<tr>
<td>Asn</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>BCDVA</td>
<td>Best corrected distance visual acuity</td>
</tr>
<tr>
<td>BDS</td>
<td>Bulk Drug Solution</td>
</tr>
<tr>
<td>BID</td>
<td>twice a day</td>
</tr>
<tr>
<td>bps</td>
<td>Base pairs</td>
</tr>
<tr>
<td>BSE</td>
<td>Bovine spongiform encephalopathy</td>
</tr>
<tr>
<td>CCI</td>
<td>Container Closure Integrity</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
</tr>
<tr>
<td>CE</td>
<td>Conformité Européenne</td>
</tr>
<tr>
<td>CEX</td>
<td>Cation exchange chromatography</td>
</tr>
<tr>
<td>CIP</td>
<td>Cleaning in progress</td>
</tr>
<tr>
<td>CIPC</td>
<td>Critical in-process control</td>
</tr>
<tr>
<td>CIPT</td>
<td>Critical in-process test</td>
</tr>
<tr>
<td>Cmax</td>
<td>Maximum serum concentration</td>
</tr>
<tr>
<td>CPP</td>
<td>Critical process parameter</td>
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<tr>
<td>CSR</td>
<td>Clinical Study Report</td>
</tr>
<tr>
<td>CQA</td>
<td>Critical Quality Attribute</td>
</tr>
<tr>
<td>CTD</td>
<td>Common technical document</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVs</td>
<td>column volumes</td>
</tr>
<tr>
<td>2D/3D</td>
<td>2 dimensional/3 dimensional</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>%DO</td>
<td>% dissolved oxygen</td>
</tr>
<tr>
<td>DP</td>
<td>Drug Product</td>
</tr>
<tr>
<td>DS</td>
<td>Drug substance</td>
</tr>
<tr>
<td>EC50</td>
<td>50% (median) Effective Concentration</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EPC</td>
<td>End of production cells</td>
</tr>
<tr>
<td>EOR</td>
<td>Edge of range</td>
</tr>
<tr>
<td>ETDRS</td>
<td>Early Treatment Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>EQ-5D</td>
<td>EuroQol 5D</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FP</td>
<td>Finished product</td>
</tr>
<tr>
<td>FMEA</td>
<td>Failure modes and effects analysis</td>
</tr>
<tr>
<td>GMP</td>
<td>Good manufacturing practice</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>HCIC</td>
<td>Hydrophobic charge interaction chromatography</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
</tr>
<tr>
<td>HCP</td>
<td>Host cell protein</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human Embryonic Kidney 293 Cells</td>
</tr>
<tr>
<td>HETP</td>
<td>Height equivalent to the theoretical plate</td>
</tr>
<tr>
<td>5-HMF</td>
<td>5-hydroxymethylfurfural</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance Liquid Chromatography</td>
</tr>
<tr>
<td>IB</td>
<td>Inclusion body</td>
</tr>
<tr>
<td>IEX-HPLC</td>
<td>on exchange HPLC</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular Pressure</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol</td>
</tr>
<tr>
<td>IPC</td>
<td>In-process control</td>
</tr>
<tr>
<td>IPM</td>
<td>In-process monitoring</td>
</tr>
<tr>
<td>IPT</td>
<td>In-process test</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl ß-D-1-thiogalactopyranoside</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-to-Treat</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>Ki</td>
<td>inhibition constant</td>
</tr>
<tr>
<td>KPC</td>
<td>Key control parameter</td>
</tr>
<tr>
<td>KRS</td>
<td>Kanamycin reference standard</td>
</tr>
<tr>
<td>LAF</td>
<td>Laminar air flow</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny broth</td>
</tr>
<tr>
<td>LC/MS</td>
<td>Liquid chromatography mass spectroscopy</td>
</tr>
<tr>
<td>LDPE</td>
<td>Low density polyethylene</td>
</tr>
<tr>
<td>LOCF</td>
<td>Last Observation Carried Forward</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantitation</td>
</tr>
<tr>
<td>MCB</td>
<td>Master cell bank</td>
</tr>
<tr>
<td>4-MEP</td>
<td>4-mercaptoethyl-pyridine</td>
</tr>
<tr>
<td>MedDRA</td>
<td>Medical Dictionary for Regulatory Activities</td>
</tr>
<tr>
<td>MimD</td>
<td>mimetic nerve growth factor/neutrophin mimetic</td>
</tr>
<tr>
<td>mNGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>MO</td>
<td>Major objection</td>
</tr>
<tr>
<td>MPP</td>
<td>Monitored process parameter</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectroscopy</td>
</tr>
<tr>
<td>MSM</td>
<td>Mineral salt medium</td>
</tr>
<tr>
<td>MUC5aC</td>
<td>Goblet Cells Specific Mucin Secretion Marker</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>NEI-VFQ</td>
<td>National Eye Institute Visual Functioning Questionnaire 25</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>NK</td>
<td>Neurotrophic Keratitis</td>
</tr>
<tr>
<td>NKCP</td>
<td>Non-key process parameter</td>
</tr>
<tr>
<td>NLT</td>
<td>Not less than</td>
</tr>
<tr>
<td>NMT</td>
<td>Not more than</td>
</tr>
<tr>
<td>OC</td>
<td>Other concern</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
</tbody>
</table>
OD600  Optical density at 600 nm
p75  p75 (low affinity) neurotrophin receptor
PC12  Pheochromocytoma rat cells
PD  Pharmacodynamics
PED  Persistent epithelial damage
PEG  Polyethylene glycol
PETG  Polyethylene terephthalate
Ph. Eur.  European Pharmacopoeia
PK  Pharmacokinetics
pKa  Acid dissociation constant
pONT  Partial Optic Nerve Transection
ppb  parts per billion
proBA  operon Proline BA operon
ProNGF  Pro Nerve growth factor
PT  Preferred Term
PV  Process validation
qPCR  quantitative polymerase chain reaction
RCS  Royal College of Surgeons
RGC  Retinal ganglion cells
rhNGF  recombinant human Nerve Growth Factor
rhProNGF  Recombinant human ProNGF
(m)RNA  (messenger) ribonucleic acid
RP-HPLC  Reverse phase high performance liquid chromatography
RPM  Rotations per minute
%RSD  % relative standard deviation
SAP  Statistical Analysis Plan
SC  subcutaneous
SD  Standard Deviation
SDS-PAGE  Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE-HPLC  Size exclusion HPLC
Ser  Serine
SH-SYSY  Human neuroblastoma cell line
SIRC  Statens Seruminstitut Rabbit Cornea
SLPM  Standard litre per minute
S/N ratio  Signal to noise ratio
ssDNA  single stranded DNA
SOC  System Organ Class
TAMC  Total aerobic microbial count
TF-1  Human bone marrow erythroblast suspension cell line
TFA  Trifluoracetic acid
TFF  Tangential flow filtration
TMA  Trimethylamine
TMP  Transmemebrane pressure
TPP  Target product profile
TSE  transmissible spongiform encephalopathies
TrkA  Tropomyosin receptor kinase A
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYMC</td>
<td>Total yeasts and moulds count</td>
</tr>
<tr>
<td>UF/DF</td>
<td>Ultrafiltration diafiltration</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopoeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>4-VP</td>
<td>4-Vinylpyridine</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>WCB</td>
<td>Working cell bank</td>
</tr>
<tr>
<td>WFI</td>
<td>Water for injection</td>
</tr>
</tbody>
</table>
1. Background information on the procedure

1.1. Submission of the dossier

The applicant Dompé farmaceutici S.p.A. submitted on 3 November 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Oxervate, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 17 November 2016.

Oxervate was designated as an orphan medicinal product EU/3/15/1586 on 14 December 2015 in the following condition: neurotrophic keratitis.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Oxervate as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency’s website: ema.europa.eu/Find medicine/Human medicines/Rare disease designation.

The applicant applied for the following indication:

'Treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) neurotrophic keratitis in adults.'

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that cenegegmin was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants’ own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0280/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0280/2016 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised
orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

**Applicant’s request(s) for consideration**

**Accelerated assessment**

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

**New active Substance status**

The applicant requested the active substance cenegermin contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

**Scientific Advice/Protocol Assistance**


**1.2. Steps taken for the assessment of the product**

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Concepcion Prieto Yerro    Co-Rapporteur: Patrick Salmon

CHMP Peer reviewer(s): Agnes Gyurasics

- The application was received by the EMA on 3 November 2016.
- Accelerated Assessment procedure was agreed-upon by CHMP on 07 November 2016
- The procedure started on 24 November 2016.
- The Rapporteur’s first Assessment Report was circulated to all CHMP members on 25 January 2017. The Co-Rapporteur’s first Assessment Report was circulated to all CHMP members on 30 January 2017. The PRAC Rapporteur’s first Assessment Report was circulated to all PRAC members on 31 January 2017. In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- During the meeting on 09 February 2017, the PRAC agreed on the PRAC Assessment Overview and Advice to CHMP. The PRAC Assessment Overview and Advice was sent to the applicant on 10 February 2017.
- During the meeting on 21 February 2017 the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 21 February 2017.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 20 March 2017.
• The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 06 April 2017.

• During the CHMP meeting on 19 April 2017 the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.

• The applicant submitted the responses to the CHMP List of Outstanding Issues on 25 April 2017.

• The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Outstanding Issues to all CHMP members on 08 May 2017.

• During the meeting on 18 May 2017, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Oxervate.
2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Neurotrophic keratitis (NK) is a rare, degenerative corneal disease that occurs as a result of partial or total impairment of trigeminal innervation, leading to a reduction (hypoesthesia) in or loss (anaesthesia) of corneal sensation (Bonini et al., 2003; Sacchetti et al, 2014; Semeraro et al, 2014). Impairment of corneal trigeminal innervation causes a reduction in the lacrimation reflex, as well as morphological and metabolic corneal epithelial disturbances, with subsequent development of recurrent or persistent epithelial defects.

2.1.2. Epidemiology

As the underlying causes for NK are numerous and heterogeneous, accurate prevalence calculations are difficult to conduct. Based on the prevalence of a range of predisposing or accompanying conditions, NK prevalence can be estimated at less than 4.1 per 10,000 patients. Patients with stage 2 and 3 disease are estimated to constitute approximately one third of the total NK population.

2.1.3. Aetiology and pathogenesis

Several ocular and systemic conditions can be associated with damage at different levels of the fifth cranial nerve, from the trigeminal nucleus to the corneal nerve endings, resulting in the development of NK. The most common causes of impaired corneal sensation are herpetic keratitis (herpes simplex and herpes zoster viral infection), intracranial space-occupying lesions, and/or neurosurgical procedures that damage the trigeminal ophthalmic branch. Other ocular causes of impairment of corneal sensitivity include chemical burns, physical injuries, corneal dystrophy, chronic use of topical medications (topical anaesthetics, timolol, betaxolol, sulfacetamide, and diclofenac sodium), and anterior segment surgery involving nerve transection. Many systemic conditions are also associated with the development of corneal anaesthesia, including diabetes, multiple sclerosis, congenital syndromes, and leprosy.

Loss of corneal sensory innervation causes a marked change in the levels of neuromediators, and consequently dystrophic changes in corneal and conjunctival tissues. The corneal epithelium is usually the primary site of disease expression, showing a decrease in the vitality, metabolism and mitosis of epithelial cells and consequently epithelial breakdown. Epithelial breakdown can lead to ulceration, infection, melting, perforation secondary to poor healing, and, ultimately, loss of eye sight.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Patients with NK rarely complain of ocular symptoms, and there is a significant discrepancy between clinical findings and subjective symptoms. A classification based on disease severity was proposed by Mackie (1995), who distinguished three stages (1 to 3 in order of increasing severity):
· Stage 1 is characterized by punctate keratopathy and/or corneal epithelial hyperplasia and irregularity, which may be associated with superficial neovascularization and stromal scarring. In addition dry eye signs may be observed, including vital dye staining of the inferior palpebral conjunctiva and decreased tear film break-up time.

· Stage 2 is characterized by a persistent corneal epithelial defect (PED), typically oval or circular in shape, with smooth and rolled edges. An area of poorly adherent opaque and oedematous epithelium is typically found rolled-up around the margin of the epithelial defect. Oedema of the corneal stroma may also be present, and it is not uncommon to observe also an inflammatory reaction in the anterior chamber.

· In Stage 3 the corneal stroma is involved and a corneal ulcer is observed. Corneal ulceration tends to progress to perforation and/or stromal melting if not promptly and properly treated. Corneal melting and perforation can also be iatrogenic, caused by inappropriate use of topical steroids or by secondary infections of the non-healing ulcer.

The prognosis of NK depends upon the specific cause behind the corneal sensory impairment, the degree of corneal hypo-/anaesthesia, and the association with other ocular surface diseases such as dry eye, exposure keratitis, and limbal stem cell deficiency. Progression to more severe disease stages is associated with a high and imminent risk of sight loss due to anatomical loss of the eye or to permanent loss of corneal transparency. NK also predisposes to secondary bacterial infection of non-healing epithelial defects.

2.1.5. Management

Treatment of NK depends on the disease severity (Abelson and McLaughlin, 2014; Bonini et al, 2003; Giannaccare et al., 2015; Mantelli et al., 2015; Sacchetti et al, 2014; Semeraro et al, 2014). Treatment for stage 1 disease aims at improving epithelial quality and transparency and avoiding epithelial breakdown. In the presence of PED (stage 2), therapy is aimed at preventing stromal involvement and corneal ulcer formation as well as promoting corneal healing. In more severe cases, when a corneal ulcer develops (stage 3), therapy is aimed at preventing or stopping corneal melting and perforation in order to preserve eye sight.

Preservative-free artificial tears are used at all stages of disease severity to help improve corneal surface integrity. Topical steroids and nonsteroidal anti-inflammatory drugs may inhibit the healing process and should be avoided. Use of topical antibiotic eye drops to prevent infection in eyes with NK at stages 2 and 3 is recommended. Furthermore, different blood-derived eye drops obtained from autologous serum, cord blood serum, and platelet rich plasma have been used to promote corneal healing because of their high concentrations of growth factors.

Non-pharmacological treatments for NK include therapeutic corneal or scleral (bandage) contact lenses in the event of PED. Contact lens wear may however increase the risk of secondary infections.

Surgical treatments are reserved for refractory cases. Partial or total tarsorrhaphy is used to cover a PED and promote healing. Alternatively, closure of the eyelids can be achieved by using a palpebral spring or botulinum A toxin injection of the eyelid elevator muscle. Furthermore, conjunctival flap is able to restore ocular surface integrity and provide metabolic and mechanical support for corneal healing. Both tarsorrhaphy and conjunctival flap are effective surgical procedures for promoting corneal healing, but have a poor cosmetic outcome and visual function is sacrificed.
Amniotic membrane transplantation is a short-term option in the management of refractory neurotrophic corneal ulcers. In case of smaller perforations cyanoacrylate glue can be used, whereas larger defects require lamellar or penetrating keratoplasty. The success rate of corneal transplants in NK patients is however low due to the lack of trophic support, with consequent poor wound healing and risk of PED recurrence.

Although preservative-free artificial tears and therapeutic contact lenses can be effective in limiting the progression of patients from stage 1 to stage 2 and stage 3 disease, there are no pharmaceutical treatments authorised for patients with stage 2 and 3 NK who are refractory to the above treatments. Furthermore, none of the treatment options used in clinical practice can reinstate corneal innervation and improve corneal sensation.

2.1.6. About the product

Cenegermin is a recombinant form of human nerve growth factor (rhNGF) produced in Escherichia coli as a pro-peptide, which is later cleaved to mature NGF. NGF is a neurotrophin, which is naturally present in the eye and is essential for the survival and growth of sympathetic and sensory neurons and for differentiation of neurons in the central nervous system (CNS). Activation of its receptors has been shown to play a role in the trophism of the cornea (de Castro et al., 1998; Smeyne et al., 1994) and there is evidence in the scientific literature of efficacy of murine NGF (mNGF) in the treatment of patients with moderate and severe (stage 2 and 3) NK unresponsive to other non-surgical treatments participated in the studies (Bonini et al., 2000; Lambiase et al., 2007).

Oxervate contains 20 μg/ml cenegermin in a preservative-free sterile eye drop solution. It is presented in vials for daily use, with 1 drop being administered 6 times daily. Both cenegermin and rhNGF are used interchangeable in this report when referring to the active substance.

Oxervate is proposed for the use in the treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) NK in adults, corresponding to disease stages 2 and 3. The applicant proposed that treatment should be initiated and supervised by an ophthalmologist or a healthcare professional qualified in ophthalmology, which was agreed by the CHMP.

2.1.7. Type of Application and aspects on development

This was a complete and independent application made under Article 8.3 of Directive 2001/83/EC.

The CHMP agreed the applicant’s request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the understanding that the medicinal product potentially implies an innovative therapeutic approach for a clinical condition that constitutes an unmet medical need. The potential benefits of Oxervate in the treatment for moderate to severe NK patients include the control of this sight-threatening condition with a pharmacological (non-surgical) treatment without preventing subsequent surgical intervention.

During the product development, the applicant sought scientific advice and protocol assistance from the CHMP on several occasions on quality, non-clinical and clinical aspects.
2.2. **Quality aspects**

2.2.1. **Introduction**

The finished product is presented as eye drops solution containing 20 µg/ml of cenegermin as active substance.

Other ingredients are: Trehalose dihydrate, Mannitol, Disodium hydrogen phosphate anhydrous, Sodium dihydrogen phosphate dihydrate, Hydroxypropylmethyl cellulose, Polyethylene glycol 6000, L-Methionine, Hydrochloric acid, Sodium hydroxide, Nitrogen and Water for injections.

The product is available in sterile, preservative-free multi-dose Type I glass vials, closed with a rubber stopper and an aluminium overseal with a polypropylene flip-off cap, presented in cardboard cartons. 7 multi-dose vials are included per carton.

The product is to be used with a delivery system consisting of vial-adapters, disposable pipettes (used to withdraw product from the vial in order to administer one ocular drop) and disinfectant wipes, which is not part of the finished product and is supplied separately to the patient.

2.2.2. **Active Substance**

**General information**

The active substance in Oxervate, cenegermin, is a recombinant human Nerve Growth factor (rhNGF) produced in *E. coli* strain HMS174. The molecule is identical to human Nerve Growth factor (NGF), a naturally occurring human protein.

In humans, NGF is naturally produced as pre-pro-peptide, secreted into the endoplasmic reticulum and cleaved by furin protease. The pro-sequence is further cleaved during the production process by enzymatic hydrolysis. Therefore these two amino acid changes have no influence on the final active ingredient (rhNGF), which is identical to the naturally secreted human protein. The 3D structure of rhNGF is a non-covalent dimer with three intra-molecular disulphide bridges.

Cenegermin contains 118 amino acids and has a relative molecular mass of 13,266 Daltons and the following molecular formula: C\textsubscript{583}H\textsubscript{908}N\textsubscript{166}O\textsubscript{173}S\textsubscript{8}. Figure 1 shows the protein sequence of recombinant human ProNGFrh ProNGF (Figure 1A), and a map of the disulphide bridges (Figure 1B):
A: amino acid sequence of rhProNGF mutated in the trypsin cleavage site (underlined). The pro-sequence is highlighted in red. **The sequence of mature rhNGF is shown in green**

![Protein Sequence and disulphide bridges mapping](image)

**Figure 1 - Protein Sequence and disulphide bridges mapping**

NGF is essential for the survival and growth of sympathetic and sensory neurons and for differentiation of neurons in the central nervous system. NGF acts through specific high affinity (i.e. Tropomyosin receptor kinase A (Trk A)) and low affinity (i.e., p75NTR) NGF receptors. NGF receptors are expressed on anterior segments of the eye (iris, ciliary body, lens, cornea and conjunctiva) and by the lacrimal gland as well as by all the intra-ocular tissues. Activation of these receptors by NGF plays a role in the tropism of the cornea.

The NGF biological activity is evaluated **in vitro** by using human bone marrow erythroblast suspension cell line expressing the TrkA receptor. Binding of NGF to Trk A is fully functional in the cell line because it is able to induce TrkA autophosphorylation and also trigger signalling events inside the cell that lead to cell proliferation.

**Manufacture, characterisation and process controls**

Cenegermin active substance is produced by Dompé farmaceutici S.p.A., Via Campo di Pile snc 67100, L’Aquila, Italy.

The manufacturing process is a multilevel stage process, starting from thawing of five vials of a working cell bank (WCB) followed by several cell expansion steps. The manufacturing process has been adequately described. Critical process parameters (CPP), in-process controls (IPC) and critical in-process controls are highlighted, with acceptance criteria when relevant. The active substance manufacturing process is considered to be acceptable.
Cenegermin active substance is stored at -20°C ± 5°C in validated freezers until ready for further processing. Cenegermin is shipped in dry ice at not more than (NMT) -15°C following approved packaging procedures, using qualified shipping containers under conditions that have been validated.

No reprocessing is mentioned for the manufacturing process of cenegermin.

**Control of materials**

*Expression plasmid*

The expression plasmid and the procedure for its preparation have been described in sufficient detail.

*Cell bank development*

A two-tiered cell culture system is used for the production of cenegermin. The preparation of the master cell bank (MCB) and working cell bank (WCB) is described in sufficient detail. Gram test and microscopic characterisation, plasmid retention, viable cell count performed according to an approved internal analytical monograph, and gene DNA sequence, plasmid copy number and bacteriophage contamination performed by contract laboratories are adequately studied and described. The MCB and WCB have been appropriately characterised.

In general, the description of generation of the plasmid construct and primary and master cell banks is adequately described. DNA sequencing, including vector flanking regions (50 – 100 base pairs (bp)) was performed on the MCB and results presented that confirmed the correct sequence. The correct protein expression confirmation was performed by using Western Blot analysis on both MCB and WCB. This is in accordance with the requirements of the Ph. Eur. monograph on Recombinant DNA technology products and ICH Q5B for the characterisation of MCB and WCB.

During the procedure, questions were raised on the genetic stability of the cells up to and beyond full scale fermentation of the WCB and the limit for in vitro cell age. The Applicant has provided adequate documentation and has justified the maximum calculated number of duplications and a time limit proposed for the whole fermentation process, which is considered acceptable.

*Other materials*

All raw materials are purchased from qualified vendors and are pharmacopoeial grade when possible. No materials of animal/human origin are used for the production of cenegermin active substance. In addition, no raw materials of human/animal origin have been used in the production of the starting materials.

The certificates of analysis have been included and confirming that all materials are free of transmissible spongiform encephalopathies (TSE) and bovine spongiform encephalopathy (BSE).

Internal procedures, based on the good manufacturing practice (GMP) and international conference for harmonisation (ICH) requirements, describe in detail the procedures for receipt, identification, storage, handling, sampling, testing, and approval or rejection of raw materials. These procedures provide assurance that materials are maintained in a controlled manner throughout quarantine and release procedures.

The incoming raw materials and the single-use devices used during the active substance manufacturing process are classified on the basis of a risk analysis considering all the critical risk factors concerning the nature of the material that may have impact on the quality of the final product, of the process and the
process step in which the material is used. On the basis of this classification, the raw materials are completely or partially reanalysed by the Applicant.

In the original submission, no information was provided in relation to the control of single use equipment, filters/column resins used during the manufacturing process. The Applicant has provided sufficient information in relation to the single use equipment and the filters. In relation to the chromatography resins it is confirmed that these are of synthetic origin and that no materials of animal origin were used during their manufacture.

**Control of critical steps and intermediates**

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout cenergermin active substance manufacture is given. The active substance manufacturing process is controlled by critical process parameters (CPP), in-process controls (IPC) and critical in-process controls (CIPC) that have been established to ensure consistent process performance and product quality. All excursions from CPP, IPC and CIPC ranges are investigated. Excursions from CIPC may result in batch rejection, as deviation from the range is likely to affect product quality. Corrective and preventive actions (which include batch rejection) are implemented as required.

The control ranges have been established during the manufacturing process development and confirmed during the manufacturing process validation. The Pro Nerve growth factor inclusion body (ProNGF IB) Intermediate is the only process intermediate of the manufacturing process of rhNGF active substance. It is stored frozen at -70±5°C until subsequent processing in 2 L capacity sterile pyrogen-free polyethylene terephthalate copolyester, glycol modified PETG bottles, closed with a high density polyethylene (HDPE) screw cap. Each batch of ProNGF IB Intermediate is subjected to release testing. Stability data are presented that support the storage conditions claimed for the intermediate.

During the procedure a major objection was raised in relation to the proposed manufacturing process control strategy. The Applicant was requested to provide more information on the manufacturing process control strategy to ensure quality of the active substance. The applicant provided a more thorough discussion of the development of the manufacturing process control strategy with the provision of extensive background information. The basis for defining the current CQAs is now appropriately described including an updated risk assessment matrix, explanation of the relationship between CQAs and the elaboration of CPPs/MPPs/IPCs for the process. The applicant also provided a detailed explanation of how criticality of process parameters and in-process controls was determined.

**Process validation**

The cenergermin active substance manufacturing process has been adequately validated.

During the procedure a major objection was raised in relation to the proposed manufacturing process control strategy, which were considered insufficient to ensure consistent quality. A justification was requested for the control strategy explaining how criticality of process parameters and in-process controls was determined.

During the procedure the Applicant was able to resolve the major objection on process control and validation. As mentioned in the section ‘Control of Critical Steps and Intermediates,’ the Applicant provided the information requested. Relevant monitored process parameters (MPPs) or in-process monitoring (IPM), while not considered critical by the applicant, were registered in the dossier as part of the control strategy. In relation to the proposed acceptance criteria, data was provided to support the target ranges for process parameters and in-process controls examined during the process validation studies. The developmental and edge of range studies used to set these target ranges were provided. Where historical batch data was
combined with process validation (PV) batch data to set acceptance criteria for IPCs, information was
supported with results from historical batches in question. The strategy for defining acceptance criteria for
process parameters and IPCs was justified with reference to statistical evaluation of data, where relevant.
Furthermore, as the control strategy was developed using data from previous manufacturing processes (edge
of range-development studies, historical batch data) this was further justified in the context of comparability
to the proposed commercial process.

A number of specific issues were raised, all of them were solved except the control of refolding of the protein
where more data was requested, and the process related impurities where the registration and justification of
limits for control of these impurities was also requested. Now, the applicant has provided sufficient
information on both issues and they are considered solved.

The sufficiency of the critical in process control (CIPC) for culture purity during fermentation was queried and
the Applicant has introduced additional tests for its control that are considered adequate. Numerous
additional queries were raised reflecting the fundamental lack of clarity afforded by the data presented in
relation to process control and design thereof. All queries have now been sufficiently addressed.

In relation to the chromatography columns, a query was raised on the sufficiency of leachable/extractable
studies performed; the applicant provided additional data including the reports for leachables from the AEX
and CEX columns and the information is considered adequate. In addition, the compatibility of the substance
with contact materials during the manufacturing process was not considered and this point was raised. The
applicant provided the leachable/extractable studies confirming that the leachables present in the finished
product Oxervate Eye Drops coming from the active substance are not a safety concern. There is a single
intermediate during the manufacturing process; ProNGF IB intermediate is stored at -70° C for up to 1 year
prior to performance of the solubilisation step. Queries were raised in terms of the control specifications,
proposed primary packaging and shelf life for the intermediate. The requested information was provided.

**Characterisation**

The analytical package to characterise cenergermin includes SDS-PAGE, peptide mapping, SE-HPLC, RP-HPLC,
IEX-HPLC, potency assay, N-terminal sequencing by Edman degradation, amino acid composition and protein
concentration by amino acid analysis, secondary structure determination by Circular Dichroism (CD) and
intact molecular weight determination by mass spectroscopy.

Data of some reference standard assays were initially not described or presented for review. This was
summarized during the procedure as a major objection on characterisation. A more thorough characterisation
study was requested and, specifically, further information was requested on the purity profile, functional
characterisation, protein modifications and secondary/tertiary structure of the active substance.

During the procedure the Applicant provided the data from the analyses by SDS-PAGE gels, peptide mapping,
SE-HPLC, RP-HPLC and IEX-HPLC, performed on rhNGF reference standards RS1213 and RS0515, and on
rhProNGF reference standard RS0115. In addition, data obtained from the detection of aggregates by AUC
and from SDS-PAGE in reducing and non-reducing conditions in gels silver stained were provided.

The major objection on characterisation was considered to be resolved on the basis of the information
provided and taking into consideration the Applicant’s commitment to conduct a number of post-authorisation
follow-up studies as detailed under Recommendations (see recommendations 1 – 7, section 2.2.6).

In relation to the purity profile, the use of a single method for detection of product related impurities (RP-
HPLC) and the suitability of SE-HPLC, SDS-PAGE and IEX-HPLC as purity methods capable of detecting
impurities was queried. The Applicant has addressed the suitability of the purity methods by testing the
capacity of the SE-HPLC and SDS-PAGE methods to separate aggregated material from the main peak (during shaking stress degradation studies). Data were also presented to demonstrate that the SE-HPLC and SDS-PAGE methods would detect truncated species, if present. Furthermore, the applicant provided data to confirm that uncleaved proNGF, oxidated forms of rhNGF and post translation modifications would be detected by the RP-HPLC method.

During the procedure a concern was raised in relation to the capacity of the IEX-HPLC method to detect charge variants, if present. The Applicant was requested to propose a suitable amendment to the acceptance criterion so that batches with an increase in charge variants present would fail to pass the specification. The applicant has now committed to amend the acceptance criterion in relation to the main peak shape as requested in a post-authorisation recommendation (see recommendation 2, section 2.2.6). Although this technique is not suitable for control of all impurities, the method appears to be suitable for control of impurity B and as such, the applicant should register this second method (RP-UPLC-UV) for routine control of impurity B. The applicant committed as a post-approval commitment to register the RP-UPLC for routine control of impurity B and to provide information about the method, validation and specification levels for impurity B in the dossier (see recommendation 1, section 2.2.6). In addition, the Applicant was requested to provide the report describing the validation of the RP-HPLC method carried out for the specific named impurities; the report was provided and is, in general acceptable, however the applicant has committed to extend the range of the validation for impurity F1 (see recommendation 3, section 2.2.6). Furthermore, additional data from forced degradation studies (report A1491 with amendments) indicate that the current RP-HPLC method is not capable of resolving the main peak from impurities F1 and F2. Therefore, the Applicant has committed to updating section 8.5 (Impact of Forced Degradation on Functional Activity) including submitting a visible corresponding Figure (Figure 68 in Module 3) and providing the report in which these data are included (see recommendation 4, section 2.2.6). The applicant has also provided a commitment that a method capable of resolving the main peak from named and unnamed impurities will be developed and registered in the dossier (see recommendation 12, section 2.2.6).

The Applicant has provided a brief discussion to support adequate characterisation of potential post-translational modifications and has confirmed the suitability of the proposed RP-HPLC method to detect relevant post-translational modifications.

The results of the assays performed for the characterisation of the secondary and tertiary structure were presented. The following analytical methods were selected for this purpose: disulphide bond mapping, far and near UV circular dichroism, free sulphhydryls by Ellman’s assay, intrinsic tryptophan fluorescence, FT-IR (Fourier Transformed Infrared) spectroscopy, Differential Scanning Calorimetry: the analysis is ongoing, to be provided before MA. The Applicant has committed to provide several reports, including data derived from the ongoing Differential Scanning Calorimetry analyses before marketing authorization with the closing sequence (see recommendation 15, section 2.2.6).

The potency assay is a cell-based potency assay using a receptor, and is the same method as proposed for the finished product release.

The primary packaging for rhNGF is composed of 500 ml capacity sterile pyrogen-free polyethylene terephthalatecopolyester, glycol modified (PETG) bottles, filled with approximately 60% volume of product and closed with a high density polyethylene (HDPE) cap. Queries were raised in relation to the Ph. Eur. compliance of the primary packaging material and specifications. An extractable/leachable study was requested and the Applicant has committed to introduce an extractable study to assess during the in-use condition of finished product post-marketing (see recommendation 8, section 2.2.6). Therefore concerns on compliance of the primary packaging material and specifications have been resolved and the dossier was
updated to include a description of the characteristic properties of the material (mechanical/physical) and sufficient information regarding potential extractables.

Process related impurities DNA, host cell protein (HCP), Kanamycin sulfate, endotoxin and bioburden are controlled through the active substance release specifications.

**Specification**

The active substance specifications include tests for identity, purity (a single method), and potency as well as tests for the following process related impurities: host cell proteins, endotoxin, bioburden and kanamycin sulfate.

Initially the specifications were not considered sufficiently justified based on batches representative of the proposed commercial process. The Applicant was asked to justify and revise the active substance specifications accordingly and to describe the statistical basis for assigning acceptance criteria, where relevant. In this regard, the Applicant has now provided an adequate justification of specifications and acceptance criteria based on GMP batches used during clinical studies. In addition, the Applicant has provided a commitment to review the specifications for protein concentration, potency, purity SE-HPLC, IEX-HPLC, residual DNA, residual HCP, bioburden, endotoxin and potency after 10 batches and to tighten, where necessary, the acceptance criteria on the basis of this review (see recommendation 6, section 2.2.6). The acceptance criteria shall also be reviewed and tightened for identity by peptide mapping as committed to by the Applicant (see recommendation 7, section 2.2.6).

DNA, HCP, Kanamycin sulfate, endotoxin and bioburden were process related impurities controlled in the active substance release specifications. In general, the limits for these impurities were justified as part of the revised justification of specifications however it was noted the limit for residual DNA is in line with WHO recommendations.

The stated impurities have been studied in nonclinical and clinical studies.

**Analytical methods**

The analytical methods used have been adequately described and (non-compendial methods) appropriately justified.

The potency assay is a cell-based potency assay using receptor and the assay is based on cell proliferation. The same method is also proposed for the finished product release. During the procedure the Applicant provided additional information to justify the appropriateness of the chosen method. In addition the Applicant has further committed to review the potency range after manufacture of 10 batches and to tighten them in accordance with the clinically qualified limits (see recommendation 6, section 2.2.6).

The analytical tests are considered to be sufficiently validated and demonstrated to be fit for purpose. During the procedure a major concern was raised in relation to the RP-HPLC purity method as the RP-HPLC method appeared not to distinguish between impurities and potential analytical artefacts and the method validation provided was considered inadequate. The Company’s proposal to refer to SE-HPLC and IEX-HPLC as “purity” methods was not accepted as these methods appear to lack sensitivity as a method for impurities.

