Phase 2 Randomized, Double-Masked, Vehicle-Controlled Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis

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DOI:
10.1016/j.ophtha.2018.02.022

Manuscript Details

Manuscript number	OPHTHA_2017_1439_R2
Title	Phase II Randomized, Double-Masked, Vehicle-Controlled Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis
Short title	Recombinant human nerve growth factor for neurotrophic keratitis
Article type	Full Length Article

Abstract

PURPOSE: To evaluate the safety and efficacy of topical recombinant human nerve growth factor (rhNGF) for treating moderate-to-severe neurotrophic keratitis (NK), a rare degenerative corneal disease resulting from loss of corneal innervation. DESIGN: Phase II multicenter, randomized, double-masked, vehicle-controlled trial. PARTICIPANTS: Patients with stage II (moderate) or stage III (severe) NK in one eye. METHODS: The REPARO Phase II study assessed safety and efficacy in 156 patients randomized 1:1:1 to rhNGF 10 μg/ml, 20 μg/ml, or vehicle. Treatment was administered 6 drops per day for 8 weeks. Patients then entered a 48- or 56-week follow-up period. Safety was assessed in all patients who received study drug, while efficacy was by intention to treat. MAIN OUTCOME MEASURES: Corneal healing (defined as <0.5 mm maximum diameter of fluorescein staining in the lesion area) was assessed in clinical pictures by masked central readers at week 4 (primary efficacy endpoint) and week 8 (key secondary endpoint) of controlled treatment. Corneal healing was also assessed post hoc by masked central readers using a more conservative measure (0 mm staining in the lesion area and no other persistent staining). RESULTS: At week 4 (primary endpoint), 19.6% of vehicle-treated patients achieved corneal healing (<0.5 mm lesion staining), vs. 54.9% receiving rhNGF 10 μg/ml (+35.3%; 97.06% confidence interval [CI] 15.88–54.71; P<0.001) and 58.0% receiving rhNGF 20 μg/ml (+38.4%; 97.06% CI 18.96–57.83; P<0.001). At week 8 (key secondary endpoint), 43.1% of vehicle-treated patients achieved <0.5 mm lesion staining, vs. 74.5% receiving rhNGF 10 μg/ml (+31.4%; 97.06% CI 11.25–51.49; P<0.001) and 74.0% receiving rhNGF 20 μg/ml (+30.9%; 97.06% CI 10.60–51.13, P<0.002). Post hoc analysis of corneal healing by the more conservative measure (0 mm lesion staining and no other persistent staining) maintained statistically significant differences between rhNGF and vehicle at weeks 4 and 8. Over 96% of patients healed after controlled rhNGF treatment remained recurrence-free during follow-up. Treatment with rhNGF was well tolerated; adverse effects were mostly local, mild, and transient. CONCLUSIONS: Topical rhNGF is safe, and more effective than vehicle in promoting healing of moderate-to-severe NK.

Taxonomy	Cornea Ulcers, Neurotrophic Keratopathy, Ulcerative Keratitis
Manuscript category	Manuscript
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Highlights: Précis

In the largest trial conducted in neurotrophic keratitis/keratopathy patients, topical recombinant human nerve growth factor demonstrated statistically significant benefits compared to vehicle for inducing healing of persistent epithelial defects and corneal ulcers.

Phase II Randomized, Double-Masked, Vehicle-Controlled Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis

Running Head: Recombinant human nerve growth factor for neurotrophic keratitis

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Meeting Presentation: Portions of this work have been presented at: the 2017 Association for Research in Vision and Ophthalmology Annual Meeting, May 6–11, 2017, Baltimore, Maryland; and the European Society of Ophthalmology 2017 Congress, June 10–13, 2017, Barcelona, Spain.

Financial Support: Supported by Dompé Farmaceutici SpA. The sponsor participated in the design and conduct of the study; data collection for pharmacokinetics and immunogenicity assessments; management, analysis, and interpretation of the data; and preparation and review of the manuscript. The sponsor was not involved in efficacy data collection for masked central analysis.