During the procedure the Applicant was able to resolve the concerns and to demonstrate the suitability of the purity methods SE-HPLC and SDS-PAGE to separate aggregated material and fragments from the main peak (which are applied during shaking stress degradation studies). Furthermore, the applicant confirmed that
uncleaved proNGF, oxidated forms of rhNGF and post translation modifications would be detected by the RP-HPLC method.

In relation to the capacity of the IEX-HPLC method to detect charge variants, if present, the Company has agreed to a request to amend the acceptance criterion further post-marketing, this was agreed by the CHMP (see recommendation 2, section 2.2.6). Furthermore, data was presented in relation to an RP-UPLC-UV method, which is capable of detecting impurity B. Although this technique is not suitable for control of all impurities, the method appears to be suitable for control of impurity B and as such, the applicant committed as a post-approval commitment to register the RP-UPLC for routine control of impurity B and to provide information about the method, validation and specification levels for impurity B in the dossier (see recommendation 1, section 2.2.6). In addition, the Applicant was requested to provide the report describing the validation of RP-HPLC method carried out for the specific named impurities; the report was provided and is, in general acceptable, however the applicant has committed to extend the range of the validation for impurity F1 (see recommendation 3, section 2.2.6).

The Applicant has committed to specify an analytical method for step yield determination and confirm its suitability for use (see recommendation 5, section 2.2.6).

Further justification was requested for the decision to control Impurities A, B, F1 and F2 as “named” impurities (A, B, F1 & F2) in the active substance and for the proposal to control all other impurities as “unknown”. It was considered that the acceptance criteria for impurities should be justified on the basis of impurity levels that had been clinically qualified and a limit for ”Total unknown impurities” should be set. In general, the applicant provided a justification for the proposed impurity limits and proposed a limit for total unknown impurities, however, some points for clarification remained. The applicant has now clarified the decision to control impurities F1 and F2 as “named” impurities and other impurities (present at the same level as F1 and F2) as "unknown" and this issue is considered resolved. Overall the proposed limits for product related impurities in the active substance are acceptable. However, the applicant has committed to review the specifications for impurity A after manufacture of additional batches and a similar recommendation is made in relation to impurities F1 & F2, individual unknown impurities and total unknown impurities (see recommendations 9 and 11, section 2.2.6).

In addition the applicant has confirmed that a new method capable of improved resolution of main peak from named and unnamed impurities shall be developed for the purpose of quantifying product-related impurities and that this method will be registered in the dossier as a release method for both active substance and finished product (see recommendation 12, section 2.2.6).

**Batch analysis**

Batches from all four manufacturing processes were analyzed. All batches analyzed met the release acceptance criteria.

**Reference standards of materials**

The history of reference standards used during development has been presented. Preparation, release testing and stability for three of them are provided.

The strategy for qualification and retesting of new internal reference standard was not described. The applicant has now provided the strategy and it is considered acceptable.
**Stability**

The proposed shelf life for the active substance is 24 months at a storage condition of -20 °C.

Stability studies have been performed using three different storage conditions: -20±5 °C (long term), -70±10 °C (which is stated to be a potential alternative storage condition) and 2-8 °C (in-use stability).

Additional queries were raised mainly in relation to trend analysis of impurities on stability and the stability indicating nature of the RP-HPLC method for control of purity. These issues were resolved during the procedure.

**Comparability exercise for Active Substance**

During the manufacturing process development, the cenegermin manufacturing process has undergone several changes including a series of manufacturing scale changes and transfer from the early development facility to the proposed manufacturing site at Dompé. The fundamental fed batch fermentation process was not significantly changed apart from scale although some modifications were made to media supplementation. The downstream purification process was modified between different versions of the manufacturing process to accommodate the increase in fermentation scale.

Comparability is claimed primarily on the basis of release testing. Additional characterisation testing performed includes: N-terminal sequencing, amino acid composition and protein concentration, circular dichroism, intact molecular weight, free sulphhydrils and analytical ultracentrifugation. The comparability exercise did not address process performance parameters or active substance stability profiles, however, this information will not be requested in the context of the manufacturing process changes introduced and on the grounds that accelerated stability data from historical batches is comparable to commercial batches.

The approach taken to demonstrate comparability between different manufacturing processes is considered to be acceptable.

**2.2.3. Finished Medicinal Product**

**Description of the product and pharmaceutical development**

Oxervate finished product is a sterile preservative-free ophthalmic solution containing 20 µg/ml of cenegermin active substance.

The finished product contains the following excipients: Trehalose dihydrate, Mannitol, Disodium hydrogen phosphate anhydrous, Sodium dihydrogen phosphate dihydrate, Hydroxypropylmethyl cellulose (Hypromellose), Polyethylene glycol 6000, L-Methionine, Hydrochloric acid, Sodium hydroxide, Nitrogen and Water for injections.

It is confirmed that the intended commercial formulation is representative of that used during phase 2 clinical studies.

The finished product is packaged in multi-dose siliconized glass class I vials closed with a rubber stopper and an aluminium seal with a polypropylene flip-off cap. Each vial contains 1.0 ml of solution. Seven multi-dose vials are packaged in a cardboard box with the leaflet.
The product is intended to be administered with a kit of delivery system devices, including one vial adapter, disposable pipettes, and disinfectant wipes, which is not part of the finished product and is supplied separately to the Oxervate.

For the pipette, sanitising wipes the EC certificate of Conformity (CE mark) confirming compliance with EC Directive 93/42/EEC Annex V have been provided. For the vial adapted a certificate has also been provided (Certificate number 346 CT issued by AMTAC Certification Services confirming compliance with Directive 93/42/EEC Annex V).

For administration of the product, the flip-off cap is removed from the vial and a vial-adapter is connected to the glass vial. The vial is then ready for use. To administer the product, a special pipette able to deliver the solution drops of 39 μl is attached to the vial adapter. The liquid transfer is secured by the pipette luer-lock system.

**Manufacture of the product and process controls**

The manufacturing process consists of the following main steps: compounding of excipient for buffer solution, filtration of the buffer solution, dispensing and pooling of the preliminary thawed cenebergermin bulk active substance (BAS), dilution of cenebergermin BAS with buffer solution, gentle wave-mixing of the bulk FP solution, filtration of the bulk FP solution and sterile filling/stoppering of vials, crimping of the vials and FP visual inspection.

There is no design space or process-analytical technologies proposed. Being proteinaceous in nature, the product cannot be terminally sterilised. Therefore it is manufactured by aseptic processing and sterilised by sterile filtration through consecutive 0.2μm filters. Filter integrity is confirmed pre- and post-filtration and bioburden is adequately controlled prior to filtration. Compatibility studies on contact materials (i.e. filters, EVA bags) have been performed.

Validation data have been provided on 3 commercial and 3 supportive batches at the final proposed batch size of 12.0 L and a vial fill volume of 1.0ml. The finished product batches were manufactured using three active substance batches (#0615, 0815 and 1015) which are representative of the commercial manufacturing process for active substance. The process validation included validation of the manufacturing process, hold times and times of refrigeration, media fills, sterile filtration and shipping. A number of issues had been raised in relation to the validation reports, in particular the filter validation reports. The Applicant has justified during the procedure, the low levels of extractables observed in terms of route of administration. The risks associated with ocular delivery of the finished product with quantified low levels of identified extractables are negligible as supported by non-clinical and clinical studies.

The applicant had not provided adequate evidence of the control strategy for the manufacturing process, and proposed controls were not adequately justified. A more thorough discussion on the risk-evaluation used for assigning criticality was requested, including justification for selection of some control parameters as non-critical versus critical. The response provided demonstrated an appropriate approach to selection of key parameters for the control of quality of finished product production.

**Product specification**

Specifications proposed for control of Oxervate finished product are in accordance with pharmacopoeial requirements and include visual appearance, pH, osmolality, identity, particulate contamination, assay,
purity, impurities, potency, sterility and on shelf-life container integrity. There was no limit proposed for total unknown impurities, extractable volume or viscosity. The applicant has proposed to introduce a USP-based method to control for viscosity and has committed to validate this method as a post-authorisation commitment as a batch-release method (see recommendation 10, section 2.2.6). The applicant has further committed to registering a limit of NMT 2.5% for total unknown impurities in the drug product release specifications. The absence of a limit for extractable volume was justified on the basis of an overfill required for multiple doses.

In addition, a specification for methionine antioxidant, total unknown impurities and viscosity at both release and shelf-life will be implemented post-marketing, which is acceptable.

During the procedure changes to specifications were requested and the justification for finished product specifications was requested to be revised. In particular the Applicant was requested to describe the statistical basis for assigning acceptance criteria, where relevant. The Applicant has also committed to re-evaluate the limits for potency and impurities within a post-marketing commitment, which has been considered acceptable.

**Analytical methods**

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

The control of finished product was not considered sufficiently justified as the proposed quality control methods could not guarantee that a major deficiency in the quality of the product would not occur. Specifically, a major objection was raised in relation to the control of purity. The proposal to use a single purity method (RP-HPLC) was not endorsed without justification, particularly as the validation of the method for named impurities was inadequate. The response to these concerns was considered partially resolved but other concerns arose after its assessment. Thus, the applicant was requested to confirm linearity for the commercial formulation using this RP-HPLC method for identity, assay and purity, to provide a very clear table describing all the parameters which had been validated for the final proposed RP-HPLC method, to confirm that all system suitability parameters for the RP-HPLC method shall comply with Ph. Eur. 2.2.46 and to provide a validation report for the proposed commercial formulation of finished product. The above-mentioned issues have been resolved and the applicant has committed to provide full validation of the method for individually named impurities as a post-authorization commitment (recommendation 11, section 2.2.6). In addition, the applicant commits to continuing efforts to develop a more appropriate method for quantification of named impurities given the poor peak resolution demonstrated by this method. A new method capable of improved resolution of main peak from named and unnamed impurities shall be developed for the purpose of quantifying product-related impurities and that this method will be registered in the dossier as a release method for both drug substance and drug product (recommendation 12, section 2.2.6).

The cell-based potency assay using a receptor is the same method as proposed for active substance (see above).

The applicant has provided an adequate summary of the risk assessment for elemental impurities and the strategy to evaluate risk as for parenterals is endorsed. It is noted that method qualification is ongoing for the Cadmium analysis and the Applicant has committed to provide the results of the analysis of cadmium levels in the finished product including information on the qualification of the method (see recommendation 13, section 2.2.6).

**Batch analysis**
Batch analysis data (12 batches at 12 L commercial scale) have been presented for the finished product. Acceptance limits have been set based on the Applicant’s own manufacturing experience.

Now, both proposed limits for osmolality and potency have been clinically justified. In addition the applicant committed to submit a variation to tighten limits for potency if appropriate, following monitoring of the next 10 commercial batches.

Reference materials
The same reference materials used in the Active Substance are used in the Finished Product.

**Stability of the product**

*Stability and storage conditions*

The proposed shelf life for Oxervate is 2 years for the unopened vial at (-20 °C ± 5 °C).

At the pharmacy, the weekly carton containing the vials must be stored in a freezer (-20 °C ± 5 °C).

The patient will receive a weekly carton including 7 vials of OXERVATE in an insulated pack. As soon as the patient is at home (and no later than 5 hours from when the patient receives the product at the pharmacy), the weekly carton should be placed into the refrigerator, at 2-8 °C. It should be noted that the frozen medicinal product received from the pharmacy could need up to 30 minutes for thawing.

As in-use shelf life, section 6.3 of the SPC details that once opened, the product must be stored below 25 °C and used within 12 hours at 25 °C.

For use, an individual multi-dose vial of Oxervate is to be removed from the fridge for use over the course of a single day. Each opened vial can be stored in the fridge or below 25 °C, but must be used within 12 hours.

After this period of time the vial contents should be discarded irrespective of whether some residual product remains in the vial.

**Stability data**

Stability for the finished product (24 month shelf-life at -20°C ± 5°C) has been carried out using clinical batches manufactured to pilot scale. These batches are considered to be representative of commercial batches as they differ only in filling volume (0.5 ml for clinical batches and 1.0 ml for industrial batches).

Analytical methods applied were identical to those used for finished product release with the exception of Container Closure Integrity test, which has been described and validated. The method (determination of maximum absorption using a UV-Vis spectrum between 200 and 700 nm of a solution of methylene blue) has been validated for linearity, repeatability, LOD, LOQ, range, determination of maximum absorption. Methods used were considered to be stability indicating.

Date were provided for real-time (-20°C), accelerated, forced and in-use studies.

Parameters such as pH, potency, osmolality, concentration, impurities and sterility remained well within shelf-life specifications over the 24 months for which data has been provided.

The assigned in-use shelf life of 7 days at 2-8°C for unopened vials is supported by a stability study consisting of three process validation batches at commercial scale, which were stored initially at 25°C for 5
hours (to represent transport from point of dispensing to the patients home) followed by 14 days at 2-8°C and a further 12 hours at 25°C.

Vial integrity testing has been performed on the adaptor in situ with multiple product withdrawals over a 12-hour period.

During the procedure a question was raised on the minimum thaw time that is required for a vial, as this was not registered in the dossier or the product information. The applicant provided the requested supporting data and updated the product information to include this information and the issue was resolved.

**Adventitious agents**

The adventitious agent safety evaluation is supported.

The production cell line, *E. coli* does not harbour mammalian viruses. There is suitable control of raw and starting materials, including characterisation of the cell bank. The phage study presented supports the claim that the cell banks do not contain any adventitious phage or pro phage.

No materials of animal or human origin are used in the manufacturing process. The production process contains filtration steps to reduce any potential contamination and also includes in-process controls and release tests for bioburden. The AS and FP manufacturers have GMP-certified hygiene measures in place.

In conclusion, the CHMP considers that the safety of the product quality in relation to adventitious agents including viral and TSE safety is adequate.

**2.2.4. Discussion on chemical, pharmaceutical and biological aspects**

From the quality point of view the CHMP considered the quality dossier at submission, to be poorly presented and incomplete with respect to critical data to support a sufficient knowledge of active substance and an appropriate control strategy for both manufacturing process and active substance. This was reflected in the two major objections that were raised during the procedure namely (1) on the proposed manufacturing process control strategy which was considered insufficient to ensure consistent quality of the active substance and (2) on insufficient characterisation of the active substance and routine control of impurities for active substance/finished product.

The major objections are interlinked as the insufficient characterisation of active substance impacts upon defining appropriate CQAs and upon the comparability studies carried out across the different historical versions of the manufacturing process. In addition, numerous inconsistencies and omissions were noted in the data presented which has been reflected in the number of other concerns raised throughout the procedure.

The first major objections related to the proposed manufacturing process control strategy which was considered to lack transparency and which was not sufficient to ensure consistent quality of the active substance. Further information was requested in order to justify the CQAs and provide a clear rationale for those used to establish CPPs and IPCs. The classification of process parameters and IPCs as critical or non-critical was not transparent and further justification was requested. It was not clear how acceptance criteria were selected for the CPPs and IPCs defined for the manufacturing process and clarification was requested. Where the control strategy has been developed using data from a previous manufacturing process (edge of
range/development studies, historical batch data), this was requested to be justified in the context of comparability to the proposed commercial process.

Linked to this major objection was also a concern related to insufficient demonstration of comparability between commercial batches and batches used during clinical trials. The batches used during clinical trials were mostly manufactured according to historical processes although a single Phase II clinical trial was carried out with a batch manufactured according to the commercial process. A more thorough characterisation study was requested to support the claim that batches manufactured according to previous manufacturing process are representative of batches manufactured according to the proposed commercial process. Specifically, further information was sought on the purity profile, functional characterisation, post translational modification and secondary/tertiary structure of the active substance. Furthermore, process performance data and active substance stability profile were requested to be addressed as part of the comparability exercise.

A second major objection was raised in relation to control of active substance which was not considered to be sufficiently justified. The proposed RP-HPLC method, as the sole method for the detection of impurities was not considered adequate to control the quality of the product. Questions were raised in relation to the suitability and validation of the method. Furthermore, the Applicant was requested to identify additional impurity methods and to provide further information on the purity profile, functional characterisation, protein modifications and secondary/tertiary structure of the active substance.

During the procedure the Applicant provided the information requested. Relevant monitored process parameters (MPPs) or in-process monitoring (IPM), while not considered critical by the applicant, were registered in the dossier as part of the control strategy. In relation to the proposed acceptance criteria, data was provided to support the target ranges for process parameters and in-process controls examined during the process validation studies. The developmental and edge of range studies used to set these target ranges were provided. Where historical batch data was combined with process validation (PV) batch data to set acceptance criteria for IPCs, information was supported with results from historical batches in question. The strategy for defining acceptance criteria for process parameters and IPCs was justified with reference to any statistical evaluation of data, where relevant.

During the procedure the Applicant was able to resolve the concerns and to demonstrate the suitability of the purity methods SE-HPLC and SDS-PAGE.

During the procedure there were also questions raised on the delivery kit, which includes one vial adapter, disposable pipettes, and disinfectant wipes, to be provided separately to the product. The delivery kit is not part of the finished product presentation. This proposal to supply the delivery kit separately to the product was considered justified with reference to the different storage conditions necessary for the delivery kit (between 2-8°C) and the finished product vial presentation (at ~ 20°C).

The suitability of the delivery system with regard to the risk to the patient from potential contamination of the vial adapter surface was demonstrated. The applicant confirmed that the microbial integrity of the product can be maintained under normal conditions of use including cleaning of the adaptor with disinfectant wipes prior to each withdrawal. In addition, the applicant has committed to continue to monitor reports related to the device during ongoing clinical studies and pharmacovigilance reporting and to continue efforts to develop the device further (see recommendation 14, section 2.2.6).

During the procedure the major objections and other concerns were resolved by the Applicant.
Although sufficient data to support marketing authorisation have now been provided, a number of recommendations will be addressed post-marketing in the context of further quality development of this product (see section 2.2.6).

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

In conclusion, based on the review of the quality data provided, the CHMP considers that the marketing authorisation application for Oxervate is approvable from the quality point of view.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The Applicant should register the RP-UPLC-UV for routine control of impurity B. Details of the analytical method and validation and an appropriate specification for levels of impurity B should be registered to the dossier. (Timeline: 30 Dec 2017)

2. The Applicant should evaluate available batches and amend the acceptance criteria for IEX-HPLC regarding main peak parameters such that batches with increase in charge variants would fail the specification. (Timeline: Prior to marketing authorisation)

3. Validation of the RP-HPLC method for impurities should be extended to include the range for impurity F1 as a post-approval variation. (Timeline: 30 Sep 2017)

4. The Applicant should update section 8.5 (Impact of Forced Degradation on Functional Activity) to submit a visible Figure 68 (reference is made to Module 3) and provide the report in which these data are included making reference to this report in the text. (Timeline: to be submitted with the closing sequence, i.e. within 15 days after the marketing authorisation)

5. The analytical method for step yield determination should be specified and suitability for use confirmed. (Timeline: 30 September 2017)

6. Acceptance criteria for protein concentration, potency, purity SE-HPLC, IEX-HPLC, residual DNA, residual HCP, bioburden and endotoxin, will be reviewed and if necessary tightened following review of the next 10 batches of active substance. In particular, the potency range should be justified/tightened in accordance with the clinically qualified limits. (Timeline: after manufacture of 10 batches)

7. The acceptance criteria for identity by peptide mapping will be amended appropriately. (Timeline: Prior to marketing authorisation)

8. The Applicant should introduce an extractable study to assess during the in-use condition of finished product post-marketing. (Timeline: By 31 May 2017)

9. The Applicant should perform a review of the specifications regard the following impurities: A, F1, F2, individual unknown/total unknown impurities after a greater number (10 batches) is acquired. Where necessary, specification limits should be justified/tightened in line with clinically qualified levels. (Timeline: after manufacture of 10 batches)

10. The USP batch release method for control of viscosity in the finished product shall be validated. (Timeline: 30 September 2017)
11. Additional validation data in support of the RP-HPLC method for control of named impurities in the finished product shall be provided. (Timeline: 30 September 2017)

12. A new method capable of improved resolution of main peak from named (A, B, F1, F2) and unnamed impurities shall be developed (or the existing RP-HPLC method optimised) for the purpose of quantifying product-related impurities and that this method will be registered to the dossier as a release method for both active substance and finished product. (Timeline: 6 month following granting of the marketing authorisation)

13. The results of the analysis of cadmium levels in the finished product should be provided, including information on the qualification of the method. (Timeline: By 31 May 2017)

14. The Applicant should continue monitoring of reports related to the device during ongoing clinical trials and pharmacovigilance reporting, and continue to develop the delivery container further. (Timeline: none)

15. The following sections of Module 3 should be updated:

   In Characterisation: Elucidation of structure section of Module 3, the following characterisation techniques should be included: Disulphide bond mapping, Intrinsic tryptophan fluorescence; FT-IR (Fourier Transformed Infrared) spectroscopy; Differential Scanning Calorimetry: the analysis is ongoing, to be provided before MA, SDS-PAGE silver stain, Sedimentation Velocity by Analytical Ultracentrifugation, Potency by TF-1 Cell-Based Assay, Binding to Recombinant TrkA, Neurite Proliferation and Differentiation and Quantitative Evaluation of Neuronal Differentiation

   The correct limit for impurity D should be registered in section S.2.2 and S.2.4 of the dossier for the trypsin hydrolysis step.

   The specifications for control of chromatography resins should be registered in section S.2.3 of the dossier. (Timeline: to be submitted with the closing sequence i.e. within 15 days after the marketing authorisation)

2.3. Non-clinical aspects

2.3.1. Introduction

To support the present application, the applicant conducted a number of non-clinical studies including both in vitro and in vivo (rat and rabbit) primary pharmacodynamic (PD) studies investigate receptor binding, signal transduction and proliferation of neuronal and corneal epithelial cells as well as to compare effects of multiple routes of administration and explore adequate dosage on inducing healing and reparation in animal disease models. A safety pharmacology study was performed in the rat for examination of CNS effects.

Absorption, distribution and toxicokinetics of rhNGF were investigated in the same animal species used in the toxicology safety studies (rat and rabbit). Toxicological studies included single and repeat dose studies with administration via local (in the eye) and systemic routes.

Rat and rabbit were identified by the applicant as relevant rodent and non-rodent species (respectively) for the toxicology studies because both express the NGF receptors in the ocular structures. Furthermore, rat is the rodent species of choice for investigating biotechnology-derived pharmaceuticals, particularly when they express the ligand-specific receptor. The rabbit is well known as suitable species for ophthalmic investigation as well as for reprotoxicity and developmental studies. The rabbit express the NGF receptors as do all
mammals and it responds to rhNGF pharmacological activity (You et al, 2000).

### 2.3.2. Pharmacology

NGF is a neurotrophic factor important for the differentiation, regeneration and the survival of sympathetic and sensory neurons in humans and other vertebrates. NGF binds to high affinity neurotrophic tropomyosin receptor kinase A (TrkA) and the low-affinity nerve growth factor receptor p75. Although expression of TrkA alone is sufficient for cellular responses, p75 can regulate TrkA-ligand interactions and signal transductions. In the presence of p75, NGF binding affinity to TrkA is increased (Maliartchouk & Saragovi, 1997; Godfrey & Shooter, 1986).

#### Primary pharmacodynamic studies

- **In vitro studies**

  *In vitro* studies were performed in a range of cell lines which included Statens Seruminstitut Rabbit Cornea (SIRC) cells, Human erythroleukaemia (TF-1) cells, Pheochromocytoma rat cells (PC12) cells, neuroblastoma cells (SH-SY5Y), human embryonic kidney 293 cells (HEK293), and rabbit ocular epithelial cell cultures.

  Studies in SIRC cells compared cell proliferation in the presence of rhNGF and mNGF. The test showed no activity of rhNGF at the tested concentration ranges \([0.3 \text{ pM (7.96 pg/ml)} \text{ to } 6.76 \text{ nmol (179.29 ng/ml)} \text{ and } 0.7 \text{ pM (18.57 pg/ml)} \text{ to } 13.52 \text{ nmol (358.58 ng/ml)})\] in contrast to mNGF which induced increased cell proliferation (30% increase) at very high concentrations of 6760pM and 13520pM.

  Activity of rhNGF and mNGF was also tested at a range of concentrations in rat PC12-Luci cells, which are known to express high affinity TrkA receptors and low-affinity nerve growth factor receptor p75. The study also included a commercially available rhNGF (SigmaAldrich) as a comparator. The PC12-Luci cell line is stably transfected with human c-fos promoter driving a luciferase reporter gene. NGF receptor activation results in c-fos mRNA and protein expression and can be measured using a luciferase assay. The assay results were indicative of a dose-dependent increase in luminiscence (EC\(_{50}\) of 1.4 ng/ml for rhNGF produced by the applicant) reflecting TrkA receptor activation. Commercially available rhNGF displayed higher levels of luminescence in this assay when compared to the rhNGF produced by the applicant and subject to this application. The role of rhNGF in neuronal differentiation and spreading was also assessed in PC12 cells. A range of concentrations were tested using mNGF and a MimD3 as comparators. Outgrowth in neurites was observed from concentrations of 25 ng/mL for rhNGF while activity for mNGF started at much higher concentrations (50 μg/mL). No effect of MimD3 was observed.

  The responsiveness of rabbit corneal epithelial cells to rhNGF and mNGF was tested in fresh prepared cell samples. Both incubation with rhNGF and mNGF, studied at various concentrations ranging from 100 pM to 1.02 μM, resulted in an increase of cell clonogenicity and also colony size. TrkA expression was found to be induced in these cell cultures in a dose dependent manner.

  Finally, a study testing iodinated rhNGF for binding activity in solubilised rabbit corneas revealed a dissociation constant (K\(_d\)) of 0.24 nmol which was similar to that seen in a study by Banerjee et al. (1976), in which K\(_d\) for \(^{125}\text{I}\) mNGF was determined at about 0.2 nmol.

  Studies using human biomaterial are summarised in more detail in section 2.4.2. of this report. Three assays were conducted. The affinity of rhNGF and mNGF for the human TrkA receptor was studied in HEK 293 cells showing higher affinity of rhNGF compared to mNGF. Furthermore, a proliferation assay with TF-1 cells
showed that rhNGF was approximately 10 time more active compared to mNGF in inducing cell proliferation. Finally, biological activity of NGF was tested using the neuroblastoma cell line SH-SY5Y. Briefly, the assay showed biologic activity and no cytotoxicity for both rhNGF and all comparators (mNGF and MimD3).

- **In vivo studies**

The effect of NGF was also studied in vivo in a number of rat models as well as in rabbits.

The effect of rhNGF in the rat superior cervical ganglia hypertrophy test was studied in comparison to mNGF. Superior cervical ganglia is a very sensitive tissue to NGF action, and even more in pre-and postnatal development, therefore neonatal male Sprague Dawley rat pups were used. Both treatment with rhNGF and mNGF at doses of 1, 10 and 20 µg resulted in dose-dependent increases of superior cervical ganglia weight. No differences were discernible between both products, suggesting a similar pharmacological effect.

The potential therapeutic effect of ocular eye drop rhNGF administration was assessed in the rat Royal College of Surgeons (RCS) model of retinitis pigmentosa. Both eye drop and intravitreal dosing were studied. Results suggest that rhNGF dosing in rats results in the protection of photoreceptor degeneration. Ocular administration reduced apoptosis and augmented TrkA expression. Furthermore, reduction of Caspase 2 and 9 levels, two apoptotic markers, was observed, supporting a potential efficacy in protection of photoreceptors. Similar results were obtained irrespective of the route of administration. The largest effects were seen at the highest concentration which was 200 µg/ml, dosed 3 times per day for 20 days. RCS rats were furthermore used to assess the optimal dose regimen. In animals dosed with the highest concentration of rhNGF at 180 µg/ml 3 times a day during 3 days the highest level of a protective effect was observed. This is in contrast to single or twice a day (BID) administration that did not result in discernible effects.

In the Partial Optic Nerve Transection (pONT) model, 21 days of administration of rhNGF at various doses resulted in increased retinal ganglion cells (RGC) survival and decrease of secondary RGC degeneration at concentrations ≥60 µg/mL.

 Conjuntival Goblet Cells (CGC) express NGF receptors, which increase mucin secretion when stimulated. When rats were administered eye drops of rhNGF, a dose related increase in mucin production (MUC5aC) occurred. Effects were seen at doses of 10-20 µg/ml, 3 or 6 times/day.

In a rabbit model after photorefractive keratometry, animals received mNGF in the right eye and balanced salt solution in the other eye. The data did not result in significant differences between active and control. A re-analysis of the data suggested that at all time points mNGF accelerated corneal healing, in comparison to placebo. The evidence shown in this study was however very weak.

**Secondary pharmacodynamic studies**

No secondary pharmacology studies have been performed. This has been justified on the basis that rhNGF binds only the TrkA and p75 receptors and in addition the ocular administration results in very low systemic exposure to NGF.

**Safety pharmacology programme**

An Irwin safety pharmacology study was carried out to assess potential effects of NGF on the CNS. No effects were reported in this study. Considering the route of administration, cardiac, renal and respiratory safety pharmacology studies were not deemed appropriate. This was confirmed in a CHMP Scientific Advice.
**Pharmacodynamic drug interactions**

Systemic exposure is considered negligible, and unlikely to result in relevant pharmacodynamic drug interactions.

2.3.3. Pharmacokinetics

Absorption and toxicokinetics were addressed in a single and repeated dose studies carried out in rats and rabbits. Animals were dosed intravenously (IV) or by topical route (eye drops) in single dose studies while in repeated dose studies animals were dosed via topical route (eye drops) or subcutaneous (SC) route.

An Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantify the levels of rhNGF levels in rat and rabbit serum with an appropriate dynamic range of 8-2000 pg/ml. The assay was considered adequately validated, meeting the criteria of selectivity as outlined in the Guideline on Bioanalytical Method Validation (ICH EMEA/CHMP/EWP/192217/2009), with the potential of only minor interference by endogenous NGF in rat and rabbit serum.

Topical (ocular) single dose administration in rats produced highly variable toxicokinetic results. One study was conducted specifically to assess systemic absorption of rhNGF following ocular administration of 0.8 mg/mL to intact and abraded corneas in rats. In rats with intact corneas, serum concentrations at both time points were all below the limit of quantification (8 pg/mL) (with the exception of one animal with a serum concentration of 28.5 pg/mL at 6 hours). In rats with abraded corneas, levels of rhNGF varied from a minimum of 54.4 pg/mL to a maximum of 772.1 pg/mL. A mechanistic toxicokinetic study in the rat showed an about 100 times higher exposure in the sublingual vein compared to tail vein sampling after ocular rhNGF administration supporting absorption of rhNGF from the oral cavity after passage through the nasolacrimal duct. IV administration in the same species was assessed at increasing doses up to 1.2 mg/mL. In male rats, rhNGF exposure showed a tendency to augment in a dose-proportional manner while in females, exposure was greater than dose proportionate. The terminal half-life ranged from 3 to 4 hours and clearance and volume distribution were high. Exposure was generally comparable in both sexes and no significant differences were observed.

In rabbits, data after IV single dose administration was indicative of a biphasic profile. In this species, differences in exposure among sexes were seen, which increased in a more than dose proportionate manner in the males and less than dose proportional in females in the high dose range (1.2-2.4 mg/kg). The terminal half-life ranged from 4 to 6 hours and clearance and volume distribution were high. Ocular administration in rabbits resulted in low and very variable exposure to rhNGF.

In a 4 week repeated dose study rats were dosed rhNGF up to 1.2mg/kg (3 times a day). Animals were sampled sublingually and therefore the exposure levels are most likely overestimated. No antibodies against the product were reported and no gender differences. Exposure in males was more than dose proportional and dose proportional in females. In a 25 weeks in rats (up to 1.2 mg/ml, 3 times a day) also using sublingual sampling resulting in the same drawbacks, some animals (three males) displayed antibodies against rhNGF. SC administration was assessed in a rat study of 26 weeks duration in which animals were dosed of 50 and 100 μg/animal/day. Exposure was very variable at the low dose. No significant differences were observed between sexes. Only one animal displayed anti-drug antibodies (ADA) at the low dose and half of them (3/6) at 100 μg/animal/day.

In a 4-week rabbit study, animals were treated by eye drops at dose concentrations of 0.6, 0.8 and 1.2 mg/mL (each rabbit received 30 l in both eyes, three times a day) and blood sampling was done from the ear
vein. Inter-individual variability in plasma exposure was very high and no exposure dose-relationship could be determined. At many sampling times, rhNGF was below the limits of quantification. Binding antibodies at the end treatment were present in the majority of the animals but this did not significantly affect the mean levels of rhNGF plasma exposure. Rabbits were also treated with rhNGF by eye drops for 2-months (0.6 and 1.2 mg/mL, 30 l three times daily). Blood sampling from the central ear artery showed that systemic exposure to rhNGF was minimal and without a dose-response relationship. On week 4, binding antibodies against rhNGF were found in 50% of animals treated with the lowest dose and in 90% of animals treated at the highest dose, and on week 8 binding antibodies were found in 100% of animals treated with the lowest dose and in 90% of animals treated at the highest dose. SC administration of rhNGF up to 200 μg/animal/day to rabbits resulted in highly variable rhNGF levels after 90 days of daily SC administration. Half of the animals displayed ADAs against the product at the low dose and 12/14 animals at 200 μg/animal/day which may have contributed to the variability seen in exposure data.

The effect of addition of L-methionine was assessed in a 14-day and 2-month repeated dose studies carried out in rabbits. In the 14-day study all but one animal had serum levels of rhNGF below the limit of quantification and antibodies against the product were seen in a limited number of animals. When the clinical formulation was assessed for 2 months (1.2 mg/ml with and without L-methionine), there was again high variability in exposure measurements of rhNGF and a high number of animals displayed levels below the limit of quantification. ADAs against rhNGF were found in all animals by the end of the study.

Reprotoxicity studies were conducted in rats and rabbits with SC administration and included toxicokinetics and immunogenicity investigations. In the fertility and embryo-foetal development study in rats, exposure to rhNGF increased in a dose-proportional manner. Cmax values were reached 2 hours after administration. Only one animal in each rhNGF-treated group developed binding antibodies in this study.

In pre- and postnatal development study in rats, the F₀ (parental) generation showed rhNGF levels 2 hours after administration, whereas no rhNGF levels were detectable in the F₁ (first filial) generation pubs. Regarding immunogenicity, 45-65% of the animals from the F₀ generation and 55-78% of the F₁ generation were positive for anti-rhNGF antibodies. Thus there was a transfer of anti-rhNGF antibodies from mother to the F₁ generation.

In embryo-foetal development studies in rabbits, Cmax was reached at 2-4 h post-dose from gestation day 7 to 20. Accumulation of rhNGF was observed. Administration of rhNGF resulted in an immunogenic reaction in some animals but it did not affect exposure to rhNGF.

Distribution was assessed in albino rats after topical (eye drop) administration of [³H]-rhNGF 6 times at 2 hours intervals. Animals were administered 0.25 or 1.0 μg/eye/dose. Radioactivity was measured at all dose levels and no gender differences were found when adjustments including animal weight were introduced. The highest levels of radioactivity were reported in serum, urine, kidney, liver, uveal tract and retina and contents of the gastrointestinal tract. Gastrointestinal re-absorption after distribution of the product in the nasolacrimal and nasopharyngeal ducts affected the final outcome of overall exposure. The half-life at 0.25 μg/eye/dose was 77.1 and 67.5 hours in males and females, respectively, and at 1.0 μg/eye/dose half-lives were 57.6 and 68.5 hours, respectively. Elimination was faster from serum than from tissues although all measurements were below the limit of quantification at the final sampling time (24 hours). The eye surface displayed higher levels of radioactivity than internal region of the eye. Differences were more evident at the low dose tested showing radioactivity in the cornea, lens and sclera. Higher levels of radioactivity were measured at 1.0 μg/eye/dose of [³H]-rhNGF where radioactivity was also present in the optic nerve, iris, ciliary body, retina and choroid although at lower levels than in the sclera and
cornea. Very low levels of radioactivity were reported in the brain and spinal cord. Serum tissue relationship was the lowest reported among all organs and only at the highest dose tested.

No measurements were made of plasma protein binding, placental transfer or milk excretion.

Furthermore, no specific metabolism studies were conducted. As a protein, rhNGF is catabolised by standard proteolytic pathways with its constituent amino acids being added to the general body pool.

2.3.4. Toxicology

Single and repeat dose toxicity

Dose escalation studies (single dose) in rats and rabbits with ocular, IV and SC administration showed that rhNGF is well tolerated at all tested doses.

Repeat-dose toxicity studies were conducted in rats and rabbits via ocular and SC administration for up to 26 weeks in rats and 90 days in rabbits.

When rhNGF was administered as eye drops up to the dose of 1.2 mg/mL no treatment-related changes were observed in the eyes or systemically. In a 2-month study in rabbits, eyes were also investigated with electroretinography, ocular tonometry and slit lamp, without observing any differences in rhNGF treatment animals compared to the control group. No effects were observed in the nervous tissue (including optic nerve) or brain in any species.

Only in the 4-week rat study slightly dose-dependent increased glucose, chloride and phosphorous concentration were observed in both sexes, and sodium concentration was increased in females after ocular administration of rhNGF. These changes were reversible. As these changes were not observed in the 26-week study in rats, they may be attributable to biological variation.

Studies in rabbits of up to 2 months of daily ocular administration with the formulation intended for marketing but with a 100-fold higher amount of L-methionine did not show any adverse effect.

In the SC studies in rats, leucocyte and lymphocyte increases that correlated with an increased inflammatory mixed cells infiltration at the injection site were observed in males. Slighty increased phosphorous concentration in blood had no morphological correlate in any organ or function. In addition, signs were observed in the rhNGF treated groups such as local swelling of legs, paws, cheeks, nose and tail, and erythema on the ears and tail indicative of immunogenic reaction to the test item.

In the rabbit SC study, effects were seen in the ovaries of females including increase weight proliferation and haemorrhagic cysts in the highest dose group of 200 µg/animal.

The Applicant conducted a comparative study with two rhNGF formulations (with and without chlorobutanol) in rats with intact or abraded cornea. Chlorobutanol was present in some formulations used in no-clinical toxicology studies but is not included in the clinical formulation. Both formulations were well tolerated.

Genotoxicity

No genotoxicity studies have been conducted since NGF is a protein and thus not expected to interact directly with DNA.
Carcinogenicity

No carcinogenicity studies were performed.

Reproduction Toxicity

Reprotoxicity studies were conducted in rats and rabbits after SC administration of rhNGF. No effects were observed on male or female fertility in rats, embryo-foetal development in pregnant rats or rabbits or pre- and postnatal development in rats at the highest doses tested (150 µg/animal/day). Parental clinical signs were similar to the findings in the SC general toxicity studies in rats and rabbits. Despite the presence of anti-rhNGF antibodies, adequate exposure to the test item was obtained in all studies.

Although there are some deviations from the reprotoxicity studies design recommended in guideline ICH S5(R2), such as the lack of an embryo-foetal development study in another species in addition to rabbits (rat) or that males in the fertility study started treatment 2 weeks before mating (4 weeks is recommended), considering the good safety profile of rhNGF in general and reproductive toxicity studies and the fact that exposure in patients is negligible, no additional reproductive toxicity studies were considered necessary.

Toxicokinetic data

See section 2.3.3.

Local Tolerance

Local tolerance was assessed as part of the ocular administration studies in rats and rabbits.

Other toxicity studies

Immunogenicity was assessed as part of the repeat dose studies (see also section 2.3.3.). No sign of an immunotoxic potential of rhNGF according to ICH guideline S8 was observed.

rhNGF formulations containing up to 1 mg/ml of L-methionine (100 fold the concentration of the formulation proposed for commercial use) were tested in rabbits for up to 2 months. No effects on body weight, clinical observations or ophthalmic examinations were observed. In terms of immunogenicity, the profile did not seem to differ from a similar study performed without L-methionine.

The specifications for the drug-product impurities were above the ICH Q3B threshold for qualification and therefore require toxicological justification. Levels of Impurities A, B, F1 and F2 have been present in batches used in the repeat dose toxicity studies at levels close to the proposed levels. Furthermore, the absolute amount of these impurities to which animals were exposed during the repeat dose toxicity studies exceeded the amount by which they will be exposed to clinically at the specification limit proposed. Therefore, the specification levels proposed are sufficiently justified.
2.3.5. Ecotoxicity/environmental risk assessment (ERA)

In line with the Guideline on the environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00 corr 2), since rhNGF is a peptide and unlikely to result in a significant risk to the environment, there is no need for ERA studies.

2.3.6. Discussion on non-clinical aspects

Pharmacology

The primary pharmacology data presented with this application clearly showed that rhNGF manufactured by the applicant binds to the TrkA receptor with high affinity. However, no information has been provided in relation to the affinity for the p75 receptor. The applicant indicated that PC12 cells of rat origin express both TrkA and p75 receptors and that two studies in PC12 cells have been conducted showing PD activity of rhNGF. While it was not entirely clear how the studies in PC12 cells would support binding to both TrkA and p75 receptors, the CHMP acknowledged that it was well documented in the scientific literature that both TrkA and p75 are expressed in the eye and that binding of rhNGF to the p75 receptor increases binding of NGF to TrkA. Taking into account that the rhNGF doses proposed for clinical use are quite low, the known higher affinity for TrkA compared to p75 and the clinical efficacy data (see section 2.5.), absence of data showing a direct binding effect at the p75 receptor can be accepted.

Furthermore, the initial dataset provided in support of this application did not inform on the relative binding of rhNGF to TrkA of rat and rabbit origin compared to human TrkA. The applicant was not able to present data directly comparing the binding affinity of rhNGF to TrkA of rat, rabbit and human origin. Instead reference was made to the ability of rhNGF to induce pharmacological activity in the various species and in some instances in comparison to the endogenously expressed NGF of that species. In this regard published data by Ibanez et al. (1991) provides evidence that both the rat and human NGF have comparable activity in rat PC12 cells. Furthermore, Altar et al. (2001) provide some evidence that binding of rhNGF in rat and rabbit brain was similar. Finally, a new study was conducted in which iodinated rhNGF was tested for binding activity in solubilised rabbit corneas. The results suggest a similar binding affinity between murine NGF and rhNGF binding to rabbit TrkA. In the context of these data and despite the lack of a direct quantitative comparison of rhNGF binding to TrkA of rat, rabbit and human origin, it is agreed that rhNGF is likely to have comparable pharmacological activity at the aforementioned tissues.

Overall, both the in vitro and in vivo pharmacology studies performed provided evidence for pharmacological activity of rhNGF in cells of human, rat and rabbit origin as well as in several animal models. However, in any event, the final evaluation of efficacy will need to rely on the clinical data.

Pharmacokinetics

The absorption profile of the rhNGF in both rats and rabbits has been measured by toxicokinetics studies as part of the toxicology studies. In the absence of a suitable in vivo model of neurotrophic keratitis, this was considered acceptable. In most instances, following administration of rhNGF eye drops, a high interindividual variability of plasma exposure levels was observed. An unexpectedly high systemic exposure was seen in rats, which was attributed to passage of the rhNGF through the naso-lacrimal and nasopharyngeal ducts into the oral cavity where it was, at least in part, absorbed through the sublingual vein. Of note, systemic exposure levels were markedly increased in rats where the cornea was abraded when compared to those with intact cornea where levels were mostly below the level of detection.
Distribution of rhNGF was assessed in rats and showed presence of rhNGF in the serum, urine, kidney, liver, uveal tract/retina and the gastrointestinal tract after topical administration. From a therapeutic perspective relevant was the presence of rhNGF in the cornea and sclera as well as in the optic nerve and retina after administration of 1.0 μg/eye/dose.

**Toxicology**

The species used in the toxicology studies were rat and rabbit. Non-clinical pharmacology studies have shown that these species are relevant and rhNGF receptors are expressed in many tissues including eyes of all mammals. In addition the rabbit is particularly adequate for ocular toxicity and embryo-foetal development investigation. However, as already outlined above in relation to the pharmacology studies, although there is some evidence of pharmacological activity of rhNGF in rabbits, questions remained in this regards as well as in relation to the relative activity of rhNGF in the species of choice as compared to humans with the potential for masking of toxicities due to difference in binding between species.

The assessment of the activity of rhNGF in rabbits was impeded by the findings of lack of activity in a proliferation assay performed in rabbit SIRC cells. The CHMP acknowledged that SIRC cells are of fibroblastic origin and do not express TrkA (Fabricant et al., 1977), which would explain the lack of induction of cell proliferation by rhNGF. However, at the same time it was noted that Fabricant et al. showed very little binding of a $^{125}\text{I}$ radiolabelled mNGF, and mNGF actually induced SIRC cell proliferation in the present assay. The applicant argued that the mNGF extract used in the assay may have contained additional growth factors and that the observed positive response at high doses was unspecific and not related to NGF. The CHMP considered the explanation acceptable.

Only very limited additional support for biological activity of rhNGF in rabbits was derived from the scientific literature (see above). A new study using iodinated rhNGF and solubilised rabbit corneas revealed a dissociation constant (Kd) lower than in another study with human embryonic kidney cells (HEK). However, given the differences in the experimental set-ups of the two studies, it was agreed that rhNGF is likely to have similar pharmacological activity in rabbit cells as compared to human cells. In any event, given that the concentration of rhNGF used in toxicology studies is significantly higher than that proposed for the clinical setting, it was accepted that any differences in pharmacological activity should thereby be accounted for.

When administered in the form of eye drops in rats and rabbits, rhNGF was generally well tolerated. Repeat-dose toxicity studies with rhNGF eye drops concentrations up to 1.2 mg/mL no treatment-related changes were observed in the eyes or systemically. Some clinical signs occurred after SC administration of rhNGF, but all these findings were mild and reversible.

In the rabbit SC study, effects were seen in the ovaries of females including increased weight proliferation and haemorrhagic cysts in the highest dose group of 200 μg/animal. These might result from the pharmacological activity of rhNGF as NGF receptors are present in the ovaries of mammals. However, a toxic effect could not be excluded. Given that rhNGF is administered locally to patients at low doses with low or no systemic exposure, and in light of the significant margin of safety, the CHMP did not further pursue the issue.

No sign of an immunotoxic potential of rhNGF was observed. Limited immunogenicity was observed in rats after topical administration of rhNGF. In rabbits, rhNGF was considerably more immunogenic with the presence of binding antibodies found in most animals after repeated dosing for 8 weeks. The presence of anti-rhNGF antibodies in most animal studies was expected as the animals were exposed to a heterologous protein and this response has no clinical or safety implications for humans.
The effect of the addition of L-methionine to the formulation was investigated both in terms of absorption of rhNGF and local and systemic tolerability. No significant differences in local tolerability, systemic exposure or immunogenic potential were seen in rabbits treated with a formulation without L-methionine or with a 100-fold higher amount of L-methionine than intended for marketing. Furthermore, scientific literature data supported low toxicity and good tolerability of L-methionine both ingested and applied locally on skin or eyes. For example, an eye irritation study in New Zealand rabbits using a dose of 0.1 g of the additive in the conjunctival sac showed no adverse effects in the eyes of the animals, resulting in the classification of the product is classified as non-irritant to the eye [EFSA 2013].

As per ICH S6(R1) guidance, standard carcinogenicity studies are generally inappropriate for biotechnology derived produced, however, the guideline does make reference to studies being potentially required depending on the biological activity of the product with specific reference made to growth factors. NGF is a growth factor with proliferative effects and may play a role in tumourigenesis. The applicant referred to repeat dose studies in animals, in which rhNGF was administered systemically for up to 6 months. In these studies, there was no evidence indicative of proliferative changes in any organs examined. Although the duration of the studies was only 6 months, the CHMP agreed that it was unlikely that rhNGF could contribute to tumour initiation or promotion given the topic route of administration and at the dose levels proposed for use in clinical practice. Nevertheless, it was considered advisable not to use rhNGF eye drops in patients with eye tumours, and a related warning was included in SmPC section 4.4.

2.3.7. **Conclusion on the non-clinical aspects**

Overall, the CHMP was of the view that the non-clinical data presented by the applicant were adequate to support the application for Oxervate eye drops in the treatment of moderate or severe NK in adults. The available data supported pharmacological activity of rhNGF through binding to the TrkA receptor, as well as good tolerability and limited systemic exposure after ocular administration, the latter being mainly the result of absorption from the oral cavity after passage of the eyes drops through the nasolacrimal duct. Absence of certain types of studies including secondary PD studies, cardiac, renal and respiratory safety pharmacology studies, drug-drug interaction and metabolism studies, genotoxicity, and carcinogenicity studies were considered acceptable.

2.4. **Clinical aspects**

To support the present application, the applicant provided the results of one Phase I study (NGF0112) in healthy male and female volunteers, two randomized, vehicle-controlled, double-masked Phase II studies (NGF0212 and NGF0214) in NK patients, whereby study NGF0212 consisted of two segments (Phase I and Phase II) which were analysed separately. Data from two additional studies in other conditions (moderate-severe dry eye disease and retinitis pigmentosa) were also provided to support the safety database.

2.4.1. **Introduction**

**Good Clinical Practice**

The applicant confirmed that the clinical trials were performed in accordance with Good Clinical Practice as claimed by the applicant.
Table 1 - Overview of clinical studies

<table>
<thead>
<tr>
<th>Study identifier (Phase)</th>
<th>Key objectives</th>
<th>Design</th>
<th>Subjects</th>
<th>Test product, doses, duration of treatment</th>
<th>No of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGF0112 (Phase I)</td>
<td>Safety, PK, dose escalation (formulation without methionine)</td>
<td>Randomized, double-masked, combined single and multiple ascending dose study</td>
<td>Healthy volunteers</td>
<td>rhNGF at dose of 0.5, 5, 20, 60 and 180 μg/ml or vehicle 1 drop at different regimens, up to 3 times per day for 1-5 days</td>
<td>73 (57 rhNGF, 16 vehicle)</td>
</tr>
<tr>
<td>NGF0212 (Phase I and II)</td>
<td>Safety, efficacy, PK, dose-ranging (formulation without methionine)</td>
<td>Multicentre, randomized, double-masked, parallel group study including a Phase I and a Phase II segment</td>
<td>Stage 2-3 NK</td>
<td>rhNGF at doses of 10 and 20 μg/ml or vehicle 1 drop 6 times a day for 8 weeks 48 or 56 week follow-up period</td>
<td>174 (118 rhNGF, 56 vehicle): 18 (14 rhNGF, 4 vehicle) in Phase I and 156 (104 rhNGF, 52 vehicle) in Phase II</td>
</tr>
<tr>
<td>NGF0214 (Phase II)</td>
<td>Safety and efficacy (formulation containing methionine)</td>
<td>Multicentre, randomized, double-masked, parallel group study</td>
<td>Stage 2-3 NK</td>
<td>rhNGF 20 μg/ml or vehicle 1 drop, 6 times a day for 8 weeks 24 or 32 week follow-up period</td>
<td>48 (24 rhNGF, 24 vehicle)</td>
</tr>
<tr>
<td>NGF0213 (Phase II)</td>
<td>Efficacy and Safety (formulation containing methionine)</td>
<td>Open label, twogroup, two doses, single centre study</td>
<td>Moderate-severe dry eye disease</td>
<td>rhNGF at doses of 4 and 20 μg/ml twice a day every 12h for 4 weeks (4 week follow-up)</td>
<td>40</td>
</tr>
<tr>
<td>NGF0113 (Phase I/II)</td>
<td>Safety, tolerability and potential efficacy (formulation containing methionine)</td>
<td>Multicentre, randomized, double-masked, parallel-group, dose ranging study</td>
<td>Retinitis pigmentosa</td>
<td>rhNGF at doses of 60 and 180 μg/ml or vehicle 1 drop 3 times a day for 24 weeks with a 24 week follow-up period</td>
<td>50 (40 rhNGF, 10 vehicle)</td>
</tr>
</tbody>
</table>

NK = neurotrophic keratitis; PK = pharmacokinetic; rhNGF = recombinant human nerve growth factor

2.4.2. Pharmacokinetics

The Pharmacokinetic (PK) profile of Oxervate has been evaluated in two clinical studies including both healthy volunteers (study NGF0112) and NK patients (study NGF0212), as well as in three studies using human biomaterials. Immunogenicity was also assessed in these studies, as well as in the pivotal study NGF0214 and study NGF0113 in patients with retinitis pigmentosa.
**Absorption**

The primary aim of the PK evaluation was to assess the potential of systemic absorption of rhNGF after ocular instillation.

A total of 74 healthy volunteers in study NGF0112 received fractionated single and multiple doses of rhNGF (up to 18.9 µg/day [6.3 µg three times a day] up to 5 days) or vehicle control. A total of 64 subjects were considered for the PK evaluation; these were those participating in part A and B of the study receiving either 1 eye drop 3 times during 1 treatment day (part A), or 1 eye drop 3 times a day for 5 days (part B). Serial blood samples were collected pre-dose and after dosing with the rhNGF eye drops (at screening, during the study and at follow-up (Day 10 +/- 2 for Part A and Day 15 +/- 2 for Part B).

Study NGF0212 consisted of 2 phases. The Phase I segment was conducted primarily for the assessment of PK parameters and included 18 patients who were randomised to receive either rhNGF 10µg/ml (7 patients), rhNGF 20 µg/ml (7 patients) or vehicle (4 patients). The respective rhNGF doses were 2.1 µg (10 µg/ml) or 4.2 µg (20 µg/ml). During this Phase of the study, blood samples for PK profiling were collected at multiple time points from Day 1 through to the end of the 8-week controlled treatment period: on Days 1 and 55 (at 0.5 hours pre-dose, and at 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, 6, 6.5, 7, and 8 hours post-dose), on Days 2 and 8 (at 0.5 hours pre-dose, and at 0.5, 1, and 2 hours post-dose), and at Weeks 1, 2, 3, 4, and 6 (pre-initial daily dose). During the Phase II segment of the study, which was the main phase for the efficacy assessment, patients were randomised to the same three treatment groups and sparse PK blood sampling was conducted during the 8 week controlled treatment period for the first 50 patients recruited only: Baseline (pre-treatment) serum samples on Day 0 and at Weeks 1, 3, 6, and 8 (pre-initial daily dose).

A validated ELISA assay was used for PK measurements.

The majority of the subjects (both healthy volunteers and patients) had serum levels of NGF below the limit of quantification (< 32.000 pg/ml). Only 6 out of 64 subjects in study NGF0112 and 7 out of 68 patients in study NGF0212 showed quantifiable serum levels of rhNGF. Two of them were on placebo (vehicle). These serum concentrations of rhNGF have been attributed to individual physiological fluctuation of the basal NGF level, rather than treatment-related absorption.

Given the limited number and the variability of samples, no PK analysis was performed. No dose concentration relationship could be established.

**Immunogenicity**

Short and longer-term rhNGF immunogenicity after single and multiple ocular dosing of rhNGF eye drops (with and without L-methionine) at various concentrations in different populations including healthy subjects and NK patients. In two studies, NGF0212 and NGF0214, immunological data were obtained at the dose regimen proposed for NK treatment (20 µg/ml, one drop 6 times a day [4.2 µg/day] for 8 consecutive weeks). In both these studies, long-term immunogenicity was also assessed during follow-up.

A validated ELISA assay was used to detect Immunoglobulin G antibodies against rhNGF

Serum levels of rhNGF antibodies were found to be below the lower limit of quantification in all 4 studies where this parameter was examined (NGF0112, NGF0212, NGF0214 and NGF0113), suggesting that ocular rhNGF at treatment doses has no systemic immunogenic potential. This result was achieved across patient populations (healthy subjects and NK patients) and dose regimens (up to 180 µg/ml rhNGF three times a day for 24 consecutive weeks). No accumulation or long-term effects were seen.
Distribution

No clinical data were presented. Studies in rat and rabbit in which rhNGF was applied as eye drops at concentrations up to 60-fold the clinical dose indicated a variable, but overall low systemic exposure to rhNGF. Administration of radioactive rhNGF to male and female rats at a nominal dose level of 1.0 μg/eye/dose resulted in the distribution of radioactivity mostly in the cornea and to some minor extent in the posterior portion of the eye.

Elimination

No clinical data in relation to metabolism and elimination of rhNGF has been provided. The applicant stated that rhNGF is a recombinant protein which is not metabolized by the ocular metabolizing enzymes with the exception of tissue proteases that could degrade it to the corresponding amino acids. After ocular administration, a good portion of the protein through the nasolacrimal duct reaches the nasal and then the oropharyngeal cavity and is then degraded by proteases.

Dose proportionality and time dependencies

Differences in doses and administration schedules were assessed as part of the clinical efficacy and safety programme only.

Special populations

No specific studies in special populations were conducted given the lack of systemic absorption of rhNGF after ocular administration.

Pharmacokinetic interaction studies

No specific drug-interaction studies have been performed. Given the method of administration and the limited systemic exposure reached, no systemic drug-drug interactions are expected.

Pharmacokinetics using human biomaterials

To support the human PK development, the applicant conducted 3 studies with human biomaterials to determine the extent of binding of rhNGF to its associated receptors and its effects on cell differentiation.

The affinity of rhNGF and mNGF for the human TrkA receptor was measured in HEK 293 cells using a homogeneous and non-radioactive cell-based assay. The HEK cells expressed TrkA which was fused to a SNAP tag, which in turn was labelled with a fluorescent donor dye (terbium cryptate), while NGF ligands were labelled with a red acceptor dye (d2). The binding of the ligand to TrkA receptor resulted in a measurable fluorescence resonance energy transfer. A competition assay was conducted using 10 concentrations of NGFs were tested at the previously determined optimal cell density (10000 cells/well) with a reference standard ligand (NGF-d2) used at 13 nmol. The assay showed that rhNGF had a higher affinity for the human TrkA receptor expressed in HEK293 cells than mNGF (inhibition constant Ki 3.7 nmol versus 6.2 nmol and median (50%) inhibition concentration IC50 of 7.5 nmol versus 12.4 nmol, respectively). The binding reaction was somehow slow and overnight incubations were needed for reaching equilibrium.
A second study was conducted to evaluate and compare the *in vitro* biological activity (EC$_{50}$) and potency of rhNGF and mNGF using a well-established TF-1 cell proliferation assay adapted from Chevalier et al, 1994. Cells were seeded in a 96-well plate in the presence of rhNGF and mNGF in the concentration range of 0.3 pM (7.95 pg/ml) to 6.76 nmol (179.14 ng/ml) and incubated for 48 hours. Cell proliferation was evaluated by incubating cells for an additional 4 hours in the presence of a chromogenic substrate, then assessed using ELISA. The results showed that rhNGF was more active (approximately 10 fold) than mNGF in the TF-1 assay. Mean EC$_{50}$ calculated for rhNGF was $19.2 \pm 1.6$ pM ($509.44 \pm 42.45$ pg/ml), whereas EC$_{50}$ for mNGF was $237.4 \pm 59.1$ pM.

Finally, in order to evaluate and compare the *in vitro* biological activity of rhNGF, mNGF and a Neurotrophin Mimetic (MimD3), using SH-SY5Y cells. Cells were seeded in an appropriate medium, with either N2 differentiation supplement (control) or rhNGF and mNGF at concentrations of 25, 50 and 100 ng/ml (approximately 1 nmol, 2 nmol, 4 nmol) or MimD3 at 25, 50 and 100 ng/ml (36.85, 73.7 and 147.4 nmol) for a 7 day incubation period at 37 °C. The results showed that rhNGF, mNGF and MimD3 were not cytotoxic to a neuroblastoma-derived cell line at the tested concentrations. All compounds induced an increase of neurite localization of the early neuronal differentiation marker β-tubulin III, but rhNGF at 25 ng/ml was more effective than mNGF and MimD3 in promoting axonal sprouting and neurite length. MimD3 was the least active of the three compounds. At higher concentrations (100 ng/ml), the effect of both NGFs was comparable. Gap-43, a protein that it is highly expressed in processes related with axonal regeneration was mainly found in the axons of rhNGF treated cells and in the cytoplasm of mNGF cultures. Localization in axons was higher in rhNGF cultured cells at 50 ng/ml and levels were comparable at 100 ng/ml.

### 2.4.3. Pharmacodynamics

In absence of adequate clinical models of NK, no specific clinical pharmacology studies have been conducted.

**Mechanism of action**

Cenegermin (rhNGF) is a recombinant form of human nerve growth factor, an endogenous protein involved in the differentiation and maintenance of neurons. NGF is naturally present in the eye and is essential for the survival and growth of sympathetic and sensory neurons and for differentiation of neurons in the central nervous system (CNS). The protein is highly conserved across species, with human NGF and mNGF sharing 90% homology in the amino acid sequence of the mature protein.

NGF binds with two entirely distinct classes of receptors: 1) tropomyosin receptor kinase A (TrkA), a transmembrane tyrosine kinase that is also known as high-affinity NGF receptor, and 2) low-affinity NGF receptor, also called p75 neurotrophin receptor. NGF receptors are expressed on anterior segments of the eye (iris, ciliary body, lens, cornea and conjunctiva) (Lambiase et al., 2002; Qi et al., 2007) and by the lacrimal gland (Nguyen et al., 1997; Ghinelli et al., 2003; Vesaluoma et al., 2000) as well as by all the intraocular tissues including the posterior segment of eye (Lambiase et al., 2002).

NGF has been shown to play a crucial role in the pathophysiology of several ocular diseases, including NK. In NK, corneal sensory innervation is impaired, causing marked changes in the levels of neuromediators, and resulting in dystrophic changes of epithelial cells and consequently epithelial breakdown. NGF in turn has been shown to induce *in vitro* corneal epithelial cell proliferation and differentiation and it is involved in maintaining limbal epithelial stem cell potential (Kruse & Tseng, 1993; Qi et al., 2007). In addition, animal models of corneal injury (such as mechanical epithelial removal and refractive surgery) show that NGF
expression is increased following surgical procedures, and that NGF eye drop treatment stimulates corneal epithelial healing (Eskenazi et al., 2005). Evidence of NGF involvement in corneal trophism and homeostasis has been demonstrated in a molecular study on keratoconus cornea (Lambiase et al, 2005). Increasing evidence also shows that NGF is involved in the regulation of tear film production (Ghinelli et al., 2003).

**Primary and Secondary pharmacology**

No clinical PD clinical studies have been conducted by the applicant. Studies in human biomaterial demonstrating pharmacological activity of rhNGF relevant to its use in NK are summarised in section 2.4.2.

**2.4.4. Discussion on clinical pharmacology**

Limited clinical pharmacology data have been presented in support of this application. This was considered largely acceptable by the CHMP given the local route administration as well as low doses and limited duration of treatment required.

For the most part, the PK data provided were considered reassuring showing no or very low systemic absorption after ocular instillation of rhNGF eye drops. Immunogenicity results complemented the PK findings indicating that ocular rhNGF is not systemically absorbed. No antibodies to rhNGF were detected in any of the subjects using rhNGF eye drops in the 4 studies where this parameter was examined, suggesting no systemic immunogenic potential including at the dose regimen proposed for use in NK patients.

Since there is no adequate clinical model of NK, no specific clinical PD studies have been conducted, which was considered acceptable by the CHMP. Results of studies with human biomaterial (as well as from the clinical and non-clinical program) showed that rhNGF elicits bioactivity relevant to the proposed indication and has a 10-times higher affinity to the TrkA receptor when compared to mNGF. Furthermore, a better safety profile of rhNGF compared to mNGF can be assumed given that rhNGF is a recombinant form of human endogenous NGF.

The lack of any information on the possibility of interactions with other ophthalmological medicines administered via the ocular route was explained by the applicant by the low interaction potential of rhNGF as it is a recombinant protein which is not metabolized by the ocular cytochrome P 450 enzymes or by any other ocular metabolizing enzyme with the exception of tissue proteases that could degrade it to the corresponding amino acid. Furthermore, in clinical practice, NK patients are advised to discontinue other topical treatments. rhNGF. The issue was not pursued in light of the available clinical data which did not suggest any problems with the co-administration of other ocular medicines (see section 2.6. ).

**2.4.5. Conclusions on clinical pharmacology**

The clinical pharmacology of Oxervate has been sufficiently well characterised to support the present application.
2.5. Clinical efficacy

2.5.1. Dose response study(ies)

Study NGF0212 was the only clinical study that conducted a formal comparison between rhNGF doses, comparing 10 μg/ml and 20 μg/ml rhNGF to vehicle control. The rationale for selecting the 20 μg/ml dosage of rhNGF eye drops solution and the 6 times/day dosing regimen in both efficacy studies was derived from the results across the rhNGF clinical and non-clinical program as well as published literature.

Published clinical studies using mNGF 200 μg/ml eye drops, administered to NK patients 6 times a day for up to 60 days (Bonini et al., 2000; Lambiase et al., 2007) showed complete corneal healing and corneal sensitivity improvement in the majority of patients (see summary in section 2.5.2.6.). The mNGF used in these studies is closely homologous to rhNGF, so an extrapolation could be made across species. In addition, in vitro models utilizing human cells have demonstrated that rhNGF is more potent than mNGF by a factor of approximately 10 and hence supported a 10-fold reduction for the rhNGF dose.

Furthermore, a dose-response relationship was identified in in vitro studies in rabbits and rats. A set of experiments in Sprague Dawley rats performed with the aim of comparing the efficacy of 10 and 20 μg/ml rhNGF administered according to 2 different schedules: 6 times per day (i.e. 1 eye drop every 2 hours) versus 3 times per day (i.e. 1 eye drop every 4 hours) showed a dose-response relationship. The largest effect was observed in rats treated with 20 μg/ml 6 times daily. The efficacy of rhNGF tended to decrease when the number of dispensed drops per day was reduced. A dose-response effect on neurite length was also observed in human biomaterial studies, with neurite length increasing with increasing rhNGF concentrations.

Masked preliminary efficacy results of study NGF0212 showed a trend of increased efficacy of the 20 μg/ml as compared to the 10 μg/ml strength without additional safety concerns for the higher dose. Therefore, for study NGF0214, only 20 μg/ml rhNGF eye drops 6 times/day was selected for testing.

Doses higher than 20 μg/ml were tested during Phase I of the clinical development (NGF0112) but only for safety and tolerability purposes.

2.5.2. Main study(ies)

Two randomized, vehicle-controlled, double-masked, Phase II studies (NGF0212 and NGF0214) were conducted to support the efficacy of rhNGF in patients with stage 2 or 3 NK.

Study NGF0212: An 8-week Phase I/II, Multicentre, Randomized, Double-masked, Vehicle-controlled Parallel-group Study with a 48- or 56-week Follow-up Period to Evaluate the Safety and Efficacy of Two Doses (10 μg/ml and 20 μg/ml) of Recombinant Human Nerve Growth Factor Eye Drops Solution Versus Vehicle in Patients with Stage 2 and 3 of Neurotrophic Keratitis

Study NGF0212 was conducted in several EU countries as an 8-week, randomized, double-masked, vehicle controlled, parallel group study, followed by a 48- or 56-week follow-up period. The study design consisted of a Phase I segment and Phase II segment with each part analysed separately. Only the Phase II segment of NGF0212 is included in this analysis of efficacy since the Phase I segment was conducted primarily for the assessment of PK parameters (see section 2.4.2.).
All patients completely healed at week 8 (including those receiving active treatment) were eligible for another course of treatment in the event of recurrence during the follow-up period. The follow-up period was 48 weeks (approximately 12 months) for patients who were initially randomized to rhNGF (10 μg/ml or 20 μg/ml), regardless of whether the patient was completely healed or not completely healed at Week 8. The follow-up period was also 48 weeks in length for patients who were initially randomized to vehicle and who were completely healed at Week 8. The follow-up period was 56 weeks (approximately 14 months) in length for patients who were initially randomized to vehicle and who were not completely healed at Week 8. These patients were randomly assigned to treatment with rhNGF (10 μg/ml or 20 μg/ml) for 8 weeks (from Week 8 to Week 16); this was referred to as the uncontrolled treatment period.

**Study NGF0214: An 8-week phase II, multicentre, randomized, double-masked, vehicle controlled, parallel group study with a 24 or 32 week follow-up period to evaluate the efficacy of a formulation containing anti-oxidant of recombinant human nerve growth factor (rhNGF) in 20 μg/ml, eye drops solution versus vehicle containing anti-oxidant in patients with Stage 2 and 3 Neurotrophic Keratitis**

Study NGF0214 was conducted in the United States of America (USA) as an 8-week multicentre, randomized, vehicle controlled, double-masked, parallel-group study, followed by a 24- or 32-week follow-up period. The NGF0214 study was designed as a two-arm study to confirm superiority of the methionine containing rhNGF.
20 μg/ml eye drops solution 6 times a day versus vehicle containing the same amount of methionine given 6 times a day.

Similar to study NGF0212, if not completely healed at week 8, patients randomized to vehicle during the controlled treatment period were eligible for a treatment with rhNGF 20 μg/ml during the uncontrolled treatment period. Patients, completely healed at Week 8 or Week 16, with a recurrent PED or corneal ulcer electing to receive an additional course of rhNGF treatment were followed with the assessments outlined in the 8 week, randomized, double-masked, controlled treatment period visits. A maximum of one additional rhNGF treatment course of 8 weeks could be given during the uncontrolled treatment period.