Conflicts of Interest/Financial Disclosures: The author(s) have made the following disclosure(s):

S.B.: Licensed intellectual property – Dompé Farmaceutici SpA; A.L.: Consultant/advisor, Licensed intellectual property – Dompé Farmaceutici SpA; P.R.: Scientific Advisory Board, Dompé Farmaceutici SpA; F.S.: Consultant/advisor – Dompé Farmaceutici SpA; Employee – Dompé Farmaceutici SpA; M.A.: Employee – Dompé Farmaceutici SpA; W.C.: Employee – Dompé Farmaceutici SpA; F.M.: Employee – Dompé Farmaceutici SpA.

Abbreviations and Acronyms:

AE = adverse event; BCDVA = best corrected distance visual acuity; CBA = Cochet-Bonnet aesthesiometer; CI = confidence interval; CRO = clinical research organization; ETDRS = Early Treatment Diabetic Retinopathy Study; GCP = Good Clinical Practice; GLP = Good Laboratory Practice; IOP = intraocular pressure; IEC = independent ethics committee; IRB = institutional review board; ITT = intention to treat; LLQ = lower limit of quantification; LOCF = last observation carried forward; LSmean = least squares mean; mNGF = murine nerve growth factor; NGF = nerve growth factor; NK = neurotrophic keratitis; PED = persistent epithelial defect; PK = pharmacokinetics; rhNGF = recombinant human nerve

46 growth factor; SAE = serious adverse event; SE = standard error; TAE = treatment-related

47 adverse event; VAS = visual analogue scale.

This article contains additional online-only material. The following should appear online-only:

Appendix 1 (REPARO study group)

Appendix 2 (eligibility criteria)

Appendix 3 (prior treatments)

Appendix 4 (additional phase II safety results)

Figure 4 (change in Schirmer I from baseline)

Figure 6 (Kaplan-Meier uncontrolled treatment period)

Figure 7 (phase II PK)

Table 5 (corneal sensitivity improvement)

Abstract (350 words)

PURPOSE: To evaluate the safety and efficacy of topical recombinant human nerve growth factor (rhNGF) for treating moderate-to-severe neurotrophic keratitis (NK), a rare degenerative corneal disease resulting from loss of corneal innervation.

DESIGN: Phase II multicenter, randomized, double-masked, vehicle-controlled trial.

PARTICIPANTS: Patients with stage II (moderate) or stage III (severe) NK in one eye.

METHODS: The REPARO Phase II study assessed safety and efficacy in 156 patients randomized 1:1:1 to rhNGF 10 μg/ml, 20 μg/ml, or vehicle. Treatment was administered 6 drops per day for 8 weeks. Patients then entered a 48- or 56-week follow-up period. Safety was assessed in all patients who received study drug, while efficacy was by intention to treat.

MAIN OUTCOME MEASURES: Corneal healing (defined as <0.5 mm maximum diameter of fluorescein staining in the lesion area) was assessed in clinical pictures by masked central readers at week 4 (primary efficacy endpoint) and week 8 (key secondary endpoint) of controlled treatment. Corneal healing was also assessed post hoc by masked central readers using a more conservative measure (0 mm staining in the lesion area and no other persistent staining).

RESULTS: At week 4 (primary endpoint), 19.6% of vehicle-treated patients achieved corneal healing (<0.5 mm lesion staining), vs. 54.9% receiving rhNGF 10 μg/ml (+35.3%; 97.06% confidence interval [CI] 15.88–54.71; P<0.001) and 58.0% receiving rhNGF 20 μg/ml (+38.4%; 97.06% CI 18.96–57.83; P<0.001). At week 8 (key secondary endpoint), 43.1% of vehicle-treated patients achieved <0.5 mm lesion staining, vs. 74.5% receiving rhNGF 10 μg/ml (+31.4%; 97.06% CI 11.25–51.49; P<0.001) and 74.0% receiving rhNGF

20 μg/ml (+30.9%; 97.06% CI 10.60–51.13, P<0.002). Post hoc analysis of corneal healing by the more conservative measure (0 mm lesion staining and no other persistent staining) maintained statistically significant differences between rhNGF and vehicle at weeks 4 and 8. Over 96% of patients healed after controlled rhNGF treatment remained recurrence-free during follow-up. Treatment with rhNGF was well tolerated; adverse effects were mostly local, mild, and transient.