2.5.2.1. Methods

Study Participants

Key Inclusion Criteria

- Patients 18 years of age or older.
- Patients with Stage 2 PED or Stage 3 (corneal ulcer) NK. In Study NGF0212, patients with NK stage 2 or 3 involving only 1 eye were permissible. Patients with contralateral eye affected with Stage 1 NK could be enrolled. In Study NGF0214, patients with one or both eyes affected could be enrolled.
- PED or corneal ulceration of at least 2 weeks duration, refractory to one or more conventional non-surgical treatments for NK (eg, preservative-free artificial tears, gels or ointments; discontinuation of preserved topical drops and medications that can decrease corneal sensitivity; therapeutic contact lenses).
- Evidence of decreased corneal sensitivity (≤ 4 cm using the Cochet-Bonnet aesthesiometer) within the area of the PED or corneal ulcer and outside of the area of the defect in at least 1 corneal quadrant.
- Best corrected distance visual acuity (BCDVA) score ≤ 75 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (equivalent to ≥ + 0.2 LogMAR, ≤ 20/32 Snellen or ≤ 0.625 decimal fraction) in the affected eye.
- No objective clinical evidence of improvement in the PED or corneal ulceration within the 2 weeks prior to study enrolment.

Key Exclusion Criteria

- Any active ocular infection (bacterial, viral, fungal or protozoal) or active ocular inflammation not related to NK in the affected eye.
- Any other ocular disease requiring topical ocular treatment in the affected eye during the course of the study treatment period. No topical treatments other than the study medications provided by the study sponsor or allowed by the study protocol could be administered in the affected eye during the course of the study treatment periods.
- Patients with severe vision loss in the affected eye with no potential for visual improvement in the opinion of the Investigator as a result of the study treatment.
- Schirmer test without anesthesia ≤ 3 mm/5 minutes in the affected eye.
• Patients with severe blepharitis and/or severe meibomian gland disease in the affected eye.

• History of any ocular surgery (including laser or refractive surgical procedures) in the affected eye within the three months before study enrolment.

• Prior surgical procedure(s) for the treatment of NK (eg, complete tarsorrhaphy, conjunctival flap, etc) in the affected eye with the exception of amniotic membrane transplantation. Patients previously treated with Botox (botulinum toxin) injections were eligible for enrolment only if the last injection was given at least 90 days prior to enrolment in the study.

• Use of therapeutic contact lenses or contact lens worn for refractive correction during the study treatment periods in the eye with NK.

• Anticipated need for punctual occlusion during the study treatment period. Patients with punctual occlusion or punctual plugs inserted prior to the study were eligible for enrolment provided that the punctual occlusion was maintained during the study.

• Evidence of corneal ulceration involving the posterior third of the corneal stroma, corneal melting or perforation in the affected eye.

• Presence or history of any ocular or systemic disorder or condition that might have hindered the efficacy of the study treatment or its evaluation, could possibly have interfered with the interpretation of study results, or could have been judged by the Investigator to be incompatible with the study visit schedule or conduct (eg, progressive or degenerative corneal or retinal conditions, uveitis, optic neuritis, poorly controlled diabetes, autoimmune disease, systemic infection, neoplastic diseases).

• Any need for or anticipated change in the dose of systemic medications known to impair the function of the trigeminal nerve (eg, neuroleptics, antipsychotic and antihistamine drugs), unless initiated prior to 30 days before study enrolment and they remained stable throughout the course of the study treatment periods.

**Treatments**

**Test product:** rhNGF 10 μg/ml (one 35 μl drop equals to 0.35 μg of rhNGF), or rhNGF 20 μg/ml (one 35 μl drop equals to 0.70 μg of rhNGF).

**Vehicle Control:** Ophthalmic solution of the same composition as the test product but excluding rhNGF.

In contrast to study NGF0212, both test products and vehicle contained the anti-oxidant L-methionine.

The test product and vehicle were formulated as single-use preparation (frozen-solution packaged in a glass vial) and administered through a sterile polyethylene dropper (in the kit box there was a dropper per each vial). The study treatments were supplied in identical boxes. Each treatment kit included 7 boxes, each containing 6 frozen single-use vials of the randomized / assigned medication for daily treatment, i.e. a total number of 42 vials in each kit. At home the study medication was to be stored in the fridge at 2 - 8 °C for a maximum of 7 days. When the patient started to use the study medication he/she was to remove from the fridge only 1 daily box including 6 single-use vials to be used over the course of the day.

One drop (35 μl) of study medication was to be administered 6 times a day in the affected eye(s), during the 8-week, randomized, double-masked, controlled treatment period and during the 8-week, uncontrolled treatment period for qualifying study subjects (see study design above).
Objectives

Study NGF0212 primarily aimed at assessing safety and the efficacy of rhNGF eye drops solution (10 μg/ml or 20 μg/ml) compared to vehicle in inducing complete healing of Stage 2 (PED) and Stage 3 (corneal ulcer) NK as measured by the central reading centre evaluating the clinical pictures of corneal fluorescein staining. Secondary objectives of the study were to assess the complete healing as measured by the investigator, the duration of complete healing, improvement in visual acuity and improvement in corneal sensitivity, and percentage of patients achieving complete corneal clearing defined as complete absence of staining on the modified Oxford Scale.

The primary objective of study NGF0214 was to evaluate the efficacy of 20 μg/ml rhNGF eye drops solution containing anti-oxidant compared to vehicle (formulation containing anti-oxidant) in inducing complete healing of stage 2 (PED) and 3 (corneal ulcer) NK as measured by the central reading centre, evaluating the clinical pictures of corneal fluorescein staining. Secondary objectives of the study were to assess the duration of complete healing, improvement in visual acuity and improvement in corneal sensitivity, and percentage of patients achieving complete corneal clearing defined as complete absence of staining on the modified Oxford Scale.

Outcomes/endpoints

The primary efficacy endpoint was the percentage of patients experiencing complete healing, defined as the greatest diameter of corneal fluorescein staining in the area of the PED or corneal ulcer, as determined by the reading centre, being less than 0.5 mm. Corneal Fluorescein Staining using the modified Oxford scale was assessed at the slit lamp using a yellow barrier filter and cobalt blue illumination.

In study NGF0212, complete corneal healing assessed at Week 4 was considered the primary efficacy endpoint, while in study NGF0214 complete healing at 8 weeks was chosen as primary study endpoint.

Secondary efficacy endpoints:

- Percentage of patients experiencing complete healing of the PED or corneal ulcer determined by corneal fluorescein staining at 4 weeks (study NGF0212) or 8 weeks (study NGF0214) as defined by the Investigator.
- Percentage of patients experiencing complete healing of the PED or corneal ulcer at 6 and 8 weeks (NGF0212)/ 4 and 6 weeks (NGF0214) as measured by both the central reading centre and Investigator.
- Percentage of patients experiencing complete corneal clearing (Grade 0 on the modified Oxford scale) at 4, 6 and 8 weeks.
- Mean change in BCDVA from baseline to Week 8.
- Percentage of patients that achieved a ≥15 letter gain in BCDVA at 4, 6, and 8 weeks.
- Percentage of patients that achieved an improvement in corneal sensitivity as measured by the Cochet-Bonnet aesthesiometer at 4, 6 and 8 weeks (binary goal attainment variable: Week 4/6/8 corneal sensitivity – Baseline corneal sensitivity > 0 [Yes/No]).
- Percentage of patients experiencing deterioration (increase in lesion size ≥ 1mm, decrease in BCDVA by >5 ETDRS letters, progression in lesion depth to corneal melting or perforation, onset of infection) in Stage 2 or 3 NK from baseline to Weeks 4, 6 and 8.
- Time to onset of deterioration from baseline to Week 8.
- Investigator global evaluation of efficacy at 4 and 8 weeks.

**Study NFG0212 included the following secondary efficacy endpoints related to the follow-up period:**

- Percentage of patients achieving complete healing of the PED or corneal ulcer by Week 8/16 that remained healed (ie, no recurrence) at Weeks 20/28, 32/40, 44/52, 56/64.
- Mean change in BCDVA in patients achieving complete healing by Week 8/16 at Weeks 20/28, 32/40, 44/52, 56/64.
- Percentage of patients achieving complete healing by Week 8/16 that achieved ≥ 15 letter gain in BCDVA at Weeks 20/28, 32/40, 44/52, 56/64.
- Percentage of patients achieving complete healing by Week 8/16 with improved or no change in corneal sensitivity that show further improvement or no change at Weeks 20/28, 32/40, 44/52, 56/64.
- Percentage of patients achieving complete healing by Week 8/16 with no recurrence of Stage 2 (PED) or Stage 3 (corneal ulcer) at Weeks 20/28, 32/40, 44/52, 56/64.
- Time to recurrence of Stage 2 (PED) or Stage 3 (corneal ulcer) in patients achieving complete healing by Week 8/16, defined as the stage of NK recorded by the Investigator as Stage 2 or Stage 3 after healing.

**Study NFG0212 also included a number of exploratory efficacy variables**, including time to complete corneal clearing and to onset of healing (defined as a >20% reduction in the greatest diameter of the lesion), change in Schirmer’s without anesthesia score, change in tear film osmolarity and change in National Eye Institute Visual Functioning Questionnaire 25 (NEI-VFQ) and EuroQol 5D (EQ-5D) scores.

**Sample size**

The sample size for study NGF0212 was calculated based on an estimated 60% of patients achieving complete healing of the PED or corneal ulcer with rhNGF eye drops at 4 weeks as compared to 30% in patients treated with the vehicle. Based on this, the Phase II segment of study NGF0212 needed 141 evaluable patients to have 80% power to detect such a difference. Assuming a drop-out rate of 10-20%, a minimum of 156 patients were to be randomized in the Phase II segment. This sample size was considered adequate to evaluate safety for progression to continue into Phase II of the study. A minimum of 174 patients in total (including 18 patients for the Phase I segment) was planned to be randomized.

The sample size calculation for study NGF0214 was based on the assumption of the statistical superiority of rhNGF 20 μg/ml versus vehicle and on preliminary masked data from the ongoing NGF0212 study after Week 8. The proportion of patients achieving complete healing of the PED at Week 8 was estimated at 70% in the rhNGF 20 μg/ml treated group and 30% for vehicle. Thirty eight patients (19 patients in each treatment arms) were to be recruited in order to observe with 80% power a difference in the healing rate of 40% between treatments using a one-sided Chi-square test. At least 48 patients were considered necessary to be randomized to have at least 38 evaluable patients for the statistical analysis.

**Randomisation**

Eligible patients were randomized using an Interactive Web Response System. Patients were randomized in a 1:1:1 ratio (study NGF0212) or 1:1 ratio (study NGF0214) to either active or vehicle treatment.
In the phase II segment of study NGF0212, included in the baseline randomization scheme for all patients assigned to vehicle was a randomized secondary treatment assignment to active study medication (rhNGF 10 μg/ml or 20 μg/ml). This randomized secondary treatment assignment defined the regimen of the active study medication that the patients in the vehicle control arm would receive during the 56-week follow-up period if not completely healed at Week 8, or in the event of a recurrence of their PED or corneal ulcer.

**Blinding (masking)**

During the 8-week randomized, double-masked controlled treatment period, the patient, the investigator, all other site staff involved in study assessments, and the sponsor’s clinical research personnel were blinded to the study treatment. In study NGF0212, although only treatment with rhNGF was administered during the 48- or 56-week follow-up period, the rhNGF dose administered was not made known to the sponsor, patients, investigators and site personnel, and the treatment assignments during the 8-week controlled treatment period were not disclosed. Unmasking during the studies occurred only if the patient progressed to deterioration of their Stage 2 or 3 NK or in the case of medical emergencies where the knowledge of patient treatment was required to provide the patient with appropriate care.

The study treatments were supplied in identical boxes, and the vials of rhNGF (both doses) and the vials containing the vehicle were identical in appearance and the contents of the vials were indistinguishable.

**Statistical methods**

**Study Populations**

The following populations were used for the analysis and presentation of the study data.

- Intent-to-Treat (ITT) Population: The ITT population was defined as all randomized patients. The ITT population was used to present all efficacy analyses. Patients were summarized according to the treatment to which they were randomized.

- Safety Population: The Safety population was defined as all randomized patients who received at least 1 dose of study medication. The Safety Population was used to present all safety summaries.

**Primary Analysis:**

For study NGF0214, there were 2 planned comparisons of the percentage of patients achieving complete healing of the PED or corneal ulcer at Week 4, comparing the rhNGF 10 μg/ml group against vehicle and comparing the rhNGF 20 μg/ml group with vehicle. In addition, the two rhNGF treatment groups were compared against each other to test possible differences between the doses. This comparison between the active doses was considered exploratory. Each of the comparisons was conducted on the data for the Phase II segment of the study using a 2 × 2 chi-square test, based on the null hypothesis that there was no association between treatment (rhNGF or Vehicle Control) and response (Complete Healing at Week 4 [Yes/No]). In the case of missing data at Week 4, the last post-Baseline observation prior to Week 4 was carried forward for the primary analysis. An observed case analysis was also conducted. The significance level of the chi-square test was corrected for multiplicity according to the Pocock method, and the 2-sided significance level α for statistical tests was 0.0294.

For study NGF0212, the two-sided significance level for the 2x2 chi-square test was 0.10 to compare patients receiving 20 μg/ml rhNGF to patients receiving vehicle. Patients, who discontinued before Week 4 (and who
did not have a post-baseline corneal photograph with fluorescein), were assumed to have been ‘Not Completely Healed’ for primary efficacy endpoint if the investigator recorded that the ‘measurement was N/A because the greatest dimension of the PED or corneal ulcer evaluated was greater than 1 mm on the slit lamp’. If no post-baseline values were available, no imputation was performed, the patient was assumed to have a missing ‘Completely Healed’ endpoint and was not included in the analysis. All other missing evaluations were imputed by the last observation carried forward (LOCF) method up to the 8 Week visit.

Sensitivity analyses included multiple imputation methods for missing data. In addition, in study NGF0212 an analysis was performed of patients considered to have not completely healed (ie, were treated as failures) if they did not have a completely healed Yes/No response as determined by the reading centre at Week 4, irrespective of the reason for the missing data. In study NGF0214, additional sensitivity analyses were performed (i) on observed cases removing patients who were discontinued from the study before week 4, and (ii) imputing all missing completely healed statuses as failures.

In study NGF0212, as an additional exploratory analysis, the chi-square treatment comparisons described above were repeated for the subgroups of patients with evidence of punctual occlusion during the external ocular examination of the affected eye at any visit from Baseline to Week 4 and those with no such evidence.

**Secondary analysis:**

The chi-square treatment comparisons described for the primary endpoint was also conducted at the significance level of 0.0294 (study NGF0212) or 0.10 (study NGF0214) for secondary endpoints of complete healing. For endpoints concerning the percentage of patients who experienced complete corneal clearing, achieved 15 letter gain in BCDVA or improvements in corneal sensitivity, and who experienced deterioration, chi-square treatment comparisons were conducted. The mean change in BCDVA score from the Baseline visit to the Week 8 visit was analysed using an analysis of covariance (ANCOVA) model including treatment and Baseline BCDVA score as covariates. In study NGF0212, the ANCOVA analysis also included amount of corneal anaesthesia at baseline, time since diagnosis of NK and Schirmer test value as covariates. A Kaplan-Meier survival analysis was performed on the time to onset of deterioration from baseline to week 8.

For the investigator global evaluation of efficacy in study NGF0212, the number and percentage of patients with investigator responses in each of the following categories (‘Very Good’, ‘Good’, ‘Moderate’, ‘Poor’ and ‘Non-evaluable’) was recorded. Data were analysed by means of a multinomial proportional odds model. The model included treatment, visit and treatment-by-visit interaction as fixed effects with patient identifier as the repeated variable. In study NGF0214, data were collected as a categorical variable (‘Very satisfactory’, ‘Satisfactory’, ‘Not very satisfactory’ and ‘Unsatisfactory’) and analysed with a chi-square test.

No formal statistical testing of the data collected during the follow-up period of study NGF0212 was performed.

**2.5.2.2. Results for Study NFG0212**

**Participant flow**

A total of 156 patients were randomized to the controlled treatment period: 52 patients in each treatment group. Of the 156 patients randomized, a total of 109 patients (69.9%) entered the 48-week follow-up period. A total of 52 patients (33.3%) were withdrawn prematurely from the study including slightly more in the active arms compared to vehicle.
Of the 52 patients who were initially randomized to the vehicle control group during the 8-week controlled treatment period, a total of 23 patients (44.2%) were randomized to the uncontrolled treatment period at Week 8: 10 patients were randomized to rhNGF 10 μg/ml and 13 patients were randomized to rhNGF 20 μg/ml. Of these 23 patients, 22 patients (95.7%) entered the 56-week follow-up period: 9 patients in the rhNGF 10 μg/ml group and 13 patients in the rhNGF 20 μg/ml group. A total of 16 patients (69.6%) completed the study.

Table 2 - Summary of Patient Disposition (All Patients, Study NGF0212)

<table>
<thead>
<tr>
<th></th>
<th>rhNGF 10 μg/ml (N=52)</th>
<th>rhNGF 20 μg/ml (N=52)</th>
<th>Vehicle Control (N=52)</th>
<th>Total (N=156)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized at Baseline</td>
<td>52 (100.0%)</td>
<td>52 (100.0%)</td>
<td>52 (100.0%)</td>
<td>156 (100.0%)</td>
</tr>
<tr>
<td>Randomized at Week 8</td>
<td>0</td>
<td>0</td>
<td>23 (44.2%)</td>
<td>23 (14.7%)</td>
</tr>
<tr>
<td>Withdrawn from Study</td>
<td>19 (36.5%)</td>
<td>19 (36.5%)</td>
<td>14 (26.9%)</td>
<td>52 (33.3%)</td>
</tr>
<tr>
<td>Withdrawn on or before Week 8a</td>
<td>7 (36.8%)</td>
<td>13 (68.4%)</td>
<td>4 (28.6%)</td>
<td>24 (46.2%)</td>
</tr>
<tr>
<td>Withdrawn after Week 8 and on or before Week 16a</td>
<td>0</td>
<td>0</td>
<td>1 (7.1%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>Withdrawn during 48-Week or 56-Week Follow-Up Periodb</td>
<td>12 (63.2%)</td>
<td>6 (31.6%)</td>
<td>9 (64.3%)</td>
<td>27 (51.9%)</td>
</tr>
<tr>
<td>Attended Week 8 Visit</td>
<td>48 (92.3%)</td>
<td>42 (80.8%)</td>
<td>40 (76.9%)</td>
<td>130 (83.3%)</td>
</tr>
<tr>
<td>Attended Week 16 Visit</td>
<td>0</td>
<td>0</td>
<td>22 (42.3%)</td>
<td>22 (14.1%)</td>
</tr>
<tr>
<td>Entered the 48-Week Follow-Up Period</td>
<td>45 (86.5%)</td>
<td>39 (75.0%)</td>
<td>25 (48.1%)</td>
<td>109 (69.9%)</td>
</tr>
<tr>
<td>Entered the 56-Week Follow-Up Period</td>
<td>0</td>
<td>0</td>
<td>22 (42.3%)</td>
<td>22 (14.1%)</td>
</tr>
<tr>
<td>Completed the Study</td>
<td>33 (63.5%)</td>
<td>33 (63.5%)</td>
<td>38 (73.1%)</td>
<td>104 (66.7%)</td>
</tr>
<tr>
<td>Completed the Study with 48-Week Follow-Up Periodb</td>
<td>33 (100.0%)</td>
<td>33 (100.0%)</td>
<td>22 (57.9%)</td>
<td>88 (84.6%)</td>
</tr>
<tr>
<td>Completed the Study with 56-Week Follow-Up Periodb</td>
<td>0</td>
<td>0</td>
<td>16 (42.1%)</td>
<td>16 (15.4%)</td>
</tr>
</tbody>
</table>

The denominator of percentage was the number of patients randomized at Baseline in each group, and their total.

- The denominator of percentage was the number of patients who withdrew from the study.
- The denominator of percentage was the number of patients who completed the study.

Recruitment

Date of first observation: 30 Jan 2013; date of last observation: 19 May 2016.

Conduct of the study

The original protocol, dated 19 July 2012, was amended 4 times. Furthermore, there were 3 addenda to the statistical analysis plan (SAP). These included the introduction of additional post-hoc analyses. After
finalisation of the main clinical study report, the US Food and Drug Administration requested the sponsor to repeat the analyses of complete healing at Week 4 and Week 8, considering patients with persistent residual staining (ie, persistent in a specific zone of the cornea) to be not completely healed.

Overall, a total of 30 protocol violations were reported during the study. The main categories of protocol violations were: concomitant medications (occurring in 9 patients), inclusion criteria and/or exclusion criteria (occurring in 9 patients), investigational product (occurring in 4 patients), informed consent (occurring in 2 patients), and randomization, visit schedule, study procedures, and “Other” (patient did not stop preservative-free artificial tears at Day 0 and stopped at Week 2) (each occurring in 1 patient).

Baseline data

A summary of demographics for patients in the Phase II segment of the study (Safety population) is presented in Table 3. Overall, 61 patients (39.1%) were male and 95 patients (60.9%) female. The mean age was 60.6 years with a range of 18 to 95 years.

Table 3 – Summary of Patient Demographics, by Treatment and Overall (Safety Population, Study NGF0212 Phase II)

<table>
<thead>
<tr>
<th></th>
<th>Baseline Randomized Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rhNGF 10 µg/ml (N=52)</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>59.0 (17.17)</td>
</tr>
<tr>
<td>n</td>
<td>52</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>61.5</td>
</tr>
<tr>
<td>Median</td>
<td>20.87</td>
</tr>
<tr>
<td>Gender n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (42.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>30 (57.7%)</td>
</tr>
<tr>
<td>Ethnicity n (%)</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>4 (7.7%)</td>
</tr>
<tr>
<td>Hispanic, Latino or Spanish</td>
<td>6 (11.5%)</td>
</tr>
<tr>
<td>Not Hispanic, Latino or Spanish</td>
<td>42 (80.8%)</td>
</tr>
<tr>
<td>Race n (%)</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>5 (9.6%)</td>
</tr>
<tr>
<td>White</td>
<td>46 (88.5%)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (1.9%)</td>
</tr>
</tbody>
</table>

Abbreviations: Min = minimum; Max = maximum; rhNGF = recombinant human nerve growth factor; SD = standard deviation.
N/A: Not applicable (Ethnicity and race were not collected in all countries).
Percentages are calculated using the number of non-missing responses in each treatment group as the denominator.

a Age was recorded directly in the database and was not calculated separately.
Medical History

Overall, 76 patients (48.7%) had Stage 2 NK and 80 patients (51.3%) had Stage 3 NK. The duration of the disease prior to enrolment was approximately 28 months and PED or corneal ulcers had been present on average for 17 months. Herpetic keratitis (herpes simplex, herpes zoster) was the main aetiology, with surgical procedures, dry eye disease and diabetes mellitus being reported less frequently.

Table 4 - Summary of Primary Diagnosis History (Safety Population, NGF0212 Phase II)

<table>
<thead>
<tr>
<th>Underlying Cause of NK (n)</th>
<th>rhNGF 10 µg/ml (N=52)</th>
<th>rhNGF 20 µg/ml (N=52)</th>
<th>Vehicle (N=52)</th>
<th>Total (N=156)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustic neuraoma</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Chronic topical medications e.g. glaucoma drops</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Dry eye disease</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>12</td>
<td>7</td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Neurosurgical procedure</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Non-viral infection</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ocular surface injury or inflammation</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Ocular surgery procedure</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Other systemic condition</td>
<td>-</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>11</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>Systemic medication</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Topical medication use/abuse or toxicity</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Since Initial Diagnosis of NK (months)^a</th>
<th>52</th>
<th>52</th>
<th>52</th>
<th>156</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>28.14 (26.165)</td>
<td>30.25 (26.805)</td>
<td>24.32 (26.841)</td>
<td>27.57 (55.329)</td>
</tr>
<tr>
<td>Median</td>
<td>4.76</td>
<td>12.27</td>
<td>6.01</td>
<td>7.00</td>
</tr>
<tr>
<td>Min, Max</td>
<td>0.7, 350.6</td>
<td>0.8, 331.9</td>
<td>0.8, 271.6</td>
<td>0.7, 350.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time Since Initial Diagnosis of NK stage 2 or stage 3 (months)^b</th>
<th>52</th>
<th>52</th>
<th>52</th>
<th>156</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>17.66 (52.971)</td>
<td>17.50 (32.006)</td>
<td>15.52 (40.562)</td>
<td>16.89 (42.457)</td>
</tr>
<tr>
<td>Median</td>
<td>3.55</td>
<td>6.55</td>
<td>3.40</td>
<td>3.79</td>
</tr>
<tr>
<td>Min, Max</td>
<td>0.7, 350.6</td>
<td>0.4, 192.5</td>
<td>0.8, 271.6</td>
<td>0.4, 350.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classification of NK, n (%)^c</th>
<th>Stage 1</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td>21 (40.4%)</td>
<td>27 (51.9%)</td>
<td>28 (53.8%)</td>
<td>76 (48.7%)</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>31 (59.6%)</td>
<td>25 (48.1%)</td>
<td>24 (46.2%)</td>
<td>80 (51.3%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Eye, n (%)^d</th>
<th>Right</th>
<th>31 (59.6%)</th>
<th>22 (42.3%)</th>
<th>23 (44.2%)</th>
<th>76 (48.7%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>21 (40.4%)</td>
<td>30 (57.7%)</td>
<td>29 (55.8%)</td>
<td>80 (51.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Min = minimum; Max = maximum; NK = neurotrophic keratitis; rhNGF = recombinant human nerve growth factor; SD = standard deviation.

Percentages are calculated using the number of non-missing responses in each treatment group as the denominator.

^a/b | Time since initial diagnosis of NK = (informed consent date - initial NK diagnosis date) / 365.25 x 12.

^c | Stage 1: Presence of epithelial dystrophy or punctuate keratopathy; Stage 2: PED; Stage 3: Corneal ulcer.
Prior and concomitant medication

Overall, the most commonly used prior medications for NK were topical antibiotics (95 patients, 60.9%), followed by artificial tears/gels/ointments (79 patients, 50.6%), preservative free artificial tears/gels/ointments (71 patients, 45.5%), and therapeutic contact lenses (47 patients, 30.1%). A total of 66 patients (42.3%) reported the use of “Other” prior treatments for NK.

During the controlled treatment period, concomitant ocular medications were taken by 68 patients (43.6%) overall: 21 patients (40.4%) in the rhNGF 10 μg/ml group, 26 patients (50.0%) in the rhNGF 20 μg/ml group, and 21 patients (40.4%) in the vehicle control group. The most common concomitant ocular medications taken during the controlled treatment period were reported as ofloxacin (13 patients, 18.3%), hyaluronate sodium (10 patients, 6.4%), aciclovir (8 patients, 5.1%), chloramphenicol and levofloxacin (7 patients each, 4.5%), dexamphenol (6 patients, 3.8%), and ophthalmologicals (5 patients, 3.2%).

During the controlled treatment period, concomitant systemic medications were taken by 115 patients (73.7%) overall: 36 patients (69.2%) in the rhNGF 10 μg/ml group, 39 patients (75.0%) in the rhNGF 20 μg/ml group, and 40 patients (76.9%) in the vehicle control group. The most commonly used concomitant systemic medications during the controlled treatment period were beta blocking agents (38 patients, 24.4%), agents acting on the renin-angiotensin system (37 patients, 23.7%), antithrombotic agents (33 patients, 21.2%), and drugs for acid related disorders (30 patients, 19.2%). The most commonly used concomitant systemic medication during the controlled treatment period was acetylsalicylic acid (19 patients, 12.2%).

Numbers analysed

In the Phase II segment of the study, all 156 patients (52 patients in each treatment group) were included in the ITT population and in the Safety population. For the uncontrolled treatment period, all 10 patients who were randomized to rhNGF 10 μg/ml and all 13 patients who were randomized to rhNGF 20 μg/ml, were included in the ITT population and in the Safety population.

Outcomes and estimation

A summary of the most relevant results is provided below.

- **Primary Efficacy Analysis: Complete Healing at Week 4**

After 4 weeks of treatment, complete healing of the PED or corneal was achieved in a total of 67 patients overall: 28 patients (54.9%) in the rhNGF 10 μg/ml group, 29 patients (58.0%) in the rhNGF 20 μg/ml group, and 10 patients (19.6%) in the vehicle control group. The percentage of patients who achieved complete healing was found to be statistically significantly higher in the rhNGF treatment groups compared to the vehicle control group. There was no significant difference between the rhNGF 10 μg/ml and rhNGF 20 μg/ml doses at Week 4 (p=0.754).
### Table 5 - Percentage of Patients who Achieved Complete Healing at Week 4 (LOCF) as Determined by the Reading Centre (ITT Population, Study NGF0212 Phase II)

<table>
<thead>
<tr>
<th>Treatment Comparisona</th>
<th>rhNGF 10 μg/ml (N=52)</th>
<th>rhNGF 20 μg/ml (N=52)</th>
<th>Vehicle Control (N=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete Healing Achieved</strong></td>
<td>28 (54.9%)</td>
<td>29 (58.0%)</td>
<td>10 (19.6%)</td>
</tr>
<tr>
<td>No</td>
<td>23 (45.1%)</td>
<td>21 (42.0%)</td>
<td>41 (80.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100.0%)</td>
<td>50 (100.0%)</td>
<td>51 (100.0%)</td>
</tr>
</tbody>
</table>

**Exploratory Treatment Comparison** (rhNGF 20 μg/ml vs. rhNGF 10 μg/ml)

<table>
<thead>
<tr>
<th>Difference in % Complete Healing</th>
<th>3.1%</th>
<th>0.754</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.06% CI b</td>
<td>(-18.38, 24.58)</td>
<td></td>
</tr>
<tr>
<td>p-value c</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; ITT = intent-to-treat; LOCF = last observation carried forward; rhNGF = recombinant human nerve growth factor.

Top N counts relate to the number of patients randomized to each treatment at Baseline.

- rhNGF 10 μg/ml and rhNGF 20 μg/ml were each compared against the vehicle control group.
- Asymptotic (Wald) CI.
- Asymptotic p-value based on Pearson statistic from Chi-Square test.

The results of the observed case analysis for complete healing at Week 4 were consistent with the primary analysis. Furthermore, as a sensitivity analysis, the primary efficacy variable was analysed firstly by imputing missing data as failures and secondly by using multiple imputation for missing data. The findings of the sensitivity analyses were similar to the results of the primary analysis.

- **Secondary Efficacy Analyses (Controlled Treatment Period)**

**Complete Healing at Week 8 as Determined by the Reading Centre**

Complete healing of the PED or corneal ulcer at Week 8, as determined by the reading centre, was achieved in a total of 97 patients overall: 38 patients (74.5%) in the rhNGF 10 μg/ml group, 37 patients (74.0%) in the rhNGF 20 μg/ml group, and 22 patients (43.1%) in the vehicle control group. The percentage of patients who achieved complete healing was found to be statistically significantly higher in the rhNGF treatment groups compared to the vehicle control group. There was no significant difference between the rhNGF 10 μg/ml and rhNGF 20 μg/ml doses at Week 8 (p=0.953).

Furthermore, 23 patients randomized to the vehicle control arm at baseline who worsened before Week 8 or were non-completely healed at Week 8 entered the uncontrolled treatment period and received rhNGF during Week 8-16. A post-hoc subgroup analysis of these patients showed that there were 4 patients in the rhNGF 10 μg/ml group (4/10 = 40%) and 8 patients in the rhNGF 20 μg/ml group (8/13 = 61.5%) achieving complete corneal healing by Week 16.
### Table 6 - Percentage of Patients who Achieved Complete Healing at Week 8 (LOCF) as Determined by the Reading Centre (ITT Population, Study NGF0212 Phase II)

<table>
<thead>
<tr>
<th></th>
<th>rhNGF 10 μg/ml (N=52)</th>
<th>rhNGF 20 μg/ml (N=52)</th>
<th>Vehicle Control (N=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete Healing Achieved</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 (74.5%)</td>
<td>37 (74.0%)</td>
<td>22 (43.1%)</td>
</tr>
<tr>
<td>No</td>
<td>13 (25.5%)</td>
<td>13 (26.0%)</td>
<td>29 (56.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>51 (100.0%)</td>
<td>50 (100.0%)</td>
<td>51 (100.0%)</td>
</tr>
<tr>
<td><strong>Treatment Comparison</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rhNGF vs. Vehicle Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference in % Complete Healing</td>
<td>31.4%</td>
<td>30.9%</td>
<td></td>
</tr>
<tr>
<td>97.06% CI b</td>
<td>(11.25, 51.49)</td>
<td>(10.60, 51.13)</td>
<td></td>
</tr>
<tr>
<td>p-value c</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td><strong>Exploratory Treatment Comparison</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rhNGF 20 μg/ml vs. rhNGF 10 μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference in % Complete Healing</td>
<td>-0.5%</td>
<td>9.70%</td>
<td></td>
</tr>
<tr>
<td>97.06% CI b</td>
<td>(-19.46, 18.44)</td>
<td>(10.85, 39.21)</td>
<td></td>
</tr>
<tr>
<td>p-value c</td>
<td>0.953</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; ITT = intent-to-treat; LOCF = last observation carried forward; rhNGF = recombinant human nerve growth factor.

Top N counts relate to the number of patients randomized to each treatment at Baseline.

The significance level for the statistical tests is 0.0294 (adjusted according to Pocock).

a rhNGF 10 μg/ml and rhNGF 20 μg/ml were each compared against the vehicle control group.

b Asymptotic (Wald) CI.

c Asymptotic p-value based on Pearson statistic from Chi-Square test.

### Complete Healing at Week 4 and Week 8 as determined by the Investigator

Of the 127 patients who had a response available at Week 4, complete healing as determined by the Investigator was achieved in a total of 60 patients overall: 25 patients (52.1%) in the rhNGF 10 μg/ml group, 25 patients (61.0%) in the 20 μg/ml group, and 10 patients (26.3%) in the vehicle control group. The difference between the rhNGF 10 μg/ml group and the vehicle control group was 25.8% (97.06% CI: 3.66, 47.87; p=0.016). The difference between the rhNGF 20 μg/ml group and the vehicle control group was 34.7% (97.06% CI: 11.91, 57.41; p=0.002).

Of the 127 patients who had a response available at Week 8, complete healing as determined by the Investigator was achieved in a total of 90 patients overall: 37 patients (78.7%) in the rhNGF 10 μg/ml group, 33 patients (78.6%) in the rhNGF 20 μg/ml group, and 20 patients (52.6%) on vehicle control. The difference between the rhNGF 10 μg/ml group and vehicle was 26.1% (97.06% CI: 4.18, 48.01; p=0.011). The difference between the rhNGF 20 μg/ml group and vehicle was 25.9% (97.06% CI: 3.55, 48.33; p=0.014).

There was no significant difference between the 2 active treatment arms at any time point.

### Complete Corneal Clearing

Of the 131 patients with a response available at Week 4, 21 patients experienced complete corneal clearing: 10 (20.8%) patients in the rhNGF 10 μg/ml group, 8 (19.5%) patients in the rhNGF 20 μg/ml group, and 3 (7.1%) patients in the vehicle control group. The difference between the rhNGF 10 μg/ml group and vehicle
was 13.7% (95% CI: -0.19, 27.57, p=0.065). The difference between the rhNGF 20 μg/ml group and vehicle was 12.4% (95% CI: -2.05, 26.78, p=0.097).

Of the 130 patients with a response available at Week 8, 26 patients experienced complete corneal clearing: 13 (27.1%) patients in the rhNGF 10 μg/ml group, 9 (21.4%) patients in the rhNGF 20 μg/ml group, and 4 (10.0%) patients in the vehicle control group. The difference between the rhNGF 10 μg/ml group and vehicle was 17.1% (95% CI: 1.45, 32.72, p=0.043). The difference between the rhNGF 20 μg/ml group and vehicle was 11.4% (95% CI: -4.08, 26.93, p=0.157).

There was no significant difference between the 2 active treatment arms at either time point.

**Best Corrected Distance Visual Acuity (BCDVA) Score**

The results for least squares (LS) mean change in BCDVA score from Baseline are summarised in Table 7.

### Table 7 – BCDVA Score and LS Mean Change from Baseline (ITT Population, Study NGF0212 Phase II)

<table>
<thead>
<tr>
<th>BCDVA in ETDRS letters</th>
<th>rhNGF 10 μg/ml (N=52)</th>
<th>rhNGF 20 μg/ml (N=52)</th>
<th>Vehicle Control (N=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BCDVA</td>
<td>30.7 (SD= 28.35)</td>
<td>24.2 (SD=25.88)</td>
<td>32.4 (SD=26.07)</td>
</tr>
<tr>
<td>LS Mean change from Baseline at 4 Weeks</td>
<td>9.2 (SD=13.12)</td>
<td>11.3 (SD=17.24)</td>
<td>4.8 (SD=14.25)</td>
</tr>
<tr>
<td>LS Mean change from Baseline at 8 Weeks</td>
<td>15.8 (SD=16.82)</td>
<td>11.9 (SD=20.90)</td>
<td>6.9 (SD=15.44)</td>
</tr>
<tr>
<td>Treatment Difference (LS mean) (rhNGF vs. Vehicle Control)</td>
<td>8.9 (1.33, 16.50)</td>
<td>5.0 (-2.90, 12.88)</td>
<td>-</td>
</tr>
<tr>
<td>95% CI, p-value</td>
<td>0.022</td>
<td>0.213</td>
<td>-</td>
</tr>
<tr>
<td>Treatment Difference (LS Mean) (rhNGF 20 μg/ml - rhNGF 10 μg/ml)</td>
<td>-3.9 (-11.47, 3.62)</td>
<td>- - - -</td>
<td>-</td>
</tr>
<tr>
<td>95% CI, p-value</td>
<td>0.305</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

LS Mean = Least Squares Mean, N = number of patients randomized to each treatment at Baseline, SD = Standard Deviation, CI = Confidence Interval.

Of the 133 patients who had a response available at Week 4, a 15 letter gain in BCDVA score was achieved in 18 (36.7%) patients in the rhNGF 10 μg/ml group, 14 (34.1%) patients in the rhNGF 20 μg/ml group, and 9 (20.9%) patients in the vehicle control group. There was no significant difference between the rhNGF 10 μg/ml and vehicle (p= 0.097), the rhNGF 20 μg/ml and vehicle (p= 0.175), or the rhNGF 10 μg/ml and rhNGF 20 μg/ml doses (p= 0.798).

Of the 129 patients who had a response available at Week 8, a 15 letter gain in BCDVA score (or more) was achieved in 24 (50.0%) patients in the rhNGF 10 μg/ml group, 17 (41.5%) patients in the rhNGF 20 μg/ml group, and 9 (22.5%) patients in the vehicle group. The difference between rhNGF 10 μg/ml and vehicle was 27.5% (95% CI: 8.33, 46.67), and was statistically significant (p=0.008). The difference between rhNGF 20 μg/ml and vehicle was 19.0% (95% CI: -0.91, 38.83), and did not reach statistical significance (p= 0.068). There was no significant difference between rhNGF 10 μg/ml and 20 μg/ml (p= 0.421).

**Improvement in Corneal Sensitivity**

Of the 122 patients who had a response available at Week 4, an improvement in corneal sensitivity was achieved in a total of 79 patients overall: 31 patients (68.9%) in the rhNGF 10 μg/ml group, 22 patients (61.1%) in the rhNGF 20 μg/ml group, and 26 patients (63.4%) in the vehicle control group. There was no
significant difference between the rhNGF 10 μg/ml group and vehicle (p= 0.592), the rhNGF 20 μg/ml group and vehicle (p=0.835), or between the rhNGF 10 μg/ml and 20 μg/ml doses (p= 0.465).

Of the 118 patients who had a response available at Week 8, an improvement in corneal sensitivity was achieved in a total of 88 patients overall: 33 patients (78.6%) in the rhNGF 10 μg/ml group, 29 patients (76.3%) in the rhNGF 20 μg/ml group, and 26 patients (68.4%) in the vehicle control group. There was no significant difference between the rhNGF 10 μg/ml group and vehicle (p= 0.303), the rhNGF 20 μg/ml group and vehicle (p=0.442) or the rhNGF 10 μg/ml and 20 μg/ml doses (p= 0.809).

**Deterioration from Baseline**

Of the 134 patients with a response available at Week 4, one patient each in the rhNGF 10 μg/ml group and 20 μg/ml group, and 2 patients in the vehicle control group experienced deterioration. Of the 130 patients with a response available at Week 8, 11 patients experienced deterioration: 2 patients in the rhNGF 10 μg/ml group, 3 patients in the rhNGF 20 μg/ml group, and 6 patients in the vehicle control group. There was no significant difference between treatment groups in the percentage of patients with deterioration at either time point. Median time to onset of deterioration in the 3 treatment groups (Kaplan-Meier analysis) was not estimable due to the small number of events.

**Investigator Global Evaluation of Efficacy**

Of the 132 patients with a response available at Week 4, 19 patients (39.6%) in the rhNGF 10 μg/ml group, 14 patients (34.1%) in the rhNGF 20 μg/ml group, and 11 patients (25.6%) in the vehicle control group had an evaluation response of "Very Good". The odds ratio of a more favourable response at Week 4 between the rhNGF 10 μg/ml group and vehicle was 2.18 (95% CI: 1.03, 4.62; p=0.041). The odds ratio was neither statistically significant between the rhNGF 20 μg/ml group and vehicle (p=0.081), nor between the active arms (p=0.755). The results at Week 8 were consistent with the findings at Week 4 with slightly higher rates of patients with an evaluation response of "Very Good".

- **Exploratory Variables (Controlled Treatment Period)**

  The median time to onset of healing (defined as a >20% reduction in the greatest diameter of the lesion compared to Baseline) was 8 days (95% CI: 7, 14), 14 days (95% CI: 7, 14), and 14 days (95% CI: 14, 28), in the rhNGF 10 μg/ml group, 20 μg/ml group, and vehicle control group, respectively. The median time to complete healing (defined as the greatest diameter of the lesion being less than 0.5 mm) was 29 days (95% CI: 20, 55), 28 days (95% CI: 19, 55), and 56 days (95% CI: 42, not estimable), in the rhNGF 10 μg/ml group, 20 μg/ml group, and vehicle control group, respectively.

  The mean Schirmer test measurement at Baseline was 10.1 mm (SD=7.30) in the rhNGF 10 μg/ml group, 10.0 mm (SD=7.14) in the rhNGF 20 μg/ml group, and 12.6 mm (SD=11.71) in the vehicle control group. The mean change from Baseline at Week 4 was 3.7 mm (SD=8.35) for rhNGF 10 μg/ml, 3.0 mm (SD=6.18) for rhNGF 20 μg/ml and -0.9 mm (SD=8.13) for vehicle. The mean change from Baseline at Week 8 was 4.2 mm (SD=8.83) for rhNGF 10 μg/ml, 2.0 mm (SD=8.82) for rhNGF 20 μg/ml, and -1.7 mm (SD=10.48) for vehicle. The overall difference in LS mean change from Baseline (Mixed Effects Repeated Measures model) between the rhNGF 10 μg/ml group and vehicle was statistically significant (95% CI: 0.89, 6.66; p=0.011). There were no significant differences in LS mean change from Baseline between the rhNGF 20 μg/ml group and vehicle, or between the rhNGF 10 μg/ml and rhNGF 20 μg/ml doses, at Week 4, Week 8, or overall.

  The mean tear film osmolarity at Baseline was 302.4 mOsm/L (SD=15.99) in the rhNGF 10 μg/ml group (n=7), 288.3 mOsm/L (SD=9.82) in the rhNGF 20 μg/ml group (n=8), and 297.5 mOsm/L (SD=8.04) in the vehicle control group (n=6). At Week 4 and Week 8, an increase in mean tear film osmolarity was observed.
in the rhNGF 20 μg/ml group. This increase was not observed in the rhNGF 10 μg/ml group or in the vehicle control group: At Week 4, the mean change from Baseline in mean tear film osmolarity was -7.0 mOsm/L (SD=10.20) in the rhNGF 10 μg/ml group, 14.0 mOsm/L (SD=19.17) in the rhNGF 20 μg/ml group, and -8.7 mOsm/L (SD=4.62) in the vehicle control group. At Week 8, the mean change from Baseline in mean tear film osmolarity was -2.8 mOsm/L (SD=13.33) in the rhNGF 10 μg/ml group, 11.2 mOsm/L (SD=13.91) in the rhNGF 20 μg/ml group, and -3.3 mOsm/L (SD=16.29) in the vehicle control group.

The mean NEI-VFQ overall composite score at Baseline was 56.51 (SD=19.957) in the rhNGF 10 μg/ml group, 56.57 (SD=24.971) in the rhNGF 20 μg/ml group, and 58.64 (SD=23.876) in the vehicle control. The LS mean change in NEI-VFQ overall composite score from Baseline to Week 8 was 7.9 (SE=1.79) in the rhNGF 10 μg/ml group, 4.8 (SE=1.92) in the rhNGF 20 μg/ml group, and 3.2 (SE=1.94) in the vehicle control group. There was no significant difference in LS mean change from Baseline between the treatment groups.

The mean EQ-5D health state score at Baseline was 63.3 (SD=22.21) in the rhNGF 10 μg/ml group, 62.9 (SD=19.79) in the rhNGF 20 μg/ml group, and 68.8 (SD=16.41) in the vehicle control group. The LS mean change in EQ-5D health state score from Baseline to Week 8 was 4.5 (SE=2.25) in the rhNGF 10 μg/ml group, 2.8 (SE=2.43) in the rhNGF 20 μg/ml group, and 0.2 (SE=2.45) in the vehicle control group. There was no significant difference in LS mean change from Baseline between the treatment groups.

48- or 56-Week Follow-Up Period

The efficacy analysis of the follow-up period considered only patients who were completely healed at Week 8/16. The analyses were based on all patients initially assigned to one of the active treatment arms as well as all patients in the vehicle arm not healed at Week 8 and thereafter assigned to rhNGF 10 or 20 μg/ml.

Patients Remaining Healed During Follow-up

Table 8 provides an overview of the percentage of patients who remained healed during follow-up.

<table>
<thead>
<tr>
<th>Time After Completing Treatment</th>
<th>Remained Healed</th>
<th>rhNGF 10 μg/ml (N=62)</th>
<th>rhNGF 20 μg/ml (N=65)</th>
<th>Vehicle Control (N=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Weeks (Week 20/28)</td>
<td>Yes</td>
<td>34 (87.2%)</td>
<td>33 (89.2%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (12.8%)</td>
<td>4 (10.8%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 (100.0%)</td>
<td>37 (100.0%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td>24 Weeks (Week 32/40)</td>
<td>Yes</td>
<td>28 (82.4%)</td>
<td>33 (89.2%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6 (17.6%)</td>
<td>4 (10.8%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>34 (100.0%)</td>
<td>37 (100.0%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td>36 Weeks (Week 44/52)</td>
<td>Yes</td>
<td>26 (83.9%)</td>
<td>32 (84.2%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (16.1%)</td>
<td>6 (15.8%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>31 (100.0%)</td>
<td>38 (100.0%)</td>
<td>20 (100.0%)</td>
</tr>
<tr>
<td>48 Weeks (Week 56/64)</td>
<td>Yes</td>
<td>26 (83.9%)</td>
<td>28 (80.0%)</td>
<td>19 (95.0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (16.1%)</td>
<td>7 (20.0%)</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>31 (100.0%)</td>
<td>35 (100.0%)</td>
<td>20 (100.0%)</td>
</tr>
</tbody>
</table>

Abbreviations: ITT = intent-to-treat; rhNGF = recombinant human nerve growth factor.

Top N counts relate to the number of patients randomized to each treatment – this will be the Baseline randomized treatment for patients completely healed at Week 8 and will be the Week 8 randomized treatment for patients completed healed at Week 16. Patients without a Yes/No response available are not included in the summary of that time point.
Recurrence of PED or Corneal Ulcer During Follow-up

Table 9 provides an overview of the results for patients with a response available at the various time points.

The mean time from complete healing to recurrence of PED or corneal ulcer was 73.3 days (SD=60.79) in the rhNGF 10 μg/ml group (n=7), 132.8 days (SD=113.71) in the rhNGF 20 μg/ml group (n=10), and 219.5 days (SD=197.28) in the vehicle control group (n=2).

Table 9 - Percentage of Patients who Achieved Complete Healing at Week 8/16 With Recurrence of PED or Corneal Ulcer During Follow-up (ITT Population, NGF0212)

<table>
<thead>
<tr>
<th>Time After Completing Treatment</th>
<th>Recurrence</th>
<th>rhNGF 10 μg/ml (N=62)</th>
<th>rhNGF 20 μg/ml (N=65)</th>
<th>Vehicle Control (N=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Weeks (Week 20/28)</td>
<td>Yes</td>
<td>2 (5.1%)</td>
<td>4 (10.0%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>37 (94.9%)</td>
<td>36 (90.0%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 (100.0%)</td>
<td>40 (100.0%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td>24 Weeks (Week 32/40)</td>
<td>Yes</td>
<td>3 (8.6%)</td>
<td>2 (5.4%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>32 (91.4%)</td>
<td>35 (94.6%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35 (100.0%)</td>
<td>37 (100.0%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td>36 Weeks (Week 44/52)</td>
<td>Yes</td>
<td>0</td>
<td>4 (10.5%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>33 (100.0%)</td>
<td>34 (89.5%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>33 (100.0%)</td>
<td>38 (100.0%)</td>
<td>21 (100.0%)</td>
</tr>
<tr>
<td>48 Weeks (Week 56/64)</td>
<td>Yes</td>
<td>1 (3.1%)</td>
<td>3 (8.6%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>31 (96.9%)</td>
<td>32 (91.4%)</td>
<td>20 (95.2%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32 (100.0%)</td>
<td>35 (100.0%)</td>
<td>21 (100.0%)</td>
</tr>
</tbody>
</table>

Abbreviations: ITT = intent-to-treat; rhNGF = recombinant human nerve growth factor.
Top N counts relate to the number of patients randomized to each treatment – this will be the Baseline randomized treatment for patients completely healed at Week 8 and will be the Week 8 randomized treatment for patients completely healed at Week 16.
Patients without a Yes/No response available are not included in the summary of that time point.

Best Corrected Distance Visual Acuity Score During Follow-up

For patients who achieved complete healing at either Week 8/16, the mean BCDVA score at Baseline was 32.7 (SD=28.83) in the rhNGF 10 μg/ml group (n=40), 28.7 (SD=26.82) in the rhNGF 20 μg/ml group (n=43), and 38.2 (SD=26.55) in the vehicle control group (n=22). An increase from Baseline in the mean BCDVA score was observed across the treatment groups at Weeks 20/28, 32/40, 44/52, and 56/64.

In the 10 μg/ml group, the mean change from Baseline in BCDVA score was 16.7 (SD=21.43), 17.6 (SD=22.96), 22.3 (SD=20.68), and 18.6 (SD=23.66) at Weeks 20/28, 32/40, 44/52, and 56/64, respectively. In the rhNGF 20 μg/ml group, the mean change from Baseline in BCDVA score was 15.5 (SD=21.51), 17.4 (SD=20.28), 16.3 (SD=23.18), and 16.9 (SD=24.31) at Weeks 20/28, 32/40, 44/52, and 56/64, respectively. In the vehicle control group, the mean change from Baseline in BCDVA score was 9.1 (SD=12.58), 6.5 (SD=15.56), 6.1 (SD=17.59), and 9.1 (SD=17.99) at Weeks 20/28, 32/40, 44/52, and 56/64, respectively.
The percentage of Phase II patients who achieved complete healing at Week 8/16 and achieved a 15 letter gain in BCDVA score during the follow-up period (ITT population) was:

- After 12 weeks of follow-up (at Week 20/28) 51.3% of patients in the rhNGF 10 μg/ml group, 45.0% in the rhNGF 20 μg/ml group, and 33.3% in the vehicle control group achieved a 15 letter gain in BCVA.
- After 24 weeks of follow-up (at Week 32/40) the figures are 47.1% of patients in the rhNGF 10 μg/ml group, 45.9% in the rhNGF 20 μg/ml group, and 23.8% in the vehicle control group.
- After 36 weeks of follow-up (at Week 44/52), 67.7% of patients in the rhNGF 10 μg/ml group, 47.4% in the rhNGF 20 μg/ml group, and 28.6% in the vehicle control group achieved a 15 letter gain in BCDVA.
- After 48 weeks of follow-up (at Week 56/64), 58.1% of patients in the rhNGF 10 μg/ml group, 37.1% in the rhNGF 20 μg/ml group, and 28.6% in the vehicle control group achieved a 15 letter gain in BCDVA.

**Corneal Sensitivity During Follow-up**

Of the 73 patients who had a response available after 24 weeks of follow-up (at Week 32/40), 28 patients (100.0%) in the rhNGF 10 μg/ml group, 29 patients (96.7%) in the rhNGF 20 μg/ml group, and 14 patients (93.3%) in the vehicle control group showed an improvement or no change in corneal sensitivity at this time point during the follow-up period.

Of the 68 patients who had a response available after 48 weeks of follow-up (at Week 56/64), 24 out of 25 patients (96.0%) in the rhNGF 10 μg/ml group, 27 out of 28 patients (96.4%) in the rhNGF 20 μg/ml group, and all 15 patients (100.0%) in the vehicle control group showed an improvement or no change in corneal sensitivity at this time point during the follow-up period.

**Complete Corneal Clearing During Follow-up**

Overall, 25 patients had a response available after 24 weeks of follow-up (at Week 32/40), of which 16 patients maintained complete corneal clearing at this time point: 8 patients (80.0%) in the rhNGF 10 μg/ml group, 6 patients (54.5%) in the rhNGF 20 μg/ml group, and 2 patients (50.0%) in the vehicle control group. After 48 weeks of follow-up (at Week 56/64), 22 patients had a response available, of which 11 patients maintained complete corneal clearing at this time point: 6 patients (66.7%) in the rhNGF 10 μg/ml group, 3 patients (33.3%) in the rhNGF 20 μg/ml group, and 2 patients (50.0%) in the vehicle group.

**Ancillary (post-hoc) analyses**

*Corneal Healing defined as no corneal fluorescein staining in the area of the PED or corneal ulcer, and non-persistent lesions in the surrounding area of the cornea*

At Week 4, 49.0% (25/51) of patients in the rhNGF 10 μg/ml group and 58.0% (29/50) of patients in the rhNGF 20 μg/ml group were completely healed with zero staining as definition of complete healing compared to 13.7% (7/51) in the vehicle group. Statistical significance against vehicle was achieved for both 10 and 20 μg/ml rhNGF groups (p<0.001). There was no significant difference between the rhNGF doses (p=0.366).

At Week 8, complete corneal healing with no residual staining was achieved in 32/51 patients (62.7%) in the rhNGF 10 μg/ml group, 36/50 patients (72.0%) in the rhNGF 20 μg/ml group, and 17/51 patients (33.3%) in the vehicle control group. The difference was 29.4% (97.06% CI: 8.82, 50.01; p=0.003) between rhNGF 10 μg/ml and vehicle and 38.7% (97.06% CI: 18.72, 58.62; p<0.001) between rhNGF 20 μg/ml and vehicle.
Time from End of Treatment to Recurrence

Of the Phase II patients who achieved complete healing at either Week 8/16, a total of 19 patients had a recurrence of PED or corneal ulcer during follow-up: 7 patients in the rhNGF 10 µg/ml group, 10 patients in the rhNGF 20 µg/ml group, and 2 patients in the vehicle control group. The mean time from end of treatment regimen including complete healing to recurrence of PED or corneal ulcer during follow-up for completely healed patients was 54.9 days (SD=53.60) in the rhNGF 10 µg/ml group (n=7), 114.4 days (SD=106.08) in the rhNGF 20 µg/ml group (n=10), and 188.5 days (SD=222.74) in the vehicle control group (n=2).

Patients with Specific NK aetiologies

An exploratory post-hoc efficacy analysis was conducted to determine the effect of rhNGF on complete healing (as determined by the reading centre) at Week 4 and 8 in Phase II patients with specific aetiologies (acoustic neuroma, neurosurgical procedure, meningioma, and schwannoma). This subgroup of patients represents a NK population with isolated damage to the trigeminal nerve or to its main trunks, who are likely to experience corneal disease progression. Of the 21 patients with specific aetiologies of NK, complete healing (as determined by the reading centre) at Week 4 was achieved in a total of 11 patients overall: 4/6 patients (66.7%) in the rhNGF 10 µg/ml group, 6/8 patients (75.0%) in the rhNGF 20 µg/ml group, and 1/7 patients (14.3%) in the vehicle control group. No statistically significant difference was observed between treatment groups. At Week 8, complete healing was achieved in a total of 12 patients due to one more patient in the vehicle arm; the difference between treatment groups remained statistically not significant.

Additional post-hoc analyses were performed in response to a request by the CHMP for the most representative local and systemic NK aetiologies.

Table 10 - Percentage of Patients by NK Aetiology with Complete Healing at the End of Treatment (Stud NGF0212 Phase II)

<table>
<thead>
<tr>
<th>NK etiology</th>
<th>Study NGF0212</th>
<th>Study NGF0214</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rhNGF 10 µg/ml</td>
<td>rhNGF 20 µg/ml</td>
</tr>
<tr>
<td>Diabetes</td>
<td>67% (2/3)</td>
<td>75% (3/4)</td>
</tr>
<tr>
<td>Dry Eye</td>
<td>100% (6/6)</td>
<td>100% (6/6)</td>
</tr>
<tr>
<td>Herpes*</td>
<td>85% (11/13)</td>
<td>70% (7/10)</td>
</tr>
<tr>
<td>Iatrogenic eye damage</td>
<td>50% (6/12)</td>
<td>64% (7/11)</td>
</tr>
<tr>
<td>Innervation alterations b</td>
<td>67% (4/6)</td>
<td>75% (6/8)</td>
</tr>
</tbody>
</table>

*a”Herpes” includes both Simplex and Zoster;  
b“Innervation alterations” include both neurosurgical procedures and isolated diseases of the trigeminal/cranial nerves.

Patients with Punctual Occlusion

Only 4 patients (2 patients in the rhNGF 20 µg/ml group and 2 patients in the vehicle control group) had evidence of punctual occlusion and had a response available at Week 4. Of these 4 patients, 1 patient in each group achieved complete healing as determined by the reading centre at Week 4. There was no significant difference between the rhNGF group and the vehicle control group (p>0.999).
2.5.2.3. Results for Study NFG0214

Participant flow

A total of 48 patients were randomized to study NGF0214: 24 patients in each treatment group. One patient was randomised although he was not eligible for the study and therefore discontinued the study before receiving any study medication and was not included in the Safety Population.

A total of 33 randomized patients (33/48= 68.8%) completed the 8-week Controlled Treatment Period: 18 (18/24=75.0%) patients in the rhNGF treatment arm and 15 (15/24=62.5%) patients in the vehicle arm.

Seven vehicle patients were considered to be not completely healed at week 8 and continued directly into the Uncontrolled Treatment Period with rhNGF for 8 weeks. Further, 6 vehicle patients terminated the Controlled Treatment Period prematurely and continued directly into the Uncontrolled Treatment Period with rhNGF.

Figure 3 – Patient Disposition (Study NGF0214)

A total of 33 randomized patients (33/48= 68.8%) completed the 8-week Controlled Treatment Period: 18 (18/24=75.0%) patients in the rhNGF treatment arm and 15 (15/24=62.5%) patients in the vehicle arm.

Seven vehicle patients were considered to be not completely healed at week 8 and continued directly into the Uncontrolled Treatment Period with rhNGF for 8 weeks. Further, 6 vehicle patients terminated the Controlled Treatment Period prematurely and continued directly into the Uncontrolled Treatment Period with rhNGF.
Therefore, 13 patients, randomized to initial treatment with vehicle, received at least a single dose of rhNGF in the Uncontrolled Treatment Period.

Recruitment
First patient randomized: 1 May 2015; Last patient completed: 06 Aug 2016 (date of last observation).

Conduct of the study
Three (3) protocol amendments and a number of refinements to the statistical analyses were implemented. SAP version 3.0, which was used for present analysis, was finalised and signed off prior to database lock for the main objective analysis. The following main changes to the planned analysis were introduced following unmasking:

• An additional sensitivity analysis of 'Complete Healing as assessed by the Central Reading Centre' at Week 8 was added post-hoc, which imputed 'Not completely Healed' for all treated patients with a missing value at Week 8. This amended planned Worst-Case analysis, which imputed only missing values for patients who did not discontinue before Week 8.
• The endpoint "Time to recurrence" was redefined as "Time to first Intake of Re-Treatment due to Recurrence".
• P-values and 95% confidence intervals were calculated for the endpoints Complete Corneal Clearing, change in Schirmer’s tear test and change in intraocular pressure as post-hoc analysis. These inferential results must be interpreted as purely exploratory.

GCP findings
In March 2016, one study site was temporarily suspended by their independent review board for GCP non-compliance. The suspension was lifted in May 2016 after corrective measures were implemented. The Applicant has performed a sensitivity analysis (including/excluding this site’s patients), which were reassuring and showed no major impact of these data on the overall study outcome.

Protocol Deviations
A total of 57 protocol deviations in 31 patients occurred during the whole study, with 17 classified as major and 35 classified as minor. Nineteen deviations occurred at the study site which was suspended for GCP non-compliance including 5 major deviations concerning 3 patients allocated to rhNGF and 1 patient allocated to vehicle who were randomized before they had undergone all study entry procedures. In addition, the patient in the vehicle group did not comply with inclusion and criteria.
Baseline data

The demographic characteristics are summarised below.

Table 11 - Demographics of ITT population (Study NGF0212)

<table>
<thead>
<tr>
<th>Statistic</th>
<th>rhNGF (N=24)</th>
<th>Vehicle (N=24)</th>
<th>Overall (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>65.9 (13.85)</td>
<td>64.5 (14.15)</td>
<td>65.2 (13.87)</td>
</tr>
<tr>
<td>Median</td>
<td>66.5</td>
<td>65.0</td>
<td>65.5</td>
</tr>
<tr>
<td>Min, Max</td>
<td>33, 94</td>
<td>35, 92</td>
<td>33, 94</td>
</tr>
<tr>
<td>Gender</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>10 (41.7)</td>
<td>9 (37.5)</td>
<td>19 (39.6)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (58.3)</td>
<td>15 (62.5)</td>
<td>29 (60.4)</td>
</tr>
<tr>
<td>Race</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (4.2)</td>
<td>0 (0.0)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>3 (12.5)</td>
<td>2 (8.3)</td>
<td>5 (10.4)</td>
</tr>
<tr>
<td>Native Hawaiian or Other Pacific Islander</td>
<td>0 (0.0)</td>
<td>1 (4.2)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>White</td>
<td>20 (83.3)</td>
<td>20 (83.3)</td>
<td>40 (83.3)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0)</td>
<td>1 (4.2)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>N/A</td>
<td>4 (16.7)</td>
<td>4 (16.7)</td>
<td>8 (16.7)</td>
</tr>
<tr>
<td>Hispanic, Latino or Spanish</td>
<td>0 (0.0)</td>
<td>1 (4.2)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Not Hispanic, Latino or Spanish</td>
<td>20 (83.3)</td>
<td>19 (79.2)</td>
<td>39 (81.3)</td>
</tr>
</tbody>
</table>

Age was calculated as the integer part of \((\text{Date of Informed Consent} - \text{Date of birth})/365.25\)

Medical History

Nearly all patients had a history of other eye disorders (100.0% in the rhNGF group and 95.8% in the vehicle group). The most common were cataract (87.0% and 62.5%, respectively), meibomian gland dysfunction (82.6%; 58.3%), dry eye (73.9%; 41.7%) and blepharitis (47.8%; 33.3%). Many had undergone surgical procedures on the eye (78.3% and 79.2%), mainly intraocular lens implant (30.4%; 16.7%), amniotic membrane graft (21.7%; 12.5%), and cataract operation (13.0% and 20.8%)

Other common disorders were systemic hypertension (56.5% in the rhNGF group, 54.2% in the vehicle group), ophthalmic herpes simplex (39.1% and 20.8%, respectively), anxiety (34.8% and 20.8%), depression (26.1% and 16.7%), hypercholesterolemia (21.7% and 25.0%), diabetes mellitus (13.0% and 4.2%), gastroesophageal reflux disease (13.0% and 20.8%), drug hypersensitivity (30.4% and 37.5%), rosacea (21.7% only in the rhNGF group) and asthma (21.7% only in the rhNGF group).

Right eye was the study eye in 27 patients (56.3%), left eye in 21 patients (43.8%). Among those, for 3 patients both eyes were affected. The majority of patients (33 patients; 68.8%) had stage 2 NK including 15 (62.5%) in the rhNGF group versus 18 (75.0%) in the vehicle group. There were slightly more Stage 3 patients in the rhNGF group: 9 (37.5%) in the rhNGF group versus 6 (25.0%) in the vehicle group. Mean time since NK stage 2, 3 diagnosis was 7.5 (SD: 14.51) in the rhNGF group and 7.9 (SD: 8.59) in the vehicle group.
The underlying cause of NK in the study eye was identified primarily as ‘other’ (58.3% in the NGF group and 41.7% in the vehicle group), dry eye disease (12.5% each), herpes zoster (8.3%; 12.5%) and ocular surgery procedure (8.3% each).

**Concomitant Medications**

Most patients had already received prior ophthalmologicals: 91.3% in the rhNGF group and 79.2% in the vehicle group. The most common prior medications were: artificial tears (39.1% in the rhNGF group and 33.3% in the vehicle group), ganciclovir (26.1% only in the rhNGF group), moxifloxacin (17.4% and 25.0%), and prednisolone acetate (39.1% and 16.7%).

Most patients received concomitant ophthalmological medication during the Controlled Treatment Period: 87.0% in the rhNGF group and 83.3% the vehicle group. The most common were: artificial tears (26.1% and 29.2%), acyclovir (39.1% and 20.8%), moxifloxacin hydrochloride (30.4% and 20.8%), and prednisolone acetate (17.4% and 12.5%).

The most common concomitant non-ophthalmological medications during the Controlled Treatment Period were: cardiovascular drugs, especially agents acting on the renin-angiotensin system (30.4% and 29.2%, respectively) and beta-blocking agents (43.5% and 16.7%), analgesics (39.1% and 25.0%), systemic antibacterials (43.5% and 16.7%), antihistamines (13.0% and 20.8%), anti-inflammatory and antirheumatic products (13.0% and 25.0%), antithrombotic agents (30.4% and 20.8%), antidiabetics (30.4% and 12.5%), drugs used for obstructive airway diseases (21.7% and 8.3%), drugs for acid related disorders (17.4% and 29.2%), lipid-modifying agents (47.8% and 50.0%), psychoanaleptics (34.8% and 37.5%), psycholeptics (34.8% and 20.8%), and vitamins (43.5% and 66.7%).

Concomitant medications were similar in the Uncontrolled Treatment Period.

**Numbers analysed**

A total of 48 were randomized into the study and formed the ITT population including 24 patients in the rhNGF group, and 24 patients in vehicle group. The Safety Population consisted of 47 patients including 23 in rhNGF and 24 in vehicle control treatment group. One patient in rhNGF group was randomized but then immediately discontinued and never treated.

**Outcomes and estimation**

- **Primary efficacy analysis**

  There was a statistically significant difference in favour of rhNGF between the percentages of patients reaching complete healing as determined by the Central Reading Centre at week 8: 69.6% in the rhNGF-treated group versus 29.2% in the vehicle-treated group; $p=0.006$ (see Table 12).

  The results of primary analysis were supported by planned sensitivity analysis, which demonstrated similar results. Sensitivity analyses excluding patients from site 09 also showed a statistically significant difference in favour of rhNGF for complete healing as determined by the Central Reading Centre: 70.0% (14/20) in the rhNGF group versus 25.0% (14/20) in the vehicle group ($p=0.004$).