CONCLUSIONS: Topical rhNGF is safe, and more effective than vehicle in promoting healing of moderate-to-severe NK.

INTRODUCTION

With approximately 7000 nerve endings/mm^2, the cornea is the most densely innervated tissue in humans.[1] Corneal nerves (deriving from the trigeminal ganglion) help maintain transparency in this avascular tissue and participate in ocular surface homeostasis by producing neurotrophins and facilitating sensory-dependent corneal and tearing reflexes.[1, 2] Trigeminal nerve damage may cause neurotrophic keratitis/keratopathy (NK) with partial or total loss of corneal sensation, leading to visual impairment and potentially permanent blindness. NK is a rare disease (estimated prevalence: 1.6–4.2 cases per 10,000)[3, 4] with various underlying etiologies (most commonly herpetic infections and ocular or neurological surgeries) that impair corneal innervation.[5, 6] NK diagnosis, prognosis, and treatment (reviewed elsewhere)[3, 6] are based on disease severity, which is classified broadly into three stages.[7] Briefly, stage 1 (mild) NK exhibits ocular surface irregularity and reduced vision. Stage 2 (moderate) NK exhibits a nonhealing persistent epithelial defect (PED), and stage 3 (severe) NK exhibits corneal ulceration involving sub-epithelial (stromal) tissue, which may progress to corneal perforation. All disease stages cause some vision loss; however, if untreated, moderate NK progresses to severe disease with associated risks of profound vision loss due to scarring and corneal perforation. Conventional therapy for stage 1 aims to prevent epithelial breakdown, generally by administering preservative-free artificial tears and discontinuing toxic topical medications. Stage 2/3 therapies aim to facilitate corneal healing and prevent corneal thinning (which may lead to perforation); these include surgeries and procedures (e.g., tarsorrhaphy, botulinum-induced ptosis, conjunctival flap, amniotic membrane transplantation) to restore ocular surface integrity, but potentially sacrificing vision and cosmesis.

Strong evidence supports the treatment of NK with neurotrophic factors.[8] Nerve growth factor (NGF) has demonstrated important roles in maintaining corneal homeostasis *in vitro*, *ex vivo*, and in animal models.[9, 10] NGF is highly conserved among vertebrates,[11] and small uncontrolled, open-label studies with murine NGF (mNGF) produced promising results for the treatment of corneal neurotrophic ulcers.[12, 13] Confirmation of results obtained with mNGF have been highly anticipated[14]; however, nearly two decades passed with no approved treatments for NK, and no NGF-based treatments available for any indication. For NK therapies in general, clinical development has been hindered by the paucity of adequately sized and rigorously designed studies; indeed, only one randomized controlled trial of NK patients exists in the published literature to date, and the investigative treatment (topical fibronectin ophthalmic solution) was not superior to placebo for healing PEDs.[15] Thus, the natural history of NK is not completely understood, and approved treatments are not available for use as comparators for further studies. For NGF in particular, translational development has been mired by its complex tertiary structure, which complicates the manufacturing of recombinant human NGF (rhNGF) suitable for clinical use. To this end, we developed an *E. coli*-derived rhNGF formulation for topical ophthalmic use and demonstrated it to be safe and well tolerated in phase I randomized, double-masked, vehicle-controlled studies in healthy volunteers[16] and in NK patients.[17] Here, we report phase II study results of topical rhNGF treatment for moderate-to-severe NK.