  Healing was also reflected by percentage change from baseline in the greatest dimension of PED or corneal ulcer. The percentage change (LOCF, ITT) at Week 4 was -80.05% in the rhNGF group versus -47.42% in the vehicle group, and at Week 8 - 88.58% in the rhNGF group versus -15.69% in the vehicle group.
Table 12 – Analysis of Complete Healing as Determined by the Central Reading Centre at 8 weeks (LOCF, ITT population, Study NGF0214)

<table>
<thead>
<tr>
<th>Visit</th>
<th>Stats</th>
<th>rhNGF (N=24)*</th>
<th>Vehicle (N=24)</th>
<th>Overall (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-missing</td>
<td>n</td>
<td>23</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Completely Healed</td>
<td>n (%)</td>
<td>16 (69.6)</td>
<td>7 (29.2)</td>
<td>23 (48.9)</td>
</tr>
<tr>
<td>Not Completely Healed</td>
<td>n (%)</td>
<td>7 (30.4)</td>
<td>17 (70.8)</td>
<td>24 (51.1)</td>
</tr>
</tbody>
</table>

Treatment Comparison

- Difference in % Complete Healing (rhNGF vs. Vehicle Control)
  - 90% CI: 18.4, 62.4
  - 95% CI: 14.2, 66.6
  - p-value [1]: 0.006

* One Patient (randomized to rhNGF although not eligible) had no post-baseline data, was assumed to be missing, and was excluded from the analyses. Percentages are calculated accordingly.

[1]: p-value is from a 2x2 Chi-squared test.

- Secondary efficacy analyses

Complete healing at 8 weeks as measured by the Investigator

Completely healed patients (LOCF), as determined by the Investigator, at Week 8 with corneal fluorescein staining (ITT population) were 65.2% (15/23) in the rhNGF group, and 29.2% (7/24) in the vehicle group. The difference of 36.1% was statistically significant in favour of rhNGF (95% CI: 9.4, 62.7; p=0.013).

There were furthermore 7 vehicle-treated patients considered to be not completely healed at Week 8 and 6 vehicle-treated patients who terminated the controlled treatment period prematurely. These 13 patients continued into the uncontrolled treatment period with rhNGF 20 μg/ml, and 11 of them completed the treatment cycle until Week 16. As photos were not collected for all of these patients in this period, the analysis of complete healing as determined by the Central Reading Centre could not be performed. However, the investigator’s evaluation concluded that all 11 patients who completed the 8 week treatment cycle with rhNGF 20 μg/ml were completely healed at Week 16.

Complete healing at 4, and 6 weeks as measured by the Central Reading Centre and by the Investigator

The percentages of patients reaching complete healing of the PED or corneal ulcer (LOCF, ITT) at Week 4 and 6, as measured by the Central Reviewer, were as follows:

- Week 4: 56.5% (13/23) in the rhNGF group versus 37.5% (9/24) in the vehicle group;
- Week 6: 56.5% (13/23) in the rhNGF group versus 41.7% (10/24) in the vehicle group.

The between treatment difference of 19.0% (95% CI: -9.0, 47.1; p=0.191) at Week 4 and 10.7% (95% CI: 17.7, 39.1; p=0.464) was no statistically significant.

The percentages of patients reaching complete healing of the PED or corneal ulcer (LOCF, ITT) at Week 4 and 6, as Determined by the Investigator, were as follows:

- Week 4: 56.5% (13/23) in the rhNGF group versus 41.7% (10/24) in the vehicle group;
- Week 6: 65.2% (15/23) in the rhNGF group versus 41.7% (10/24) in the vehicle group.
The between treatment difference of 14.9% (95% CI: -13.4, 43.1; p=0.308) at Week 4 and 23.6% (95% CI: -4.2, 51.3; p=0.106) was no statistically significant.

**Complete corneal clearing at weeks 4, 6, and 8 defined as grade 0 on the modified Oxford scale**

The percentages of patients with complete corneal clearing, defined as grade 0 on the modified Oxford scale, at Weeks 4, 6, and 8, were as follows:

- Baseline: 8.3% (2/24) in the rhNGF group versus no patients (0%) in the vehicle group;
- Week 4: 13.6% (3/22) in the rhNGF group versus 4.2% (1/24) in the vehicle group;
- Week 6: 9.1% (2/22) in the rhNGF group versus 8.3% (2/24) in the vehicle group;
- Week 8: 22.7% (5/22) in the rhNGF group versus 4.2% (1/24) in the vehicle group.

A post-hoc analysis showed no statistically significant difference between the two treatment groups at week 4 and week 6 (p=0.255 and p=0.927, respectively) but there was a trend in favour of rhNGF at week 8 (p=0.062)

**Mean change in BCDVA from baseline to Week 8**

For the study eye, mean BCDVA at baseline was substantially higher in the vehicle group than in rhNGF group (17.6 letters versus 8.3 letters). Vision slightly improved in both arms by Week 8, but there was no statistically significant difference between the two treatments (p=0.745).

**Table 13 - Analysis of BCDVA Change from Baseline in Study Eye (LOCF, ITT Population, Study NGF0214)**

<table>
<thead>
<tr>
<th>Visit</th>
<th>Statistic</th>
<th>rhNGF (N=24)</th>
<th>Vehicle (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 8</td>
<td>Non-missing (n)</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>4.48 (9.825)</td>
<td>4.33 (10.399)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Min, Max</td>
<td>-7.0, 31.0</td>
<td>-9.0, 35.0</td>
</tr>
<tr>
<td></td>
<td>LSMEANS Estimate (95% CI) [1]</td>
<td>5.0 (0.4, 9.5)</td>
<td>3.9 (-0.5, 8.3)</td>
</tr>
<tr>
<td></td>
<td>rhNGF vs. Vehicle difference (95% CI) [1]</td>
<td>1.1 (-5.6, 7.7)</td>
<td>0.745</td>
</tr>
</tbody>
</table>

Best Corrected Distance Visual Acuity consists of letters read at 4m only.

[1] Analysis results from an ANCOVA with treatment as factor controlling for baseline value, time since diagnosis of NK (months) and baseline value of Schirmer test (mm)

Only few patients achieved a 15 letter gain in BCDVA at Week 8: 13.0% (3/23) in the rhNGF group versus 16.7% (4/24) in the vehicle group. There was no statistically significant difference between the two treatment groups (p=0.727).

**Corneal sensitivity as measured by the Cochet-Bonnet aesthesiometer**

Corneal sensitivity inside PED or ulcer at baseline was 0.81 in rhNGF group and 0.65 in the vehicle group. At Week 8, corneal sensitivity inside PED or ulcer was 2.91 in the rhNGF group and 1.83 in the vehicle group. The adjusted mean change (SD) from baseline in corneal sensitivity were 1.88 (1.401) in the rhNGF group.
and 1.00 (1.254) in the vehicle group. The between treatment difference of 0.6 was not statistically significant (95% CI: -0.4, 1.5; p=0.207).

Deterioration in stage 2 or 3 NK from baseline

The percentage of patients with deterioration from baseline at weeks 1 to 8 ranged from 0.0% (none) patient to 10.0% in the rhNGF group and from 5.9% to 25.0% in the vehicle group. A total 6 patients in the rhNGF group and 11 patients in the vehicle arm experienced deterioration during the active phase of treatment. Statistically significant differences between the treatments were seen at Week 2 (p= 0.023), but not at Week 1 (p=0.446), Week 3 (p= 0.077), Week 4 (p=0.647), Week 6 (p=0.112) or Week 8 (p=0.110).

Investigator global evaluation

Investigator global evaluation was compared at the Week 4 and Week 8 time point. Amongst the patients without missing data, the ratings of “very satisfactory” and “satisfactory”, combined, were assigned to 18/19 rhNGF patients (94.7%) versus 11/16 vehicle patients (68.8%) at Week 4 (p=0.111), and in 14/18 rhNGF patients (77.8%) versus 8/15 vehicle patients (53.3%) at Week 8 (p=0.164).

Completely staining free (no corneal fluorescein staining in the area of the corneal lesion and non-persistent lesions elsewhere in the cornea) as assessed by Central Reading Centre

At Week 4, 56.5% (13/23) of patients in the rhNGF group were completely healed compared to 33.3% (8/24) in the vehicle group (p=0.11). At Week 8, 65.2% (15/23) of patients in the rhNGF group were completely healed compared to 16.7% (4/24) in the vehicle group (p<0.001).

Summary of Re-treatment due to Recurrence

Data were available for 14 patients who received rhNGF during the controlled treatment period, and were completely healed at the last controlled treatment period visit. Of these, 14.3% (2/14=14.3%) were treated for recurrence.

Data were available for 10 patients who received vehicle during the controlled treatment period and rhNGF during the uncontrolled treatment period, and were completely healed at the last uncontrolled treatment period visit. Of these, 30.0% (3/10=30.0%) were treated for recurrence.

In the 7 patients who received vehicle during the controlled treatment period, and who did not undergo uncontrolled treatment, none was treated for recurrence.

2.5.2.4. Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 14 – Summary of Study NGF0212

<table>
<thead>
<tr>
<th>Study identifier</th>
<th>NGF0212</th>
</tr>
</thead>
</table>

Title: An 8-week Phase I/II, Multicentre, Randomized, Double-masked, Vehicle-controlled Parallel-group Study with a 48- or 56-week Follow-up Period to Evaluate the Safety and Efficacy of Two Doses (10 μg/ml and 20 μg/ml) of Recombinant Human Nerve Growth Factor Eye Drops Solution Versus Vehicle in Patients with Stage 2 and 3 of Neurotrophic Keratitis
Design
Multicentre, Randomized, Double-masked, Vehicle-controlled Parallel-group

<table>
<thead>
<tr>
<th>Duration of main phase:</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Run-in phase:</td>
<td>not applicable</td>
</tr>
<tr>
<td>Duration of Extension phase:</td>
<td>48/56 weeks</td>
</tr>
</tbody>
</table>

Hypothesis
Superiority

Treatments groups

<table>
<thead>
<tr>
<th>Treatments groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhNGF 10 μg/ml</td>
<td>1 drop rhNGF 10 μg/ml six times a day for 8 weeks, n= 52</td>
</tr>
<tr>
<td>rhNGF 20 μg/ml</td>
<td>1 drop rhNGF 20 μg/ml six times a day for 8 weeks, n= 52</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1 drop six times a day for 8 weeks, n= 52</td>
</tr>
</tbody>
</table>

Endpoints and definitions

<table>
<thead>
<tr>
<th>Primary endpoint</th>
<th>Complete healing at Week 4</th>
<th>Percentage of patients experiencing complete healing, defined as the greatest diameter of the corneal fluorescein staining in the area of the PED or corneal ulcer, as determined by the reading centre, being less than 0.5 mm at the Week 4 visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary endpoint</td>
<td>Complete healing at Week 8</td>
<td>Percentage of patients experiencing complete healing, defined as the greatest diameter of the corneal fluorescein staining in the area of the PED or corneal ulcer, as determined by the reading centre, being less than 0.5 mm at the Week 8 visit</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Complete healing (Investigator) at Week 4</td>
<td>Percentage of patients experiencing complete healing of the PED or corneal ulcer at 4 weeks as measured by the Investigator</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Complete healing (Investigator) at Week 8</td>
<td>Percentage of patients experiencing complete healing of the PED or corneal ulcer at 8 weeks as measured by the Investigator</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Complete corneal clearing</td>
<td>Percentage of patients experiencing complete corneal clearing (Grade 0 on the modified Oxford scale) at 8 weeks</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>BCDVA</td>
<td>Mean change in BCDVA from Baseline to Week 8</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Corneal sensitivity</td>
<td>Percentage of patients that achieve an improvement in corneal sensitivity as measured by the Cochet-Bonnet aesthesiometer at 4, 6 and 8 weeks</td>
</tr>
</tbody>
</table>

Database lock
The database was locked after the last Phase II patient had completed 12 weeks of the follow-up period

Results and Analysis

Analysis description

<table>
<thead>
<tr>
<th>Primary Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intent to treat (ITT): All randomized patients</td>
</tr>
</tbody>
</table>

Descriptive statistics and estimate variability

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>rhNGF 10 μg/ml</th>
<th>rhNGF 20 μg/ml</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subject</td>
<td>52</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Complete healing at Week 4, n/N (%)</td>
<td>28/51 (54.9%)</td>
<td>29/50 (58.0%)</td>
<td>10/51 (19.6%)</td>
</tr>
<tr>
<td></td>
<td>Comparison groups</td>
<td>(1) rhNGF 10 μg/ml vs. Vehicle</td>
<td>(2) rhNGF 20 μg/ml vs. Vehicle</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------------------------------------------</td>
<td>---------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Complete healing at Week 4</td>
<td>Difference in % Complete Healing</td>
<td>(1) 35.3%</td>
<td>(2) 38.4%</td>
</tr>
<tr>
<td></td>
<td>97.06% CI</td>
<td>(1) 15.88, 54.71</td>
<td>(2) 18.96, 57.83</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1)/(2) &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Complete healing at Week 8</td>
<td>Difference in % Complete Healing</td>
<td>(1) 31.4%</td>
<td>(2) 30.9%</td>
</tr>
<tr>
<td></td>
<td>97.06% CI</td>
<td>(1) 11.25, 51.49</td>
<td>(2) 10.60, 51.13</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.001</td>
<td>(2) 0.002</td>
</tr>
<tr>
<td>Complete healing (Investigator) at Week 4</td>
<td>Difference in % Complete Healing</td>
<td>(1) 25.8%</td>
<td>(2) 34.7%</td>
</tr>
<tr>
<td></td>
<td>97.06% CI</td>
<td>(1) 3.66, 47.87</td>
<td>(2) 11.91, 57.41</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.016</td>
<td>(2) 0.002</td>
</tr>
<tr>
<td>Complete healing (Investigator) at Week 8</td>
<td>Difference in % Complete Healing</td>
<td>(1) 26.1%</td>
<td>(2) 25.9%</td>
</tr>
<tr>
<td></td>
<td>97.06% CI</td>
<td>(1) 4.18, 48.01</td>
<td>(2) 3.55, 48.33</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.011</td>
<td>(2) 0.014</td>
</tr>
<tr>
<td>Complete corneal clearing</td>
<td>Difference in % Complete Healing</td>
<td>(1) 17.1%</td>
<td>(2) 11.4%</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>(1) 1.45, 32.72</td>
<td>(2) -4.08, 26.93</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.043</td>
<td>(2) 0.157</td>
</tr>
<tr>
<td>BCDVA</td>
<td>Difference in letters (LS mean)</td>
<td>(1) 8.9</td>
<td>(2) 5.0</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>(1) 1.33, 16.50</td>
<td>(2) -2.90, 12.88</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.022</td>
<td>(2) 0.213</td>
</tr>
<tr>
<td>Corneal sensitivity</td>
<td>Difference in % with Improvement</td>
<td>(1) 10.2%</td>
<td>(2) 7.9%</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>(1) -9.15, 29.45</td>
<td>(2) -12.13, 27.92</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>(1) 0.303</td>
<td>(2) 0.442</td>
</tr>
</tbody>
</table>

Notes
See section 2.5.2. for details on the statistical analysis including handling of missing values and multiplicity.

n=number of responders.
N=Number of study subjects with a response available.

Table 15 – Summary of Study NGF0214

**Title:** An 8-week phase II, multicentre, randomized, double-masked, vehicle controlled, parallel group study with a 24 or 32 week follow-up period to evaluate the efficacy of a formulation containing anti-oxidant of recombinant human nerve growth factor (rhNGF) in 20 μg/ml, eye drops solution versus vehicle containing anti-oxidant in patients with Stage 2 and 3 Neurotrophic Keratitis

<table>
<thead>
<tr>
<th>Study identifier</th>
<th>NGF0214</th>
</tr>
</thead>
</table>

**Design**
An 8-week phase II, multicentre, randomized, double-masked, vehicle controlled parallel group study
- **Duration of main phase:** 8 weeks
- **Duration of Run-in phase:** not applicable
- **Duration of Extension phase:** 24/32 weeks

**Hypothesis**
Superiority

**Treatments groups**
- **rhNGF 20 μg/ml**
  - 1 drop six times a day for 8 weeks, n=24
- **Vehicle**
  - 1 drop six times a day for 8 weeks, n=24

**Endpoints and definitions**

<table>
<thead>
<tr>
<th>Primary endpoint</th>
<th>Complete healing at Week 8</th>
<th>Percentage of patients experiencing complete healing, defined as the greatest diameter of the corneal fluorescein staining in the area of the PED or corneal ulcer, as determined by the reading centre, being less than 0.5 mm at the Week 8 visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary endpoint</td>
<td>Complete healing (Investigator) at Week 8</td>
<td>Percentage of patients experiencing complete healing of the PED or corneal ulcer at 8 weeks as measured by the Investigator</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Complete healing at Week 4</td>
<td>Percentage of patients experiencing complete healing, defined as the greatest diameter of the corneal fluorescein staining in the area of the PED or corneal ulcer, as determined by the reading centre, being less than 0.5 mm at the Week 4 visit</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Complete corneal clearing</td>
<td>Percentage of patients experiencing complete corneal clearing (Grade 0 on the modified Oxford scale) at 8 weeks</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>BCDVA</td>
<td>Mean change in BCDVA from Baseline to Week 8</td>
</tr>
<tr>
<td>Secondary endpoint</td>
<td>Corneal sensitivity</td>
<td>Percentage of patients that achieve an improvement in corneal sensitivity as measured by the Cochet-Bonnet aesthesiometer at 8 weeks</td>
</tr>
</tbody>
</table>

**Database lock**
The database was locked after the last patient had completed 4 weeks of the follow-up period.

**Results and Analysis**
### Analysis description

**Primary Analysis**

Intent to treat: All randomized patients

### Descriptive statistics and estimate variability

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>rhNGF 20 μg/ml</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of subjects</strong></td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Complete healing at Week 8, n (%)</td>
<td>16/23 (69.6)</td>
<td>7/24 (29.2%)</td>
</tr>
<tr>
<td>Complete healing by Investigator at Week 8, n (%)</td>
<td>15 (65.2)</td>
<td>7 (29.2)</td>
</tr>
<tr>
<td>Complete healing at Week 4, n (%)</td>
<td>13 (56.5)</td>
<td>9 (37.5)</td>
</tr>
<tr>
<td>Complete corneal clearing, n/N (%)</td>
<td>5/22 (22.7)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>BCDVA, ETDRS letters mean change (SD)</td>
<td>4.48 (9.825)</td>
<td>4.33 (10.339)</td>
</tr>
<tr>
<td>Corneal sensitivity, mean (SD)</td>
<td>1.88 (1.401)</td>
<td>1.00 (1.254)</td>
</tr>
</tbody>
</table>

### Effect estimate per comparison

<table>
<thead>
<tr>
<th>Comparison groups</th>
<th>rhNGF vs. Vehicle Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary endpoint</strong>&lt;br&gt;Complete healing at Week 8</td>
<td>Difference in % Complete Healing 40.4%&lt;br&gt;90% CI 18.4, 66.6&lt;br&gt;P-value 0.006</td>
</tr>
<tr>
<td>Secondary&lt;br&gt;Complete healing by Investigator at Week 8</td>
<td>Difference in % Complete Healing 36.1&lt;br&gt;95% CI 9.4, 62.7&lt;br&gt;P-value 0.013</td>
</tr>
<tr>
<td>Secondary&lt;br&gt;Complete healing at Week 8</td>
<td>Difference in % Complete Healing 19.0&lt;br&gt;90% CI -4.5, 42.5&lt;br&gt;P-value 0.191</td>
</tr>
<tr>
<td>Secondary&lt;br&gt;Complete corneal clearing</td>
<td>Difference in % Complete Clearing 18.6&lt;br&gt;95% CI -0.7, 37.8&lt;br&gt;P-value 0.062</td>
</tr>
<tr>
<td>Secondary&lt;br&gt;BCDVA</td>
<td>Difference in letters Mean (SD) 1.1&lt;br&gt;95% CI -5.6, 7.7&lt;br&gt;P-value 0.745</td>
</tr>
<tr>
<td>Secondary&lt;br&gt;Corneal sensitivity</td>
<td>Difference (cm) Complete Clearing 0.6&lt;br&gt;95% CI -0.4, 1.5&lt;br&gt;P-value 0.207</td>
</tr>
</tbody>
</table>
Notes  See section 2.5.2. for details on the statistical analysis including handling of missing values and multiplicity.

n=number of responders.
N=Number of study subjects with a response available.

2.5.2.5. Analysis performed across trials

NGF0212 and NGF0214 Inter-Study Analysis

An inter-study comparison was performed for studies NGF0212 (rhNGF and vehicle both without methionine, Phase II) and NGF0214 (rhNGF and vehicle both with added methionine). The comparison aimed at evaluating efficacy and safety of 20 μg/ml 6 times a day of rhNGF eye drops solution formulation containing methionine compared to 20 μg/ml rhNGF eye drops formulation without methionine 6 times a day. The study group receiving 10 μg/ml rhNGF in study NGF0212 was not considered for the inter-study analysis.

The primary efficacy variable for the inter-study comparison was complete healing of the PED or corneal ulcer determined by corneal fluorescein staining at the end of controlled treatment period (8 weeks) as determined by the Central Reading Centre evaluating the clinical picture. Patients, who discontinued before Week 4 (and who did not have a post-baseline corneal photography with fluorescein), were assumed to have been ‘Not Completely Healed’ for primary efficacy endpoint if the Investigator recorded that the ‘measurement are N/A because of the greatest dimension of the PED or corneal ulcer evaluated was greater than 1 mm on the slit lamp’. If no post-baseline values were available, no imputation was performed, the patient was assumed to have a missing ‘Completely Healed’ endpoint and was not included in the analysis. The analyses were performed on the safety population.

In addition to the NGF0212/ NGF0214 inter-study SAP, the results of the 2 studies were also compared for corneal clearing in line with an additional analysis (where corneal healing was defined as “no corneal fluorescein staining in the area of the PED or corneal ulcer, and non-persistent lesions in the surrounding area of the cornea”). This was a post-hoc analysis for study NGF0212, whereas the analysis was included with an addendum to the SAP for study NGF0214 prior to database lock and unblinding. The results are shown in previous sections 2.5.2.2. and 2.5.2.3.

Results

Across the two studies, overall, 115/152 (75.7%) patients completed the controlled 8 week treatment period; 32 (21.1%) were withdrawn and 118 (77.6%) entered the follow-up period. The withdrawal rate was identical (25%) in the two rhNGF arms with and without methionine (6/24 patients in study NGF0214 [+methionine] and 13/52 patients in study NGF0212 [-methionine]), whereas it was higher in the vehicle arm with methionine as compared to the vehicle arm without methionine (9/24 [37.5%] vs 4/52 [7.7%]).

Patient demographics and baseline characteristics were overall comparable between the 4 treatment groups. Overall, there were more patients in study NGF0212 (vehicle and 20 μg/ml rhNGF) that had stage 3 NK (49/104 [47.1%] compared to study NGF0214 (15/47 [31.9%]). At the same time, patients in NGF0212 had a shorter average disease period (27.3 months) compared to a mean of 32.7 months for patients in study NGF0214.

Furthermore, during the controlled treatment period, comparatively more patients in study NGF0214 than in study NGF0212 took concomitant ocular preparations: 82.6% in the rhNGF + methionine arm and 75.0% in the vehicle + methionine arm versus 50.0% in the rhNGF arm and 40.4% in the vehicle arm.
**Primary endpoint: Complete Corneal Healing at Week 8 as Assessed by the Central Reading Centre**

The percentage of patients that achieved complete healing at Week 8 was similar for the rhNGF 20 \( \mu \text{g/ml} \) group in both studies and was significantly higher compared to the vehicle group, as shown in Table 16. The odds ratio (NGF0212/NGF0214) was 1.47 (95% CI: 0.73; 2.94). Sensitivity analyses with respect to handling of missing data showed similar results and confirmed the robustness of the data.

Table 16 – Study NGF0212/NGF0214 Inter-Study Comparison: Percentage of Patients who Achieved Complete Healing at Week 8 as Determined by the Reading Centre (ITT)

<table>
<thead>
<tr>
<th></th>
<th>NGF0212</th>
<th>NGF0214</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle( (N=52) )</td>
<td>rhNGF( 20 \mu \text{g/ml} )( (N=52) )</td>
</tr>
<tr>
<td>Complete Healing Achieved (Observed Cases)</td>
<td>22 (56.4%)</td>
<td>35 (83.3%)</td>
</tr>
<tr>
<td>No</td>
<td>17 (43.6%)</td>
<td>7 (16.7%)</td>
</tr>
<tr>
<td>Missing</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Odds-ratio (NGF0212/NGF0214) Value (95%-CI)</td>
<td>1.24 (0.52 - 2.97)</td>
<td>1.56 (0.78 - 3.10)</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; ITT = intent-to-treat; LOCF = last observation carried forward; M = methionine; rhNGF = recombinant human nerve growth factor

Results after 4 weeks of treatment revealed a difference between active and vehicle groups of 38% (NGF0212) and 19% (NGF0214), respectively, resulting in an OR of 0.71 (95% CI: 0.36; 1.44).

*Post-hoc pooled analysis by disease severity (stage 2 and 3)*

In response to a request by the CHMP, in order to investigate consistency of the effect by disease severity stage, the applicant presented pooled data by disease stage at baseline. In the Phase II segment of study NGF0212, 76 patients (49%) had stage 2 NK and 80 patients (51%) had Stage 3 NK. In study NGF0214, 33 patients (69%) had stage 2 NK and 15 patients (31%) had stage 3 NK.

When pooling all patients who received rhNGF in study NGF0212 and NGF0214, 37 out of 60 (62%) patients with stage 2 NK and 53 out of 64 (83%) patients with stage 3 NK achieved epithelial closure at the end of the 8 weeks controlled treatment period. By pooling all patients who received vehicle in the two studies, 15 out of 46 (33%) patients with stage 2 NK and 14 out of 29 (48%) patients with stage 3 NK achieved epithelial closure at the end of the 8 weeks controlled treatment period. The difference between rhNGF and vehicle was statistically significant in both stage 2 (p=0.003) and stage 3 (p<0.001) disease. When only considering patients who received rhNGF 20 \( \mu \text{g/ml} \) in the two studies, 25 out of 40 (63%) patients with stage 2 NK and 28 out of 33 (85%) patients with stage 3 NK achieved epithelial closure at the end of the 8 weeks controlled treatment period. This difference versus vehicle maintained statistical significance in both stage 2 (p=0.006) and stage 3 (p=0.002) disease.
2.5.2.6. Supportive study(ies)

Prior to the initiation of the clinical development program for Oxervate, reports from open-label, uncontrolled clinical trials using native mNGF in the form of eye drops had been published in the scientific literature. Two studies by Bonini et al. (2000) and Lambiase et al. (2007) using 200 μg/ml mNGF eye drops are briefly summarised.

Bonini et al. (2000) reported on 43 patients (45 eyes) with moderate (stage 2, n=17) to severe (stage 3, n=8) neurotrophic keratitis unresponsive to other nonsurgical therapies, who were treated with mNGF (200 mg/ml) every 2 hours for 2 days followed by one drop six times daily. Patients were treated until the defect had resolved and thereafter continued on a maintenance dose of one drop mNGF (100 mg/ml) four times daily for 2 weeks.

The mean duration of the follow-up period was 15.8 ± 11.5 months (9.1 ± 7.2 months in stage 2 and 19.9 ± 11.7 months in stage 3). All patients had a complete resolution of the PED (with or without an ulcer). Complete epithelial healing was observed after approximately 1 month of treatment (26.6 ± 9.0 days in stage 2; 22.8 ± 10.0 days in stage 3). Furthermore, a corneal sensitivity test was performed by touching the patient’s central corneal zone with the tip of a cotton swab. Following treatment with mNGF, corneal sensitivity was improved in 59% of the eyes with stage 2 keratitis and in 89% of the eyes with stage 3 NK. Visual acuity was also significantly improved at the end of the follow-up period from BCVA 18/300 ± 18/200 to BCVA 20/55 ± 20/100 (p<0.001) in patients with stage 2 keratitis and from BCVA 10/300 ± 10/300 to BCVA 20/50 ± 20/80 (p<0.001) in patients with stage 3 keratitis. The improvement in visual acuity in patients with stage 3 was significantly correlated to the duration of follow-up (rho 5 0.602; p<0.001).

Lambiase et al. (2007) studied further 11 patients (13 eyes) affected by NK unresponsive to conventional therapies primarily with a view to investigate anti-NGF antibody development. All patients presented with a neurotrophic corneal ulcer, stromal involvement and complete corneal anaesthesia. Patients were treated with mNGF eye drops (same dosing schedule as in Bonini et al., 2000). All patients had complete resolution of the corneal ulcer after 26 ± 11 days of treatment with mNGF (mea ± SD; range: 9–43 days). The mean follow-up was 33 ± 16 months (range: 16–72 months). In 2 patients an ulcer recurred after two months and 6 months, respectively, and healed after 2-3 weeks of mNGF treatment. Corneal sensitivity improved to hypoesthesia in all the eyes and was maintained until the last follow up visit. When compared with the values at base line, BCVA significantly improved at the end of follow up in all the eyes (mean± SD, 0.11 ± 0.13 versus 0.30 ± 0.23; p<0.05).

Overall, therapeutic efficacy was independent from the underlying cause of NK as patients with different aetiologies showed a similar response. Side effects were in both studies only local, mild and transient (within a follow-up period of 18-72 months).

2.5.3. Discussion on clinical efficacy

The evidence of the efficacy of rhNFG (cenegermin) eye drop solution in the treatment of stage 2 and 3 NK was mainly derived from data of two randomized, vehicle controlled, double-masked, parallel group clinical trials, study NGF0212 (Phase II) and study NGF0214.

During the clinical development, the eye drop solution formulation was changed and L-methionine was added as an anti-oxidant due to concerns that oxidation could have affected the stability of rhNGF in the previous formulation. Only patients included in study NGF0214 received the L-methionine formulation proposed to be marketed. An integrated comparison of study NGF0212 and NGF0214 was provided in order to support
comparability of the L-methionine free and L-methionine containing formulation. Only the 20 µg/ml dose strength was included in the comparison, as this dose was evaluated in both efficacy studies and is proposed for commercial use. The approach had previously been agreed by CHMP in a scientific advice whereby a formal bridging study to investigate differences between the two formulations was considered unfeasible.

Finally, the applicant informed that a new clinical study (NGF0215) in the intended indication is planned to be conducted with the rhNGF 20 µg/ml with methionine formulation. This study will provide additional data on the prolonged use of rhNGF eye drops in a subset of NK patients who were not completely healed after 8 weeks of treatment.

**Design and conduct of clinical studies**

The selection of the rhNGF target dose and dosing schedule (20 µg/ml rhNGF, 6 drops/eye/day for 8 weeks) to be investigated in the Phase II studies was based on results from *in vitro* and *in vivo* non-clinical studies, *in-vitro* human biomaterial studies and reports on mNGF in the scientific literature. In study NGF0212 a lower dose of 10 µg/ml rhNGF was also included to explore the dose response relationship. Doses higher than 20 µg/ml rhNGF were tested during Phase I but only for safety (tolerability) purposes. The rationale for the dose selection was considered acceptable by the CHMP.

Both Phase II studies were similar in design (randomised, double-masked, parallel-group), patient populations and endpoints, which had previously been agreed by the CHMP in the context of scientific advice and protocol assistance. Adult patients with moderate and severe clinical stage of NK were recruited, i.e. those with PED (NK stage 2) or with corneal ulcer (NK stage 3) and documented decreased corneal sensitivity of at least moderate severity. Further criteria of severity, such as refractoriness to previous treatment and reduction of visual acuity were considered reasonable to define a patient population in need of treatment and unlikely to experience spontaneous healing.

The primary endpoint was the percentage of patients experiencing complete healing, defined as the greatest diameter of CFS in the area of the PED or corneal ulcer being less than 0.5 mm, as determined by the reading centre. The primary endpoint was assessed after 4 weeks (study NGF0212) or 8 weeks (study NGF0214) of treatment. Secondary endpoints were complementary and included further responder analyses of complete healing or corneal clearing, time to corneal healing, as well as improvement in visual acuity and corneal sensitivity and percentage of patients experiencing deterioration. Overall, the choice of endpoints was considered acceptable.

After an 8 week-controlled treatment phase, patients entered a follow-up phase of either 48 weeks (study NGF0212) or 24 weeks (study NGF0214) duration. Patients initially assigned to vehicle received active treatment from week 8 to week 16 if they were not completely healed at week 8. All patients were eligible for another course of treatment in the event of recurrence during the follow-up period. Given that NK patients may require long-term/repeated treatment, data from the follow-up periods were considered highly relevant to comprehensively assess the efficacy of Oxervate.

**Efficacy data and additional analyses**

**Study NGF0212**

Phase II of study NGF0212 recruited a total of 156 patients (52 patients in each of the 3 treatment arms). A total of 109 patients (69.9%) entered the 48-week follow-up period. A rather high number of patients
withdraw from the study and did not continue until the need of the follow-up period: 24 patients withdrew at Week 8 (7/52 in rhNGF 10 µg/ml, 13/52 in rhNGF 20 µg/ml, 4/52 in vehicle arm) and 18 additional patients during the follow-up phase (8 in rhNGF 10 µg/ml, 3 in rhNGF 20 µg/ml, 7 in vehicle arm). Most of the withdrawals occurred in the active groups were reported to be due to adverse events and in the case of the high dose group many of these took place early during the 8-week controlled period of treatment (see section 2.6.1. for the discussion of safety). The CHMP noted the low retention rate in the study. The additional analyses based on observed cases, using imputation of missing data as failures or multiple imputation method were considered sufficient to address the possible impact on the study outcome (see below).

Overall, treatment groups were well balanced with respect to demographic and baseline characteristics. There were slightly more women than men (60 and 40% of the total population). Nearly half of the enrolled patients were diagnosed with to Stage 2 (48.7%) and the remainder with Stage 3 (51.3%) NK. Patients in the rhNGF 10 µg/ml dose group included a higher percentage of severe (stage 3) patients compared to rhNGF 20 µg/ml and vehicle [31/52 (59.6%), 25/51 (48.1%) and 24/52 (46.2%)]. Patients reported a wide spectrum of aetiologies as cause of NK.

After 4 weeks of treatment 67 of the 156 patients treated achieved complete healing of the corneal lesion as determined by the Reading Centre (primary endpoint). Patients on 20 μg/ml dose reached the highest rate of cure (58%; 29/52) and slightly less patients treated with 10 µg/ml (54.9%, 28/52) achieved complete corneal healing. In comparison, complete healing was achieved by 10 out of 52 of patients in the vehicle group (19.6%). The difference of 38.4% and 35.3%, respectively, was statistically significant (p<0.001). At the end of the 8 week treatment period, there was an increase in the rates of completely healed patients in all three treatment arms. Significantly more subjects receiving either rhNGF 10 or 20 µg/ml than those treated with the vehicle achieved complete corneal healing [38 patients (74.5%) in the rhNGF 10 µg/ml group, 37 (74.0%) in the rhNGF 20 µg/ml group, and 22 (43.1%) in the vehicle control; p=0.001 and 0.002]. No statistically significant differences between doses were observed at either time point. The results from other analyses conducted (observed case analysis, sensitivity analyses) were in line with the primary analysis. Likewise, the results for corneal healing measured by the clinical investigator were consistent with the cure rates observed by the Reading Centre, thus supporting robustness of the primary study outcome.