METHODS

Clinical Trial Design

REPARO (Latin, "repair") was a phase I/II, double-masked, randomized, multicenter, vehicle-controlled, parallel group study, which was designed to evaluate the safety and efficacy of rhNGF eye drops (10 or 20 μg/ml, 6 drops/day for 8 weeks) in patients with stage 2 or 3 NK. Phase I assessed safety in 18 patients to support proceeding to phase II, and was conducted, analyzed, and reported separately.[17] Phase II randomized 156 patients 1:1:1 to rhNGF 10 μg/ml, rhNGF 20 μg/ml, or vehicle for an 8-week controlled treatment period. Follow-up duration (48 or 56 weeks) was determined by baseline group assignment and corneal healing status during controlled treatment. For vehicle-treated patients, baseline randomization included the possibility of secondary rhNGF treatment (10 or 20 μg/ml) in the event of treatment failure during the 8-week controlled treatment period, pre-defined as failure to achieve corneal healing, recurrence of NK after healing, or deterioration (lesion size increase of ≥1mm; best corrected distance visual acuity [BCDVA] decrease of >5 Early Treatment Diabetic Retinopathy Study [ETDRS] letters; progression to corneal melting/perforation; or onset of infection). This patient subset received 8 weeks of uncontrolled treatment before continuing follow-up (total follow-up: 56 weeks).

The phase II study design is diagrammed in Figure 1. The REPARO study group is listed in Appendix 1, and the trial was registered at ClinicalTrials.gov (identifier NCT01756456).

Patients

Patients (≥18 years of age) with NK were diagnosed with stage 2 (PED) or stage 3 (corneal ulcer) using published criteria.[7] The main inclusion criteria were: evidence of decreased corneal sensitivity within the corneal lesion and ≥1 corneal quadrant outside the lesion; BCDVA score of ≤75 ETDRS letters (≥ +0.2 logMAR, ≤ 20/32 Snellen or ≤ 0.625 decimal fraction) in the affected eye; and no objective clinical evidence of improvement of the PED or corneal ulcer within 2 weeks prior to study enrollment. The main exclusion criteria were stage 2/3 NK affecting both eyes; active ocular infection or inflammation unrelated to NK; and other ocular disease or severe vision loss in the affected eye. For complete inclusion/exclusion criteria, see Appendix 2 (available at www.aaojournal.org).

Efficacy Assessments

The primary efficacy variable was corneal healing, defined as <0.5 mm fluorescein staining (the lower limit of reliable slit-lamp assessment) in the lesion area, assessed in clinical pictures by masked central readers as a yes/no binary variable at week 4 (primary endpoint) and week 8 (prespecified secondary endpoint). Other secondary variables included visual acuity (BCDVA measured in ETDRS letters); corneal sensitivity measured using the Cochet-Bonnet aesthesiometer (CBA); and duration of corneal healing through follow-up.

Exploratory efficacy variables included reflex tearing (Schirmer test wetting distance after

5 minutes); time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from baseline); and time to corneal healing (<0.5 mm lesion staining) during the controlled/uncontrolled treatment periods. Post hoc efficacy variables included change in lesion size, and the primary endpoint of corneal healing reassessed more conservatively as 0 mm lesion staining and no other persistent staining outside of the lesion.

Safety Assessments

The primary safety variable was incidence of adverse events (AEs). Ocular tolerability was recorded by patients on a visual analogue scale (VAS) from 0–100 mm (0 = no symptoms; 100 = worst possible discomfort) for each of 7 different symptoms: foreign body sensation, burning/stinging, itching, ocular pain, sticky feeling, blurred vision, and photophobia. An overall VAS score was calculated as the mean of individual symptom scores. Other safety parameters included visual acuity (BCDVA measured in ETDRS letters), intraocular pressure (IOP), dilated fundus ophthalmoscopy, vital signs, hematology, and clinical chemistry.