The CHMP considered the improvement in corneal surface integrity over 4 and 8 weeks to be clinically meaningful in a patient population with impaired wound healing at risk of corneal perforation and loss of eyesight. The observed increase in the responder rate (31-38%) with rhNGF compared to vehicle provided robust support of a clinically relevant treatment benefit of rhNGF.

By comparison, uncontrolled studies conducted with topically administered mNGF reported a complete treatment response (healing) in all the study participants (Bonini et al., 2000; Lambiase et al., 2007). However, in these studies treatment was administered until healing. This raised the question if some patients may obtain further benefit from prolonged treatment. Notably, in study NGF0212, the corneal defect had healed in a total of 67 patients by Week 4 and in 97 patients by Week 8 (i.e. 30 additional patients from Week 4 to 8). An extended treatment period beyond 8 weeks was not investigated and it is thus not known if some patients would have benefited from continued treatment. The CHMP recommended that the planned study NGF0215 should aim to address this question.

Results for complete corneal clearing, i.e. Grade 0 staining on the Oxford scale after instillation of fluorescein, numerically also favoured active treatments over control (20.8%, 19.5% and 7.1% responders for rhNGF 10 µg/ml, rhNGF 20 µg/ml, and vehicle, respectively) although the difference was not statistically significant (p=0.065 and p= 0.097).
Furthermore, long-term data showed that the majority of patients healed at Week 8 after rhNGF treatment, remained healed during the 48-week follow-up period (83% of patients in the rhNGF 10 µg/ml group and 80% in the rhNGF 20 µg/ml group with a response available). This was overall supportive of maintenance of the effect. Additional long-term data are expected to be generated post-approval with study NGF0215.

Compared to vehicle, complete healing was achieved earlier in the active groups, with a median time of 29 days (95% CI: 20, 55), 28 days (95% CI: 19, 55), and 56 days (95% CI: 42, not estimable), in the rhNGF 10 µg/ml group, 20 µg/ml group, and vehicle control group, respectively. However, due to the exploratory nature of this variable, these results should be considered with caution.

Only few patients experienced deterioration, i.e. increase of the size or depth of the corneal lesion, decrease of visual acuity or presence of corneal infection, with a trend in favour of active treatment with less patients worsening compared to vehicle.

With regards to visual acuity, patients presented with severe impairment of vision (mean BCDVA between 24.2-32.4 letters), which was slightly worse in the 20 µg/ml dose group compared to the other two study arms. A trend towards an improvement in vision over time was shown in all 3 treatment groups. At Week 8, the mean improvement in BCDVA was 15.8 letters for rhNGF 10 µg/ml and 11.9 letters for rhNGF 20 µg/ml and 6.9 letters for vehicle. Only the difference between the 10 µg/ml dose and vehicle was statistically significant (p=0.022). The proportion of rhNGF patients who gained at least 15 letters in BCDVA by Week 8 was 50% (24/48) in the 10 µg/ml dose arm and 41.5% (17/41) in the 20 µg/ml dose arm, which represent a 27.5% (p=0.008) and 19.0% (p=0.068) increase, respectively, over vehicle (22.5%, 9/40). The trend towards a clinical improvement in vision was acknowledged by the CHMP. However, the lack of statistical significance of the between treatment difference of rhNGF 20 µg/ml versus vehicle created uncertainty with regards to the true benefit of rhNGF treatment.

Similar to the finding for visual acuity, corneal sensitivity improved in all three study groups, with only a small numerical advantage being apparent in the active treatments compared to vehicle.

It is possible that residual corneal damage which had not completely healed at the time of assessment may have delayed or restricted improvements of functional outcomes and that both vision and corneal sensitivity will continue to improve over time. However, while the improvement in vision achieved by Week 8 persisted in the follow-up period, no further improvement in vision was observed during the 48-week extension phase. Results from the 48-week extension period also showed slightly more patients on rhNGF (both doses) than on vehicle with an improvement or no change in corneal sensitivity. While this finding was in favour of rhNGF, the response definition (improvement or no change in corneal sensitivity) did not distinguish those patients who experienced a real gain in corneal sensitivity. Furthermore, no trend of further improvement in corneal sensation over time was observed. Whether additional functional improvements could be achieved with longer or repeated courses of rhNGF treatment remained unclear at the time of this report.

When comparing the two rhNGF doses tested in study NGF0212, both appeared to have similar efficacy profiles. The applicant stated that, while there were no statistically significant differences between the 10 µg/ml and the 20 µg/ml doses, there were some endpoints which showed a trend towards better efficacy for the higher dose, e.g. the number of patients with corneal healing at Week 4, and the number of patients with residual fluorescein staining following complete healing in study NGF0212. However, the opposite is true for other endpoints and no clear advantage of the 20 µg/ml strength over 10 µg/ml could be deduced from the study data. The CHMP acknowledged that the efficacy studies were not designed or powered to show a statistically significant difference between the dosing groups. Given that efficacy of the 20 µg/ml strength has been established including in the context of the commercial formulation containing L-methionine (see study...
NGF0214 below) and subject to the assessment of the safety profiles of the two doses (see section 2.6.), the CHMP considered the applicant’s choice of rhNGF 20 μg/ml acceptable.

**Study NGF0214**

A total of 48 patients were randomized in this study (24 patients in the rhNGF 20 μg/ml and 24 patients in the vehicle group). A total of 33 patients completed the controlled treatment period: 18 patients (75%) in the rhNGF 20 μg/ml group and 15 patients (62.5%) in the vehicle control group. Most of the withdrawals occurred in the active group and were due to adverse events (4 out of 6). In the vehicle group 6 out of 9 patients terminated prematurely and entered the uncontrolled treated period.

Similar to study NGF0212, females represented approximately 60% of the population. The treatment groups were similar with regards to demographic characteristics and NK aetiology. The majority of patients had developed stage 2 (68.8%) and there were slightly more stage 3 patients in the rhNGF group (9; 37.5%) compared to vehicle (6; 25.0%).

After 8 weeks of treatment 23 of 48 patients achieved complete healing of the corneal lesion as determined by the Reading Centre (primary endpoint): 16/23 patients on rhNGF (69.6%) and 7/24 patients receiving vehicle (29.2%). The difference of 40.4% was statistically significant (p=0.006). Cure rates determined at Week 4 as well as when assessed by the investigator were overall consistent with findings for the primary endpoint, although the between-treatment difference was less pronounced at Week 4 compared to Week 8. Furthermore, complete corneal clearing (zero staining) was achieved in more patients on rhNGF that on vehicle (22.7% versus. 4.2%), although the difference did not reach statistical significance (p=0.062). Overall few, but more patients receiving rhNGF than those in the vehicle group experienced disease deterioration.

Similar to study NGF0212, little or no improvement was observed in functional outcomes at the end of the treatment period. Patients in both treatment arms improved in visual acuity, but no advantage of rhNGF compared to vehicle was observed (mean change in BCDVA from baseline 4.48 letters vs. 4.33 letters, respectively). Corneal sensitivity was numerically better in the rhNGF group than in the vehicle group but the clinical relevance of the difference in the mean change from baseline at Week 8 (0.6 cm) was uncertain. At the end of treatment all patients remained within the range of the qualification criteria of hypoesthesia/anaesthesia at study entry, i.e. ≤4 cm measured with the Cochet-Bonnet aesthesiometer.

**Inter-study comparison NGF0212/NGF0214**

Demographic and baseline characteristics suggest that the populations of both studies using L-methionine free (NGF0212) and L-methionine containing (NGF0214) eye drop formulations, respectively, were similar. Both studies included patients with different disease severity (stage 2 and 3) and a wide spectrum of aetiologies as cause of NK. When comparing disease severity, patients from study NGF0212 appeared to have been slightly more severely affected than in study NGF0214 (47.1% versus 31.9% of stage 3 patients). While these factors could be expected to have impact on the treatment response to rhNGF, subgroup analyses for stage 2 and 3 disease and by main NK aetiologies showed no relevant differences in cure rates.

The two studies differed in the inclusion criteria with regards to involvement of the contralateral eye. Only study NGF0214 allowed the entry of patients with both eyes affected. However, only 3 patients were enrolled with both affected eyes which was not considered of relevance for the study outcome.

In both studies, the response rates in the vehicle arms were rather large. The applicant argued that good patient management according to best standard of care including close monitoring as applied during the study is known to help corneal healing and the response rates were in line with the estimated rates used for
study size and power calculations (approximately 30%). While this justification was considered acceptable, the CHMP noted a difference in the response rates for patients in the vehicle arms with and without methionine. At Week 8, only 7/24 (29.2%) of the patients receiving vehicle in study NGF0214 had achieved complete corneal healing compared to 22/52 (43.1%) in study NGF0212. Furthermore, when comparing the 2 studies, it emerged that more patients receiving vehicle with methionine withdrew prematurely during the active 8 week treatment phase, needed treatment during the uncontrolled period and reported adverse events (see also discussion on clinical safety in section 2.6.). The applicant was of the view that this discrepancy was unlikely due to a detrimental effect of the excipient. Rather, the result of this indirect comparison of two studies, one of which was conducted in the US and the other in Europe, may reflect cultural differences including clinical practice, perception of and ability to describe symptoms, patients’ willingness to participate in trials, etc. Regional difference in the prescribing patterns were also considered the main reason for the difference in reported previous treatment with artificial tears or other lubricants (less than 40% of patients in study NGF0214 compared to 96.1% in study NGF0212). In general, prevalence, diagnosis and therapeutic approach differ in the US and Europe, which may be relevant to the treatment of this condition. In this context, additional analyses presented by the applicant were reassuring, showing that use of concomitant medication did not affect the treatment effect of rhNGF. The CHMP considered the explanations satisfactory.

Overall, the inter-study analyses supported the efficacy conclusions from the individual studies. Positive and consistent results have been observed with regards to corneal healing in patients after 8 weeks of treatment with rhNGF 20 µg/ml. The difference in cure rate for rhNGF 20 µg/ml with respect to vehicle was 31% and 40% at week 8, for studies NFG0212 and NFG0214, respectively (OR: 1.47[0.73-2.94]). Results from the initial 4 weeks of treatment were also reassuring although less consistent across studies (difference between active and vehicle groups were 38% and 19% (OR: 0.71 [0.36-1.44] in study NGF0212 and NGF0214).

Importantly, no major discrepancies between disease progressions as a potential reflection of a deleterious effect on the cornea of methionine have been observed. Thus, while some uncertainties remained given the limited experience with this excipient in topical eye products (see also discussion on clinical safety in section 2.6.), the CHMP considered that the methionine-containing formulation could be acceptable from an efficacy point of view given that a clear treatment benefit with this formulation has been shown. Additional long-term data with the commercial formulation are expected to be generated post-approval in study NGF0215.

### 2.5.4. Conclusions on the clinical efficacy

In conclusion, the available clinical data demonstrate a clear benefit of an 8-week treatment course with Oxervate in re-establishing ocular surface integrity in patients with stage 2 and 3 NK. The observed difference of 30-40% in the rate of corneal healing with respect to vehicle control represents robust proof of a clinically meaningful treatment benefit, as corneal health reduces the risk of eye perforation and potential sight loss. Long-term data were overall supportive, showing maintenance of the treatment effect in the vast majority of patients up to 1 year. Furthermore, a trend in favour of rhNGF over vehicle in improving visual acuity was observed, although some uncertainties regarding functional outcomes remained. The CHMP recommended that the applicant pursued the plans for an additional study (NGF0215) to generate further long-term data with the commercial, methionine-containing formulation, to help address the remaining uncertainties with the use of Oxervate including (functional) long-term outcomes and a possible additional benefit of prolonged treatment.
2.6. Clinical safety

For the purpose of the safety evaluation, safety data from the clinical development programme were aggregated in three separate pools:

- the **Primary Safety Pool**, which included data in patients with stage 2 and 3 NK from studies NGF0212 (European population) and NGF0214 (US population);

- the **Secondary Safety Pool** comprising all 5 studies with rhNGF sponsored by the applicant (see table 2) including data from the two trials in NK patients, one trial in healthy volunteers, one in moderate-severe dry eye disease and another one in retinitis pigmentosa with higher dose and longer duration of treatment in other indications.

The Primary Safety Pool was the main basis for the safety assessment, with other data being considered as supportive. All analyses were based on the Safety Set, i.e. all randomized patients who received at least one dose of study medication, with patients summarized according to the actual treatment received.

In addition to the safety analysis based on pooled study data, a comparative safety analysis was conducted based on data from study NGF0212, in which two different doses of rhNGF were compared. Data from study NGF0212 and NGF0214 were furthermore compared to assess the safety of a methionine-free versus a methionine-containing eye drops formulation.

Pooled data analyses were based on the available data at the time of database lock for this application. Databases of studies NGF0212 and NGF0214 were locked after 3 months and 4 weeks follow-up, respectively. Data for the full follow up periods (12 months for study NGF0212 and 6 months for study NGF0214) were provided in response to a CHMP request during the course of this procedure.

In addition to the review of adverse event (AE) reporting and laboratory results for haematology, clinical chemistry, and vital signs, the following ocular assessments were conducted: visual analogue scale (VAS) for ocular tolerability, BCDVA, intraocular pressure (IOP), and dilated fundus ophthalmoscopy. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 15.1.

In the pivotal trials, an adverse drug reaction was defined as an untoward and unintended response to an investigational medicinal product related to any dose administered. The definition implied a reasonable possibility of a causal relationship between the event and the investigational medicinal product. Relationship/causality of an AE to study drug was assessed by the investigator using the following terms: none (intercurrent event), unlikely (remote), possible, probable, or highly probable.

**Patient exposure**

A total of 315 subjects were exposed to rhNGF at any concentration/dose (see Table 17). Of these, 177 were NK patients, whereby only 82 (59 in phase II segment of study NGF0212 and 23 in study NGF0214) were treated with the concentration proposed for commercial use (20 µg/ml, 6 drops per day in the affected eye). Furthermore, only 23 patients from study NGF214 were treated with the proposed formulation containing methionine as an excipient. There were some additional data available from patients initially assigned to vehicle, who did not heal during the 8-week double-blind period and subsequently received rhNGF during Week 8-16 (23 and 13 additional patients from study NGF0212 and NGF0214).
Furthermore, during the follow-up period, a total of 18 patients had unscheduled exposure to study treatment following a recurrence of PED or corneal ulcer including 13 patients in study NGF0212 (6 patients in the rhNGF 10 μg/ml group and 7 patients in the rhNGF 20 μg/ml group) and 5 patients in study NGF0214.

Table 17 – Overview of the Safety Database for rhNGF

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Total subjects</th>
<th>Healthy Subjects</th>
<th>Stage 2-3 NK Patients</th>
<th>Patients with other Ocular Pathologies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NGF0112</td>
<td>NGF0212</td>
<td>NGF0214</td>
</tr>
<tr>
<td>Subjects recruited in any rhNGF study</td>
<td>386</td>
<td>74</td>
<td>174</td>
<td>48</td>
</tr>
<tr>
<td>Subjects treated with rhNGF at randomization</td>
<td>279</td>
<td>58</td>
<td>118</td>
<td>23</td>
</tr>
<tr>
<td>NK patients initially randomized to vehicle and then rescue treated with rhNGF</td>
<td>36</td>
<td>0</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Total of NK patients exposed to rhNGF</td>
<td>177</td>
<td>0</td>
<td>141</td>
<td>36</td>
</tr>
<tr>
<td>Total of patients exposed to rhNGF (any indication)</td>
<td>257</td>
<td>0</td>
<td>141</td>
<td>36</td>
</tr>
<tr>
<td>Total of subjects exposed to rhNGF</td>
<td>315</td>
<td>58</td>
<td>141</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Posology and duration</th>
<th>Unit</th>
<th>rhNGF concentrations used</th>
<th>Regimen</th>
<th>Eyes treated</th>
<th>Treatment duration</th>
<th>Inclusion in Safety Pools</th>
<th>Primary Safety Pool</th>
<th>Secondary Safety Pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit</td>
<td>(μg/ml)</td>
<td></td>
<td>(eye/day)</td>
<td>(weeks)</td>
<td>Subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5-180</td>
<td>1-3x</td>
<td>1</td>
<td>up to 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10-20</td>
<td>6x</td>
<td>1 or 2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>6x</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-20</td>
<td>2x</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60-180</td>
<td>3x</td>
<td>2</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: μg = microgram(s); ml = millilitre(s); NK = neurotrophic keratitis; rhNGF = recombinant human nerve growth factor; pts = patients

* Patients treated with 20 μg/ml rhNGF or vehicle in NGF0212 (Phase II segment) and in NGF0214

* Patients treated with rhNGF or Vehicle in all clinical studies conducted

In patients with NK, the treatment duration was 8 weeks, whereas the treatment duration for subjects enrolled in other clinical trials ranged from 4 to 24 weeks. All NK patients included were adults. The majority were white with more than 60% being women. The average age ranged between 60 and 70 years for the Primary Safety Pool. Patients from the Secondary Safety Pool were younger with a mean age ranging from 35 to 52.1 years reflecting the different indications included in this Pool.

As for disease characteristics for the Primary Safety Pool (clinical trials in NK population), the main cause of NK was herpes simplex infection. Dry eye and surgery were other common causes. The difference in percentage of patients with stage 3 of the disease (47.1% in study NGF0212 versus 31.9% in study NGF0214) suggests that patients from study NGF0212 were slightly more severely affected. Time from diagnosis was very similar in both studies (27 and 32 months, respectively) while time since diagnosis of stage 2 or 3 was double for study NGF0212 (16 versus 7.8 months respectively) what would be in line with the more advanced disease of NGF0212 patients. See section 2.5.2.2. and 2.5.2.3. for further details.

Adverse events

Primary Safety Pool

Table 18 provides an overview of the AEs observed in the Primary Safety Pool during the 8 weeks controlled treatment period.
Table 18 – Primary Safety Pool: Overview of Adverse Events and Deaths Occurring During the Controlled Treatment Period - Safety Population

<table>
<thead>
<tr>
<th>Controlled Treatment Period</th>
<th>Phase segment II NGF0212</th>
<th>NGF0214</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event</td>
<td>Vehicle (N=52)</td>
<td>rhNGF 20 µg/ml (N=52)</td>
</tr>
<tr>
<td>Patients with at least 1 adverse event n (%)</td>
<td>20 (38.5%)</td>
<td>27 (51.9%)</td>
</tr>
<tr>
<td>Number of adverse events</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Patients with at least 1 serious adverse event n (%)</td>
<td>5 (9.6%)</td>
<td>9 (17.3%)</td>
</tr>
<tr>
<td>Number of serious adverse events</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Patients with at least 1 adverse event leading to discontinuation of study drug n (%)</td>
<td>4 (7.7%)</td>
<td>9 (17.3%)</td>
</tr>
<tr>
<td>Number of adverse events leading to discontinuation of study drug</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Patients with at least 1 adverse event with relation to study drug n (%)</td>
<td>10 (19.2%)</td>
<td>9 (17.3%)</td>
</tr>
<tr>
<td>Number of adverse events with relation to study drug</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Number of deaths</td>
<td>0 (0.0%)</td>
<td>1 (1.9%)</td>
</tr>
</tbody>
</table>

Key: µg = microgram(s); ml = millilitre(s); n = number of patients in a particular category; N = number of patients in Safety population; rhNGF = recombinant human nerve growth factor.

Data for the controlled phase of the studies (see table above) suggest a worse adverse safety profile for patients receiving methionine-containing eye drops (both vehicle and rhNGF). Patients receiving vehicle plus methionine (study NGF0214) experienced a higher rate of AEs compared to those receiving vehicle alone (study NGF0212): patients with at least 1 AE (75% versus 38.5%), patients with at least 1 severe AE (16.7% versus 9.6%), patients with at least 1 AE leading to discontinuation (29.2% versus 7.7%) and patients with at least 1 AE in relation to the study drug (33.3% versus 19.2%). Similarly, for patients on rhNGF plus methionine compared to those on rhNGF alone higher percentages of patients with at least 1 AE (91.3% versus 51.9%) and with at least 1 AE in relation to the study drug (43.5% versus 17.3%) were observed.

Data for the uncontrolled phase of the studies were too limited in the pooled analyses (due to the early database lock) to draw any sound conclusions (see long-term safety section below).

A summary of AEs (occurring in ≥5% of patients in either study overall by preferred term) for the Safety population is presented in Table 19 for the controlled treatment period.
### Table 19 – Primary Safety Pool: Summary of AEs by MedDRA SOC or PT occurring in ≥5% of Patients During the Controlled Treatment Period - Safety Population

<table>
<thead>
<tr>
<th>System Organ Class/Preferred Term</th>
<th>Phase II segment NGF0212</th>
<th></th>
<th>NGF0214</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (N=52)</td>
<td>rhNGF 20 μg/ml (N=52)</td>
<td>Vehicle+methionine (N=24)</td>
<td>rhNGF+methionine 20 μg/ml (N=23)</td>
</tr>
<tr>
<td>Any Adverse Event, n (%)</td>
<td>20 (38.5%)</td>
<td>27 (51.9%)</td>
<td>18 (75.0%)</td>
<td>21 (91.3%)</td>
</tr>
<tr>
<td>Eye disorders</td>
<td>16 (30.8%)</td>
<td>13 (25.0%)</td>
<td>14 (58.3%)</td>
<td>18 (78.3%)</td>
</tr>
<tr>
<td>Cataract</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Corneal epithelium defect</td>
<td>1 (1.9%)</td>
<td>0 (0.0%)</td>
<td>2 (8.3%)</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Corneal thinning</td>
<td>0</td>
<td>0</td>
<td>2 (8.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Eye inflammation</td>
<td>0</td>
<td>1 (1.9%)</td>
<td>2 (8.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Eye pain</td>
<td>4 (7.7%)</td>
<td>5 (9.6%)</td>
<td>2 (8.3%)</td>
<td>7 (30.4%)</td>
</tr>
<tr>
<td>Foreign body sensation in eyes</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>0</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Lacrimation increased</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>1 (4.2%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Ocular discomfort</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>2 (8.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Ocular hyperemia</td>
<td>1 (1.9%)</td>
<td>1 (1.9%)</td>
<td>1 (4.2%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Photophobia</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>2 (8.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Visual acuity reduced</td>
<td>2 (3.8%)</td>
<td>3 (5.8%)</td>
<td>5 (20.8%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>0</td>
<td>1 (1.9%)</td>
<td>2 (8.3%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>7 (13.5%)</td>
<td>2 (3.8%)</td>
<td>6 (25.0%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Disease progression</td>
<td>6 (11.5%)</td>
<td>2 (3.8%)</td>
<td>4 (16.7%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Sensation of foreign body</td>
<td>0</td>
<td>0</td>
<td>2 (8.3%)</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>2 (3.8%)</td>
<td>7 (13.5%)</td>
<td>2 (8.3%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Injury, poisoning and procedural complications</td>
<td>2 (3.8%)</td>
<td>0</td>
<td>0</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Investigations</td>
<td>1 (1.9%)</td>
<td>2 (3.8%)</td>
<td>2 (8.3%)</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Intracocular pressure increased</td>
<td>0</td>
<td>1 (1.9%)</td>
<td>2 (8.3%)</td>
<td>3 (13.0%)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>2 (3.8%)</td>
<td>2 (3.8%)</td>
<td>2 (8.3%)</td>
<td>1 (4.3%)</td>
</tr>
<tr>
<td>Headache</td>
<td>2 (3.8%)</td>
<td>2 (3.8%)</td>
<td>2 (8.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>1 (1.9%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: μg = microgram(s); ml = millilitre(s); n = number of patients; N = number of patients in the Safety population; rhNGF = recombinant human nerve growth factor; PT = Preferred Term; SOC = System Organ Class.

Percentages (%) are calculated using the population number in each treatment group (N) as the denominator. The table shows only the SOC classes where PT occurred in ≥5% of patients overall in either study and shows only the PTs occurring in ≥5% of patients overall in either study.

The most common AEs were eye-related and were more frequent in study NGF0214 both for patients on vehicle and rhNGF (study NGF0212: 30.8% versus 25% of patients on vehicle and rhNGF, respectively; study rhNGF0214: 58.3% versus 78.3% of patients on vehicle+methionine and rhNGF+methionine, respectively). They included mainly eye pain (the most common), reduced visual acuity, increased lacrimation and ocular hyperaemia. Corneal epithelium defect, cataracts, ocular discomfort, foreign body sensation, photophobia.
and hyperaemia were also recorded by more than 8% of patients (although notably, this corresponds to only 2 patients in study NGF0214). Disease progression was reported mainly in the groups receiving vehicle. Headache was also commonly reported.

Systemic AEs were very rare in the clinical trial, and those which did occur in patients being treated with rhNGF (neutropenia, arrhythmia, blood pressure increased, joint swelling, and paresthesia) were considered unlikely to be related to the study medication, given the very limited systemic absorption of rhNGF.

In the Phase II segment period of study NGF0212 during the controlled treatment phase, 19 patients (18.3%) reported treatment-related AEs. Eye pain was the most frequently reported treatment-related AE, occurring in 6 patients (5.8%) overall: 4 patients (7.7%) in the rhNGF 20 μg/ml group and 2 patients (3.8%) in the vehicle control group. Other frequently reported treatment-related AEs included blepharitis, corneal neovascularization, eye pruritus and headache each reported by 2 patients (1.9%), one patient in each of the treatment groups. In addition, the events instillation site pruritus and erythema of eyelid (considered symptoms of blepharitis) were reported in one patient (0.8%) each. Disease progression was reported by 2 patients (3.8%) in the in the vehicle control group. In the uncontrolled treatment phase, overall, 2 patients (15.4%) reported 6 treatment-related AEs. Of the 13 patients receiving rhNGF 20 μg/ml, two patients experienced a treatment related event of blepharitis, conjunctival hyperaemia, erythema of the eyelid, eye discharge, eye irritation and eye pain (7.7% each).

Eighteen patients (18/47 = 38.3%) reported treatment-related AEs in study NGF0214: 10 (10/23 = 43.5%) patients receiving rhNGF and 8 (8/24 = 33.3%) patients receiving vehicle. Again, eye pain was the most frequently reported treatment-related AE, occurring in 4 patients (8.5%) overall: 3 patients (13.0%) in the rhNGF 20 μg/ml group and 1 patient (4.2%) in the vehicle control group. Eye inflammation and the related terms anterior chamber inflammation and hyphaemia, as well as lacrimation increased and the related term eye discharge were each reported once in the rhNGF group (4.3%). Other treatment-related AEs reported for rhNGF included eyelid pain, foreign body sensation (2 patients each), ocular hyperaemia and photophobia (1 patient each). Disease progression was reported by one patient in each treatment group.

**Secondary Safety Pool**

The Secondary Safety Pool encompassed 315 subjects who were treated with any dose of rhNGF. The pool included non-NK subjects treated for longer period of time (up to 24 months) and at higher doses than that proposed for the treatment of NK.

Most of the AEs observed were ocular events; subjects with ocular events accounted for nearly three quarters (73.9%) of subjects reporting AEs and ocular events accounted for two thirds of AEs (66.2%) reported in the rhNGF groups. The types of ocular AEs was similar in this pool compared to the Primary Safety Pool, although percentages of AEs were generally lower compared to the Primary Safety Pool. In general, more AEs were reported in subjects treated with rhNGF compared to vehicle with slightly higher percentages in patients receiving the recommended dose or higher compared to lower dosages.

**Study NGF0212 comparison of rhNGF 20 μg/ml versus 10 μg/ml**

Data for study NGF0212, in which two different concentrations of rhNGF were tested, showed a trend of a higher frequency of AEs with the higher dose. During the controlled treatment period of Phase II of the study, 70 of the 156 patients (44.9%) experienced at least 1 AE: 23 patients (44.2%) in the rhNGF 10 μg/ml group, 27 patients (51.9%) in the rhNGF 20 μg/ml group, and 20 patients (38.5%) in the vehicle control group. In total, 16% patients in this Phase had a treatment-related AE, with only 10.9% experiencing a serious AE and 9.6% discontinuing due to an AE.
Eye pain was the most frequently reported AE, occurring in a total of 11 patients (7.1%): 2 patients in the rhNGF 10 μg/ml group, 5 patients in the rhNGF 20 μg/ml group, and 4 patients in the vehicle control group. Other frequently reported AEs included disease progression occurring in a total of 10 patients (6.4%): 2 patients each in the 10 μg/ml and 20 μg/ml groups and 6 patients in the vehicle control group; reduced visual acuity occurring in a total of 7 patients (4.5%); 2 patients in the 10 μg/ml group, 3 patients in the rhNGF 20 μg/ml group and 2 patients in the vehicle control group and headache, occurring in a total of 6 patients (3.8%); 2 patients in each treatment group.

Long-term safety (Follow-up)

- Study NGF0212

Overall, 54 patients (34.6%) experienced at least 1 AE during the follow-up period: 22 patients (35.5%) in the 10 μg/ml group, 21 patients (32.3%) in the rhNGF 20 μg/ml group and 11 patients (37.9%) in the vehicle control group. Overall, a total of 163 AEs were reported during the follow-up period: 77 AEs were reported in 22 patients in the 10 μg/ml group, 65 AEs were reported in 21 patients in the rhNGF 20 μg/ml group, and 21 AEs were reported in 11 patients in the vehicle control group. Overall, 2 patients (1.3%), 1 patient each in the 10 μg/ml group and the vehicle control group reported at least 1 AE during the follow-up period that was considered by the Investigator to be related to study treatment.

Amongst the 13 patients who received an additional cycle of treatment with rhNGF during the follow-up period, 7 subjects experienced ocular TEAEs during the treatment cycle or in close temporal relationship with the treatment. In particular, 4 patients had a total of 10 ocular TEAEs with an onset date falling during re-treatment: 1 patient with ophthalmic herpes, conjunctival hemorrhage, and conjunctivitis, 1 patient with corneal erosion, keratitis, keratitis herpetic, and belpharitis, 1 patient with increased IOP (recovered the same day without treatment), 1 patient with dry eye and eyelid pain.

- Study NGF0214

A total of 23 (48.9%) patients reported 68 AE in the follow-up period: 14 (60.9%) patients randomized to rhNGF reported 47 AE and 9 (24.4) patients randomized to vehicle reported 21 AE. The majority of the AEs were of mild severity: 9 (19.1%) subjects reported 38 AEs of mild severity, 9 (19.1%) subjects reported 25 AEs of moderate severity, and 5 (10.6%) subjects reported 5 AEs of severe severity.

The most frequently reported AEs were eye disorders, with 15 (31.9%) patients reporting 41 AE from this MedDRA SOC, 9 (39.1) patients randomized to treatment with rhNGF reporting 9 AE, and 6 (25.0%) patients randomized to treatment with vehicle reporting 12 AE.

Five patients underwent a second course of treatment. Of these, 4 patients experienced 16 AEs. However, all 4 patients had a complicated ocular history (several ocular comorbidities including corneal dystrophies, surgery and glaucoma) that predisposed them to ocular AEs. In spite of this, the AEs reported during retreatment were very limited. Specifically, the most common AE was eye pain (in 3 patients). The only other events were a recurrence of herpes infection, a case of disease progression, a case of cataract (in a patient who already had a history of cataract), and isolated cases of AEs related to eye irritation (ocular discomfort and hyperaemia).

**Serious adverse event/deaths/other significant events**

A total of 9 deaths occurred during the clinical development of rhNGF in NK patients although none was considered related to treatment.
Serious adverse events (SAE) were mainly eye-related. The majority were reported as mild or moderate and transient in nature. Fourteen patients (13.5%) experienced a SAE in the Phase II segment of study NGF0212 during the controlled treatment period: 9 patients (17.3%) in the rhNGF 20 µg/ml group, and 5 patients (9.6%) in the vehicle control group. In study NGF0214, 8 SAEs were reported in 7 patients (14.9%): 3 patients (13.0%) in the rhNGF 20 µg/ml group, and 4 patients (16.7%) in the vehicle control group. No clear difference between patients receiving the methionine-containing formulation (plus vehicle or rhNGF) and those receiving the methionine-free formulation.

Disease progression was reported as a SAE and was observed in 3.8% of patients in study NGF0212 and 6.4% in study NGF0214. In NGF0214, the percentage of patients reporting disease progression was higher for those receiving vehicle (8.3% versus 4.3%). Furthermore, 2 patients in study NGF0212 (one each in the rhNGF 20 µg/ml and the vehicle group) experienced the SAE reduced visual acuity. All other SAEs occurred in 1 patient each.

During the follow-up period of the Phase II segment of study NGF0212, 22 patients (14.1%) overall experienced an SAE: 10 patients (16.1%) in the rhNGF 10 µg/ml group, 8 patients (12.3%) in the rhNGF 20 µg/ml group, and 4 patients (13.8%) in the vehicle control group. None of the SAEs reported during the follow-up period were considered related to study treatment. Corneal opacity and respiratory failure occurred in 2 patients each (1.3%). All other SAEs occurred in 1 patient each. In study NGF0214, 7 patients (7/47=14.9%) reported 8 SAE during the follow-up period.

**Laboratory findings**

Laboratory findings and vital signs were only evaluated in the Primary Safety Pool. No notable trends or clinically significant changes over time or between treatment groups were observed in haematology and serum chemistry parameters during rhNGF treatment. No clinically significant changes from Baseline or notable differences between treatment groups were observed for any vital signs measurements for any patients in the Safety Pool.

No anti-drug antibodies were detected at any time point for all patients in any of the Safety Pools.

Ocular tolerability, intraocular pressure and dilated fundus ophthalmoscopy were considered to be AEs of special interest. In general no relevant changes were seen during the clinical trials.

**Safety in special populations**

Safety has not been specifically studied in special populations e.g. patients with renal or hepatic insufficiency.

No data on the use of the medicinal product in children were available.

A total of 104 elderly patients (≥65 years of age) were included in the NK clinical trial program. Out of these, 77 were exposed to at least one cycle of active treatment (48 to rhNGF 20 µg/ml and 29 to rhNGF 10 µg/ml). Overall, the proportions of elderly patients experiencing adverse drug reactions as well as SAEs were similar to what was reported in the general NK patient population. No serious related adverse reaction was reported. Fatal cases reported during the study were not considered related to study treatment.