Pharmacokinetics and Immunogenicity Assessments

Blood samples were collected for pharmacokinetics (PK) profiling and immunogenicity assessments (anti-NGF antibody shifts from baseline to post-baseline), performed using enzyme-linked immunosorbent assay (ELISA) as described previously.[16]

Masking and Statistical Analysis

Patients, investigators, and site/sponsor staff were masked to primary randomized treatment and to the dosage of randomized secondary treatment. Indistinguishable kits for dispensing rhNGF or vehicle were randomly assigned according to numbers generated by Statistical Analysis System programmers not directly involved in study analysis. The sponsor was not involved in efficacy data collection for masked central analysis. Assessments by the central reading center were masked to treatment assignment and duration. Unmasking was restricted to final statistical analysis (after database lock) and medical emergencies, including NK recurrence or deterioration. A clinical research organization (CRO) maintained the masked database and performed statistical analyses.

Based on the only published randomized controlled trial of NK[15] and uncontrolled studies of mNGF-treated NK patients,[12, 13] 60% of rhNGF-treated patients were estimated to achieve <0.5 mm lesion staining at 4 weeks (vs. 30% in vehicle-treated patients). Although the study's exploratory nature did not warrant adjustment for multiple comparisons, 2-sided significance of chi-square testing was corrected according to Pocock,[18] yielding a 97.06% confidence interval (CI) for the primary efficacy endpoint of corneal healing. According to this methodology, phase II required 141 evaluable patients to have 80% power to detect this difference in the primary efficacy variable, and 156 patients assuming 10–20% dropout. Efficacy analyses were performed on intention-to-treat (ITT) populations, with missing data imputed using post-baseline last observation carried forward (LOCF). Also conducted were observed-case and sensitivity analyses (missing

post-baseline observations imputed as failures, and by multiple imputation methods MI and MIANALYZE).

For binary secondary and exploratory efficacy endpoints, two-sided significance was set at 0.05. Change in BCDVA score from baseline to week 8 was analyzed by an analysis of covariance (ANCOVA) model using treatment group and baseline BCDVA score. Mixed effects repeated measures models using treatment, visit, and baseline measurements were used to assess changes in lesion size (maximum dimension) and reflex tearing (Schirmer test wetting distance) from baseline to week 4 and week 8. The time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from baseline) and corneal healing (<0.5 mm maximum diameter of fluorescein staining) were analyzed using Kaplan-Meier methods and the log-rank test (for the controlled treatment period) and descriptive statistics (for the uncontrolled treatment period). Data collected during follow-up were also analyzed using descriptive statistics.

Study Oversight

Approval was obtained for the study protocol, amendments, and study-related documents (including informed consent) from the institutional review board (IRB) of Sapienza University of Rome and an independent ethics committee (IEC) from each country with one or more participating sites (listed in Appendix 1). The study complied with the Declaration of Helsinki, relevant parts of Code of Federal Regulations Title 21, and Good Clinical Practice (GCP)/Good Laboratory Practice (GLP) guidelines. Written informed consent was

obtained prior to study-related procedures. Compliance was assessed at each visit and verified by study monitors during onsite visits.

RESULTS

Patients and Treatment

REPARO investigators (Appendix 1) represented 39 sites in 9 European countries (Belgium, France, Germany, Hungary, Italy, Poland, Portugal, Spain, and the United Kingdom); 32 sites in 6 countries enrolled ≥1 patient. Figure 1 provides an overview of patient disposition (including reasons for withdrawal). Of 186 patients screened January 2013–May 2015, 174 were enrolled—18 in phase I,[17] and 156 in phase II. Patient demographics and baseline characteristics were well balanced in the REPARO phase II study, with no clinically notable differences between treatment groups (Table 1). Consistent with published literature,[5, 6, 13, 19] common underlying etiologies included herpetic eye disease (44 patients) and ocular or neurological surgery (21 patients each). Prior treatments for NK (most commonly artificial tears/gels/ointments and topical antibiotics) are shown in Appendix 3 (available at www.aaojournal.org).