**Safety related to drug-drug interactions and other interactions**

No interaction studies with other medicinal products have been performed.
In study NGF0212 a total of 81 patients used concomitant ocular medications in the controlled treatment period and 69 of them reported at least one adverse event (most being mild-to-moderate and transient): 21/26 (81%) patients in the rhNGF 10 µg/ml, 27/30 (90%) patients in the rhNGF 20 µg/ml group, and 21/25 (84%) patients in the vehicle group. In the rhNGF 10 µg/ml group a total of 69 AEs was reported, as compared to 120 in the rhNGF 20 µg/ml group and 102 in the vehicle group. Amongst the ocular AEs, 8/48 (17%) in the rhNGF 10 µg/ml group, 15/71 (21%) in the rhNGF 20 µg/ml group, and 28/77 (36%) in the vehicle group were considered possibly related to study drug.

An overview of the safety data in patients with concomitant medication in study NGF0214 is provided in the below table.

**Table 20 - Safety Population NGF0214 - Patients without concomitant ocular medication during controlled treatment phase**

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>rhNGF 20 µg/ml</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Adverse Events</td>
<td>10 (91%)</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Adverse Events Leading to Withdrawal of Study Treatment</td>
<td>2 (18%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Adverse Events Leading to Study Discontinuation</td>
<td>2 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Treatment-Related Adverse Events</td>
<td>6 (54%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>Any Serious Adverse Events</td>
<td>1 (9%)</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Adverse Event of Special Interest</td>
<td>1 (9%)</td>
<td>1</td>
</tr>
<tr>
<td>Adverse Events by Worst Severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Mild</td>
<td>4 (36%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>- Moderate</td>
<td>3 (27%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>- Severe</td>
<td>3 (27%)</td>
<td>2 (17%)</td>
</tr>
</tbody>
</table>

**Discontinuation due to adverse events**

In the Primary Safety Pool, overall 13/104 (12.5%) patients in study NGF0212 and 12/47 (25.5%) patients in study NGF0214 discontinued study drug due to an AE occurring during the controlled treatment period. “Eye disorders” were the primary cause of treatment discontinuation in studies NGF0212 and NGF0214 (8.7% and 14.9%, respectively), followed by "disease progression" (4.8% and 10.6%) that was more frequent in those patients treated with vehicle compared to rhNGF 20 µg/ml (5.8% versus 3.8% and 12.5% versus 8.7%, respectively).

In the Secondary Safety Pool, a total of 12 AEs led to discontinuation of the study in 8 patients allocated to any dose of rhNGF. Ten AEs in 6 patients led to discontinuation during treatment with the recommended dosage of rhNGF. A total of 4 AEs in 4 patients in vehicle groups led to discontinuation. The rates of patients discontinuing study drug because of AEs was slightly lower with rhNGF than with vehicle (2.5-3.6% vs 3.8%).

During the follow-up period of study NGF0212, 4 patients (2.6%) reported at least 1 AE that led to discontinuation of study treatment during the follow-up period: 1 patient in the rhNGF 10 µg/ml group, 2 patients in the rhNGF 20 µg/ml group, and 1 patient in the vehicle control group. During the follow-up of study NGF0214, 1 (2.1%) patients randomized to vehicle reported 1 AE that led to study discontinuation.

**Post marketing experience**

The product had not been marketed by the time of this report.
2.6.1. Discussion on clinical safety

The main basis for the safety assessment was derived from the Primary Safety Pool, including data from both pivotal trials NGF0212 (European subjects, L-methionine-free formulation) and NGF0214 (US subjects, L-methionine-containing formulation) in NK patients. This included a comparison of the safety of methionine-free and –containing eye drops, the latter of which was proposed for commercial use. While methionine is a very common food ingredient with no reported toxicity issues related to its use, it has scarcely been used so far in ophthalmological preparations.

The Secondary Safety Pool, including data from all 5 studies with rhNGF including the 2 studies in NK patients, one trial in healthy volunteers, one in moderate-severe dry eye disease and one in retinitis pigmentosa, was considered supportive. Furthermore, both a comparison of safety data for methionine-free and methionine-containing eye drops formulations (i.e. study NGF0212 versus NGF0214) as part of the Primary Safety Pool and for rhNGF doses (10 µg/ml versus 20 µg/ml) from study NGF0212 were performed.

In general, the applicant’s approach for the safety assessment was considered acceptable by the CHMP.

The total number of subjects exposed to any concentration of rhNGF was 315, including 177 NK patients. Of these, only 82 NK patients (59 in study NGF0212 and 23 in study NGF0214) were treated with rhNGF 20 µg/ml, 6 drops per day in the affected eye, as proposed for commercial use. Furthermore, only 23 patients from study NGF0214 were treated with the proposed formulation containing methionine as an excipient. Additional data were available from patients initially assigned to vehicle who did not heal during the 8 weeks controlled treatment period and therefore received rhNGF during Week 8-16 (23 and 13 additional patients from study NGF0212 and NGF0214). Treatment duration was also limited with 8 weeks for NK patients. For subjects included in other clinical trials in other indications treatment duration ranged from 4 to 24 weeks.

The small number of exposed patients, in particular those receiving the formulation intended for commercial use (rhNGF 20 µg/ml methionine-containing eye drops) and duration of exposure impeded an accurate safety assessment, whereby almost all AEs will be frequent or very frequent despite being reported in very few patients and, in addition, less frequent AEs may not be reliably detected. However, given the low prevalence of NK, the drug exposure was considered acceptable by the CHMP for the purpose of the short-term safety assessment of rhNGF. With regards to long-term safety however, the CHMP was of the view that the data included in the Safety Pools (database lock after 3 months and 4 weeks follow-up) were too limited. In response to this concern, the applicant provided full long term results for both clinical trials for the entire follow-up periods (12 months for study NGF0212 and 6 months for study NGF0214, see discussion below).

The most common AEs in the Safety Pools were eye-related, mainly eye pain, reduced visual acuity, increased lacrimation and ocular hyperaemia. Corneal epithelium defect, cataracts, ocular discomfort, foreign body sensation, photophobia and hyperaemia were also recorded by more than 8% of patients. Although the applicant states that the reporting of ocular surface symptoms could be sign of corneal re-innervation during the re-epithelialization period, many of the observed AEs affect sites outside the cornea, such as the conjunctiva. In addition, in the efficacy assessment, corneal sensitivity did not significantly improve during the trials. Conjunctival hyperaemia and photophobia had already been reported in a previous study in which topical murine NGF had been evaluated. Therefore, a causal relationship with the use of Oxervate could not be ruled out per se.

There were 9 deaths during the clinical development in NK patients. None was considered related to treatment. Furthermore, only few SAEs were reported (14 [13.5%] patients in study NGF0212 and 7 (14.9%)
patients in study NGF0214). Most of SAE were eye-related, and mild or moderate and transient in nature. This was altogether reassuring although the limited number of patients exposed to rhNGF prevented any firm conclusions.

Eye disorders was the first cause of treatment discontinuation in studies NGF0212 and NGF0214 (8.7% and 14.9%, respectively) followed by disease progression (4.8% and 10.6%) the latter being more frequent in patients treated with vehicle versus rhNGF (5.8% versus 3.8% and 12.5% versus 8.7%, for studies NGF0212 and NGF0214, respectively).

Safety data from study NGF0212, in which two different concentrations of rhNGF were tested, suggested a dose-AEs relationship (for 10 and 20 µg/ml). The applicant argued that most of the AEs observed were single episodes as well as transient and non-serious in nature. More frequently reported events were ocular surface symptoms such as eye pain (or discomfort such as increased lacrimation and photophobia) and were considered by the applicant a result of the healing process. While the latter argument was considered speculative in the absence of more solid evidence, the CHMP overall agreed that from a safety perspective the choice of the 20 µg/ml dose could be accepted.

When comparing study NGF0212 and NGF0214 within the Primary Safety Pool analysis, the data suggested a worse safety profile for patients receiving methionine-containing eye drops (study NGF0214) compared to the methionine-free formulation (study NGF0212). Differences in the incidence of AEs (percentage of patients with at least 1 AE) were observed both for vehicle plus methionine versus vehicle (75% versus 38.5%) and for rhNGF plus methionine versus rhNGF (91.3% versus 51.9%). Furthermore, the percentage of patients discontinuing was also higher in study NGF0214 compared to study NGF0212. The applicant presented several arguments including that US patients and physicians involved in study NGF0214 tend to report AEs more frequently than Europeans, that no significant differences between SAEs were observed in patients receiving methionine-containing eye drops and those receiving methionine-free formulation and none of the SAEs was considered related to study drug. Reference was also made to the experience gained with an ophthalmic product registered since 1956 in Spain for the treatment of infections after extraction of foreign bodies or corneal erosions, which contains methionine at a much higher concentration (5mg/g) than in Oxervate. While uncertainties remained in relation to the tolerability of methionine as an excipient, the CHMP was of the view that the safety profile of the methionine-containing formulation was overall acceptable. However, the safety information in SmPC section 4.8 should reflect the higher AE frequencies observed in study NGF0214.

For the purpose of labelling in the SmPC, the applicant applied the following criteria:

- incidence had to be higher (even by only one event) in the patients treated with rhNGF than with placebo;
- the events were not to be closely related to the underlying disease;
- isolated ADRs were not considered if they were justified by the presence of concomitant diseases already listed in the medical history of the patient at baseline;
- isolated systemic events since rhNGF is not absorbed into the bloodstream were excluded.

For the frequency calculation, all NK patients exposed to rhNGF 20 µg/ml (including the controlled and uncontrolled treatment periods as well as unscheduled treatment). As a result, the most commonly reported adverse reactions listed in the SmPC were eye pain (11.1 %), eye inflammation (8.3 %), lacrimation increased (5.6 %), eyelid pain (5.6 %) and foreign body sensation in the eye (5.6 %).
Safety data for the complete follow-up period of studies NGF0212 and NGF0214 included 18 patients who received a second treatment with rhNGF due to recurrence of PED or ulcer during the follow-up. Overall, these long-term data showed no new or unexpected AEs compared to the short term safety profile, including for the methionine-containing formulation. Nevertheless, long-term safety data remained limited. In this context, the CHMP appreciated the applicant’s plans for an additional clinical study NGF0215 which will help enrich the safety database with long-term data for the commercial formulation.

Finally, given that Oxervate is not systemically absorbed, no impact on laboratory measurements or vital signs was expected, and none was observed during the clinical trials. Anti-drug antibodies were not detected in any studies.

For the same reason, no interaction studies with systemic medicinal products were required. However, patients are expected to receive concomitant topical ocular medications in real life. In fact, a relevant subset of the study population received concomitant ocular treatment during the studies. While common clinical practice includes discontinuation of pre-existing topical medications for NK patients to reduce the risk of drug-induced corneal toxicity, at the same time, depending on the local practice, concomitant medication may be applied to prevent corneal infections in the presence of corneal ulceration. With respect to the risk of intraocular PK or PD interactions, due to the mechanism of action and the characteristics of the metabolism of rhNGF, the risk for interactions could be expected to be low (see also sections 2.3. and 2.4.). Therefore and since a comparative analysis of AEs in patients with and without concomitant medication in the clinical trials had not revealed relevant differences, the CHMP considered the lack of interaction studies acceptable.

Absence of studies in special populations including patients with renal or hepatic insufficiency as well as in pregnant and lactating women was considered acceptable since the product is not systemically absorbed. Oxervate is only intended for use in adult NK patients. A Paediatric Investigational Plan (PIP) deferral was granted for all paediatric age groups. The PIP considered that safety in children could be inferred by considering data in adults together with the results of juvenile animal toxicology studies in the rat and rabbit. With regards to the elderly, just slightly less than half of the patients who participated in studies NGF0212 and NGF0214 were over 65 years of age. The available data supported a similar safety profile compared to the overall population.

### 2.6.2. Conclusions on the clinical safety

Despite the limited size of the safety database both in terms of the number of exposed patients and the duration of the exposure, and inherent difficulties in interpretation of the safety data, the CHMP considered the available data to be sufficient to support the present application of Oxervate for use in the treatment of moderate to severe NK in adult patients with regards to clinical safety. Adverse events were mostly mild to moderate ocular events including eye pain, increased lacrimation and ocular hyperaemia. Based on the available short-term and long-term data, the safety profiles of both the methionine-free and the methionine-containing formulations were considered acceptable, although some uncertainties on the tolerability of this excipient remained. The CHMP recommended that the applicant pursued the plans for an additional study (NGF0215) to generate further long-term data with the commercial, methionine-containing formulation, to help address the remaining uncertainties with the use of Oxervate including tolerability of the excipient methionine.
2.7. Risk Management Plan

Safety concerns

Table 21 Summary of the Safety Concerns

<table>
<thead>
<tr>
<th>Important identified risks</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Important potential risks</td>
<td>Serious corneal disorders</td>
</tr>
<tr>
<td>Missing information</td>
<td></td>
</tr>
<tr>
<td>Use in patients with active ocular cancer</td>
<td></td>
</tr>
<tr>
<td>Use in patients with active eye infections</td>
<td></td>
</tr>
<tr>
<td>Use in patients with corneal melting or impending perforation requiring immediate surgery</td>
<td></td>
</tr>
<tr>
<td>Concomitant use with topical ophthalmic products that impair the healing process including corticosteroids and eye drops containing preservatives such as benzalkonium chloride polyquaternium-1, benzododecinium bromide, cetrimide and other quaternary ammonium derivatives</td>
<td></td>
</tr>
<tr>
<td>Off label use</td>
<td></td>
</tr>
<tr>
<td>Use with contact lenses</td>
<td></td>
</tr>
<tr>
<td>Long-term safety data</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacovigilance plan

Not applicable
**Table 22 Summary Table of the Risk Minimisation Measures**

<table>
<thead>
<tr>
<th>Safety concerns</th>
<th>Routine RMMs</th>
<th>Additional RMMs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Important identified risks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td><strong>Important potential risks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious corneal disorders</td>
<td>Wording in SmPC section 4.4&lt;br&gt;Prescription only medicine&lt;br&gt;Use restricted to an ophthalmologist or a healthcare professional (HCP) qualified in ophthalmology</td>
<td>Not applicable</td>
</tr>
<tr>
<td><strong>Missing information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use in patients with active ocular cancer</td>
<td>Wording in SmPC section 4.4&lt;br&gt;Prescription only medicine&lt;br&gt;Use restricted to an ophthalmologist or a healthcare professional (HCP) qualified in ophthalmology</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Use in patients with active eye infections</td>
<td>Wording in SmPC section 4.2, 4.4&lt;br&gt;Prescription only medicine&lt;br&gt;Use restricted to an ophthalmologist or a healthcare professional (HCP) qualified in ophthalmology</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Use in patients with corneal melting or impending perforation requiring immediate surgery</td>
<td>Wording in SmPC section 4.4&lt;br&gt;Prescription only medicine&lt;br&gt;Use restricted to an ophthalmologist or a healthcare professional (HCP) qualified in ophthalmology</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Concomitant use with topical ophthalmic products that impair the healing process including corticosteroids and eye drops containing preservatives such as</td>
<td>Wording in SmPC section 4.4, 4.5&lt;br&gt;Prescription only medicine&lt;br&gt;Use restricted to an ophthalmologist or a</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Safety concerns</td>
<td>Routine RMMs</td>
<td>Additional RMMs</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>benzalkonium chloride, polyquaternium-1, benzododecinium bromide, cetrimide and other quaternary ammonium derivatives</td>
<td>healthcare professional (HCP) qualified in ophthalmology</td>
<td></td>
</tr>
<tr>
<td>Off label use</td>
<td>Wording in SmPC section 4.1</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Use with contact lenses</td>
<td>Wording in SmPC section 4.2 4.4</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Long-term safety data</td>
<td>Wording in SmPC section 5.1</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

**Conclusion**

The CHMP and PRAC considered that the risk management plan (RMP) version 1.0 (dated 16 May 2017) is acceptable.

**2.8. Pharmacovigilance**

**Pharmacovigilance system**

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

**Periodic Safety Update Reports submission requirements**

The requirements for submission of periodic safety update reports for this medicinal product are set out in
the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. **New Active Substance**

The applicant declared that cenegermin has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers cenegermin to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. **Product information**

2.10.1. **User consultation**

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use*.

2.10.2. **Additional monitoring**

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, OXERVATE (cenegermin) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore the SmPC and the PL include a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.
3. **Benefit-Risk Balance**

3.1. **Therapeutic Context**

3.1.1. **Disease or condition**

NK is a rare degenerative corneal disease that originates from an impairment of corneal trigeminal innervation and reduction in or loss of corneal sensitivity. Trophic changes within the cornea occur and can lead to recurrent or PED with poor tendency of spontaneous healing. NK can result in severe visual impairment and the progression of the disease may lead to corneal ulcers, melting, and perforation.

Therapy of NK aims at preventing progression of corneal damage and to promote epithelial healing.

Oxervate (rhNGF or cenegermin) is intended for treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) NK in adults.

3.1.2. **Available therapies and unmet medical need**

NK is a rare disease with numerous and heterogeneous underlying causes. Based on the prevalence of a range of predisposing or accompanying conditions, NK prevalence can be estimated at less than 4.1 per 10,000 patients. Moderate and severe disease (stage 2 and 3 according to the Mackie classification) are estimated to constitute approximately one third of the total NK population.

The use of preservative-free artificial tears may help to improve the corneal surface integrity at all disease stages. Use of topical antibiotic eye drops to prevent infection is recommended at NK stages 2 and 3. Experimental use of blood-derived eye drops (autologous serum, cord blood serum, and platelet rich plasma) has also been reported in some cases. Non-pharmacological treatments for more severe NK include therapeutic corneal or scleral contact lenses. Furthermore botulinum A toxin injection of the eyelid elevator muscle, and surgical treatments such as tarsorrhaphy, conjunctival flap or amniotic membrane transplantation as well as palpebral springs are used to cover the ulcer and preserve the anatomical integrity of the eye in more advanced cases. However, these measures adversely affect visual function and have a poor cosmetic outcome.

At the time of this report, no pharmaceutical treatments had been authorised for NK patients who are refractory to conventional non-surgical NK therapies and hence progressed to stage 2 and 3.

3.1.3. **Main clinical studies**

Efficacy of rhNFG (cenegermin) in the treatment of NK was investigated in two pivotal double-masked, randomized, multi-centre, vehicle-controlled, parallel group Phase II studies: NGF0212 and NGF0214.

While study NGF0212 was conducted in the EU and investigated 2 different strength of rhNGF (10 and 20µg/ml) methionine-free eye-drop formulation, study NGF0214 was performed at sites in the US using 20µg/ml rhNGF methionine-containing eye-drops intended for commercial purposes. Both studies recruited adult patients with moderate and severe clinical stage of NK, i.e. those with persistent epithelial defects (stage 2) or with corneal ulcer (stage 3) and documented decreased corneal sensitivity.
In study NGF0212, a total of 156 patients were randomized compared to 48 patients in study NGF0214. Patients initially entered an 8-week double-blind controlled period and thereafter a follow-up period of 48/56 weeks (NGF0212) and 24/32 weeks (NGF0214), respectively. During the 8-week double-blind period, patients received a single drop of rhNGF eye drops or vehicle in the affected eye 6 times a day and were monitored for healing or deterioration of the corneal defect, as well as functional outcomes including improvement in visual acuity and corneal sensitivity. Patients in the vehicle arm who were not healed after the initial 8-week double-blind phase could receive a course of rhNGF treatment. In case of recurrence in initially healed patients, a second course of treatment with rhNGF could be administered.

### 3.2. Favourable effects

After both 4 and 8 weeks, more patients treated with rhNGF compared to those treated with the vehicle achieved complete corneal healing determined by the central reading centre in the two pivotal trials. In study NGF0212, the cure rates at 8 weeks were 74.5% (38/51), 74% (37/50) and 43.1% (22/51) in the 10 μg/ml rhNGF, 20 μg/ml rhNGF, and the vehicle group, respectively. Both rhNGF doses were statistically superior to vehicle (difference of 31.4% [p<0.001] and 30.9% [p<0.002], respectively), but no differences was obvious between doses. In study NGF0214, 69.6% (16/23) of patients on rhNGF 20 μg/ml reached complete healing, compared to the 29.2% (7/24) of patients receiving vehicle (difference of 40.4% p=0.006).

Although healing rates were generally lower after 4 weeks of treatment, statistically significant results in favour of rhNGF were already achieved at this earlier time point in study NGF0212. Patients on rhNGF 20 μg/ml reached the highest cure rate (58%; 29/50) versus 54.9% of patients receiving 10 μg/ml (28/51). This compares to 19.6% (10/51) of complete healing with vehicle (difference of 38.4% [p<0.001] and 35.3% [p<0.001], respectively). Again, no difference between doses was observed. Study NGF0214 failed to show superiority of rhNGF over vehicle at 4 weeks (secondary endpoint), but a clear numerical advantage was observed (56.5% patients on rhNGF 20 μg/ml versus 35.5% on vehicle, difference 19.0%; p=0.191).

Analyses of the response rates measured by the clinical investigator were in line with the results determined by the reading centre. Robustness of the results was shown in the sensitivity analyses including different ways to handle missing data. Likewise, analyses of complete corneal clearing (i.e. zero staining) as an alternative definition for complete healing yielded results consistent with the primary efficacy analysis. Time to complete healing, although only an exploratory analysis in study NGF0212, also supported a treatment effect of rhNGF, with patients in the active groups being healed earlier than in the vehicle group, with a median time of 29 days (95% CI: 20; 55), 28 days (95% CI: 19; 55), and 56 days (95% CI: 42; not estimable), in the rhNGF 10 μg/ml group, 20 μg/ml group, and vehicle control group, respectively.

A trend towards improvement in visual acuity over time was shown in both studies irrespective of treatment (active and vehicle). In study NGF0212 at 8 weeks, the mean improvement in BCDVA was 15.8 letters (rhNGF 10 μg/ml) and 11.9 letters (rhNGF 20 μg/ml) versus 6.9 letters in the vehicle group. The differences with respect to vehicle were 8.9 letters and 5.0 letters, respectively. Only the difference between rhNGF 10 μg/ml and vehicle was statistically significant (p=0.022). In study NGF0214, no difference between treatment arms was observed; mean changes from baseline were 4.48 letters (LS mean change: 5.0 letters) and 4.33 letters (LS mean change: 3.9 letters) in patients receiving rhNGF and vehicle, respectively.

Deterioration defined as an increase in lesion size ≥ 1mm, decrease in BCDVA by >5 letters, progression in lesion depth to corneal melting or perforation or onset of infection, occurred only in few patient and numerically more often in patients on vehicle than on rhNGF.
Results from the follow-up period of study NGF0212 were reassuring, showing that for more than 80% of patients healed at Week 8/16 after treatment with rhNGF (regardless of the dose), the corneal defects remained healed during the 48-week follow-up. A positive impact of active treatment on visual acuity and at least a non detrimental effect on corneal sensitivity was also observed.

Finally, both rhNGF doses tested in study NGF0212 appeared to have similar efficacy profiles and a clinical benefit was evident for either dose. The choice of the higher dose to be marketed was justified by the applicant based on a trend towards a clinically significant improvement in efficacy for the higher dose observed in some endpoints, including an early improvement in corneal healing, an increase in the number of patients with zero residual corneal staining as well as a later recurrence after corneal healing. However, the opposite was true for other endpoints and no clear efficacy advantage of rhNGF 20 μg/ml over 10 μg/ml was obvious from the available data.

3.3. Uncertainties and limitations about favourable effects

In addition to restoring ocular surface integrity, a favourable effect in functional outcomes would be a relevant treatment objective in NK. While visual acuity improved over time in all treatment groups in both studies, the difference between rhNGF and vehicle was not statistically significant for the vast majority of analyses conducted. Similarly, there was a trend towards improvement in corneal sensitivity during the course of both studies regardless of whether patients received rhNGF or vehicle with no clinically relevant or statistically significant differences between the groups. A possible explanation might be that residual corneal damage not completely healed at the time of assessment delayed or restricted improvements of functional outcomes. However, this theory was not supported by actual data. Whether further functional improvements could be achieved with longer or repeated courses of rhNGF treatment was unclear at the time of this report.

The main support for efficacy of Oxervate was derived from data after a single course of 8 weeks treatment with rhNGF. Only very limited data for repeated treatment courses in case of recurrences was available (18 patients) and only safety data were reported for these patients. Also, while the available data suggested that patients continued to improve over time while on treatment (e.g. in study NGF0212, the corneal defect had healed in 30 additional patients by Week 8 compared to Week 4), an extended treatment period beyond 8 weeks was not investigated. Thus, whether at least some patients could obtain further benefit when continuing treatment beyond 8 weeks is currently not known, but additional data are expected form the clinical trial NGF0215, which the applicant plans to conduct post-approval.

A higher than expected number of patients withdrew from both studies (approximately 30% by week 8). Uncertainties as to the impact of the low retention rate on the study outcome were however considered to have been adequately addressed by additional analyses conducted by the applicant including analyses based on observed cases, as well as imputing missing data as failures or using multiple imputation method, all of which showed results consistent with the primary analysis (LOCF).

Furthermore, the proportion of subjects who experienced corneal healing in the control arms (43 and 29 % in NGF0212 and NGF0214, respectively) was rather large considering that subjects recruited in the studies were patients with a long duration of NK with persistent epithelial defects and ulcers. However, the high vehicle healing rate was in fact expected and can be explained by the provision of high standard of care and the close monitoring of patients during the studies.

Finally, both studies included patients with different disease severity (stage 2 and 3) and a wide spectrum of aetiologies as cause of NK. While these factors could be expected to have an impact on the treatment
response to rhNGF, subgroup analyses for stage 2 and 3 disease and by main NK aetiologies showed no relevant differences in cure rates.

3.4. Unfavourable effects

The most common adverse reactions observed with Oxervate during the clinical trials program were eye-related and included eye pain (11.1 %), eye inflammation (8.3 %), lacrimation increased (5.6 %), eyelid pain (5.6 %) and foreign body sensation in the eye (5.6 %). In addition to being local, most events were reported only once and were transient and non-serious in nature. Few SAEs were reported and most of these were eye-related, mild or moderate and transient.

Long term safety data (12 and 6 months follow-up period of study NGF2012 and NGF0214, respectively) including 18 patients receiving a 2nd course of treatment with rhNGF due to recurrence of PED or corneal ulcer, did not reveal any new or unexpected AEs.

3.5. Uncertainties and limitations about unfavourable effects

During the clinical development of Oxervate, the rhNGF formulation was changed and L-methionine was added as an antioxidant to increase the stability of the product. Experience with this excipient is limited in ophthalmological products. Only patients in study NGF0214 received the L-methionine formulation to be marketed and thus exposure was rather limited (see below). When comparing data from study NGF0212 (methionine-free formulation) and study NGF0214, it emerged that AEs were more frequent in study NGF0214. This applied to patient receiving vehicle in either study including patients with at least 1 AE (75% versus 38.5%), patients with at least 1 serious AE (16.7% versus 9.6%), patients with at least 1 AE leading to discontinuation (29.2% versus 7.7%) and patients with at least 1 AE in relation to the study drug (33.3% versus 19.2%). Similarly, for patients on rhNGF plus methionine higher percentages of patients with at least 1 AE (91.3% versus 51.9%) and with at least 1 AE in relation to the study drug (43.5% versus 17.3%) were observed compared to those receiving a methionine-free rhNGF formulation. However, overall the CHMP considered the safety profile of the methionine-containing formulation acceptable and agreed to its use provided the safety information in SmPC section 4.8 reflects the higher AE frequencies observed in NGF0214.

The total number of subjects exposed to any concentration of rhNGF was 315. However, amongst these were only 75 stage 2 or 3 NK patients treated with the concentration proposed for approval (rhNGF 20 µg/ml, 6 times a day in the affected eye). Furthermore, only 23 patients from study NGF0214 were treated with the proposed formulation for marketing containing L-methionine as an excipient. The duration of exposure was 8 weeks in NK patients, whereby 18 subjects received a 2nd course of rhNGF treatment during the follow-up period of studies NGF0212 and 0214. The small number of NK patients, in particular those receiving the formulation intended for commercial use (rhNGF 20 µg/ml methionine-containing eye drops) and the limited duration of exposure, precluded the detection of rare events and realistic frequency estimations. However, given the low prevalence of NK, the drug exposure was considered acceptable by the CHMP.

Finally, safety data for the dose-ranging study NGF0212 showed an apparent dose-AEs relationship: 20 patients (38.5%) in the vehicle control group, 23 patients (44.2%) in the rhNGF 10 µg/ml group, and 27 patients (51.9%) in the rhNGF 20 µg/ml group experienced at least 1 AE. However, given that no major safety issues with the use of Oxervate had been observed (most AEs were local, transient and reported only once), the safety profile of both dose strength were considered acceptable.
3.1. Effects Table

Table 23 – Effects Table for Oxervate for the treatment of moderate to severe NK

<table>
<thead>
<tr>
<th>Effect</th>
<th>Short Description</th>
<th>Unit</th>
<th>rhNGF 20 µg/ml</th>
<th>Vehicle</th>
<th>Uncertainties/ Strength of evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favourable Effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete corneal healing</td>
<td>Percentage of patients experiencing complete healing(^1) of the PED or corneal ulcer as measured by the central reading centre</td>
<td>%</td>
<td>74.0</td>
<td>43.1</td>
<td>Difference active vs placebo (range: 19-38.4 at week 4 and 30.9-40.4 at week 8) was statistical significant for all but week 4 findings in study NGF0214. Consistency with Investigator’s judgement, sensitivity analyses, and alternative definition of corneal healing (zero staining). At Week 48 (uncontrolled follow-up), more than 80% of the initially healed patients with a response available remain healed).</td>
<td>CSR of study NGF0212 and NGF0214</td>
</tr>
<tr>
<td></td>
<td>Week 8 - Study NGF0212 - Study NGF0214</td>
<td></td>
<td>69.6</td>
<td>29.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Week 4 - Study NGF0212 - Study NGF0214</td>
<td></td>
<td>58.0</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>56.5</td>
<td>37.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfavourable Effects(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CSR of study NGF0214</td>
</tr>
<tr>
<td>Eye pain</td>
<td>Incidence rates(^3)</td>
<td>n/N</td>
<td>7/23 (30.4)</td>
<td>2/24 (8.3)</td>
<td>A generally higher rate of AEs was observed in study NGF0214 (methionine-containing formulation) compared to study NGF0212 (methionine-free formulation). Only few SAEs were observed.</td>
<td></td>
</tr>
<tr>
<td>Lacrimation increased</td>
<td></td>
<td>n/N</td>
<td>4/23 (17.4)</td>
<td>1/24 (4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular hyperaemia</td>
<td></td>
<td>n/N</td>
<td>4/23 (17.4)</td>
<td>1/24 (4.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Complete healing was defined as the greatest diameter of the corneal fluorescein staining in the area of the PED or corneal ulcer measured at the Baseline visit being less than 0.5 mm.

\(^2\) The limited size of the safety database precludes the detection of rare events and realistic frequency estimations.

\(^3\) Frequency rates refer to the proportion of patients with an event during the controlled treatment period of study NGF0214.

**Abbreviations:** AE=adverse event; BCDVA=Best Correct Distance Visual Acuity; CSR=Clinical Study Report; ETDRS= Early Treatment Diabetic Retinopathy Study; n=number of patients with an event; N=number of patients randomized at Baseline; PED=persistent epithelia defect; SAE=serious adverse event.

**Notes:** Study NGF0212 investigated a methionine free rhNGF eye drop formulation, whereas patients in study NGF0214 received the methionine-containing formulation intended for commercial use.

3.2. Benefit-risk assessment and discussion

3.2.1. Importance of favourable and unfavourable effects

The most relevant beneficial effect observed with Oxervate (rhNGF 20 µg/ml) has been a 30-40% increase in the rate of NK patients with complete healing of their corneal defect after a single course of rhNGF treatment.
for 8 weeks compared to vehicle. This finding represents a clear benefit for the target population with stage 2 or 3 disease in whom the main treatment objective is to prevent disease progression to corneal perforation and potentially permanent loss of vision. This is of special interest for refractory forms of the disease in which the therapeutic approach includes surgical procedures with poor cosmetic outcome and negative impact on visual function. Efficacy of rhNGF with regards to corneal healing has been robustly demonstrated with a statistically significant difference compared to vehicle observed in 2 pivotal clinical trials including L-methionine-free and – containing rhNGF formulations, respectively, and across multiple endpoints using different definitions for corneal improvements. The effect on healing of the cornea is also supported by a reduced risk of deterioration reported in the studies. In addition, results from the follow-up period of up to 1 year were reassuring. The majority of patients who were healed after 8 weeks of treatment with rhNGF remained healed during the 48-week follow-up. Furthermore, a favourable trend on visual acuity and at least a non detrimental effect on corneal sensitivity were seen.

Adverse reactions were mainly ocular and transient in nature. They included eye pain, increased lacrimation and conjunctival hyperaemia. Safety data obtained with the L-methionine containing rhNGF eye drops suggested a higher frequency of AEs compared to the methionine–free formulation, but no new safety issues were detected. Given that Oxervate is not systemically absorbed, no systemic AEs were expected and those reported did not seem related to the drug. Long-term data did not reveal additional safety issues.

The main drawback with regards to the safety assessment was the small number of NK patients exposed to Oxervate in particular when considering the proposed commercial formulation of 20 μg/ml rhNGF including L-methionine as excipient. This made the interpretation of the safety data difficult as almost all AEs appear frequent or very frequent despite only been reported in very few patients. At the same time, rare events cannot be reliably detected either. However, given the low prevalence of NK, the limited drug exposure was considered acceptable by the CHMP.

### 3.2.2. Balance of benefits and risks

Clinically relevant benefits with the use of Oxervate (rhNGF 20μg/ml) have been robustly demonstrated with an improvement of 30-40% in the healing rate of corneal defects in stage 2 and 3 NK patients. These benefits outweighed the risks of mainly transient ocular adverse reactions, including eye pain, increased lacrimation and conjunctival hyperaemia. Considering all favourable and unfavourable effects, the benefit-risk balance of Oxervate in the treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) neurotrophic keratitis in adults is considered positive.

### 3.2.3. Additional considerations on the benefit-risk balance

Not applicable.

### 3.3. Conclusions

The overall benefit-risk balance of Oxervate is positive.
4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Oxervate is favourable in the following indication:

Treatment of moderate (persistent epithelial defect) or severe (corneal ulcer) neurotrophic keratitis in adults.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that cenegermin is considered to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.