Efficacy Outcomes

Table 2 summarizes efficacy analyses at weeks 4 and 8. Corneal healing (<0.5 mm lesion staining) was achieved at week 4 (primary endpoint) in 19.6% of vehicle-treated patients vs. 54.9% receiving rhNGF 10 µg/ml (+35.3%; 97.06% CI 15.88–54.71; P<0.001) and 58.0% receiving rhNGF 20 µg/ml (+38.4%; 97.06% CI 18.96–57.83; P<0.001). Corneal healing at week 8 (key secondary endpoint) was achieved in 43.1% of vehicle-treated

patients vs. 74.5% receiving rhNGF 10 μg/ml (+31.4%; 97.06% CI 11.25–51.49; P<0.001) and 74.0% receiving rhNGF 20 μg/ml (+30.9%; 97.06% CI 10.60–51.13; P<0.002). Table 3 summarizes the post hoc reanalysis of corneal healing using the more-conservative definition (0 mm lesion staining and no other persistent staining). This confirmed statistically significant differences between rhNGF and vehicle, with consistently higher percentages healed in the rhNGF 20 μg/ml group at both week 4 and week 8. Observed-case, worst-case (missing post-baseline observations imputed as failures), and multiple imputation analyses produced similar results (not shown). Differences between rhNGF groups were not statistically significant.

Figure 2A shows representative images of corneal fluorescein staining at baseline through week 8. Lesion size changes from baseline (determined by the reading center) were analyzed post hoc for clinically significant differences between treatments (Figure 2B). At week 4, least squares mean (LSmean) lesion size change from baseline was -49.8% with rhNGF 20 μg/ml, -39.5% with rhNGF 10 μg/ml, and -8.9% with vehicle. At week 8, lesion size change was -76.0% with rhNGF 20 μg/ml, -58.4% with rhNGF 10 μg/ml, and -26.2% with vehicle. Overall, rhNGF-treated patients exhibited greater (but statistically nonsignificant) lesion size reductions from baseline vs. vehicle-treated patients, trending towards significance in rhNGF 20 μg/ml vs. vehicle at week 8 (p=0.102, 95% CI -109.61–9.98).

Visual acuity outcomes were assessed as changes from baseline to week 8. As shown in Figure 3, compared to vehicle-treated patients, LSmean change in BCDVA score (ETDRS

letters) from baseline to week 8 was significantly different in patients receiving rhNGF 10 µg/ml (p=0.022) but not those receiving rhNGF 20 µg/ml (p=0.213). However, the difference between rhNGF doses was not significant (p=0.305). BCDVA assessed as gain of 15 ETDRS letters (yes/no) from baseline to week 8 produced similar results (Table 4). Compared to vehicle, 15-letter gains were achieved by more patients receiving rhNGF 10 µg/ml (+27.5%; 95% CI: 8.33–46.67; p=0.008) and rhNGF 20 µg/ml (+19%; 95% CI: 0.91–38.83; p=0.068), with no statistically significant difference between rhNGF doses (p=0.421).

Corneal sensitivity during the controlled treatment period was measured directly in the corneal lesion and outside quadrants using the CBA as secondary efficacy variable, and indirectly by Schirmer testing of reflex tearing as an exploratory variable. Compared to vehicle, more patients receiving rhNGF 10 or 20 µg/ml exhibited improvement in corneal sensitivity (cm) from baseline to weeks 4 and 8, but the differences between treatment groups were not significant (Table 5, available online at www.aaojournal.org). Figure 4 (available at www.aaojournal.org) shows results of Schirmer tests of reflex tearing. LSmean change from baseline was greater in the rhNGF-treated groups compared to those receiving vehicle, with differences reaching statistical significance between rhNGF 10 µg/ml and vehicle groups at week 4 (p=0.047) and week 8 (p=0.010). Comparisons between patients receiving rhNGF 20 µg/ml and vehicle were not significant at week 4 (p=0.234) or week 8 (p=0.201). However, comparisons between rhNGF doses were also not significant at either week 4 (p=0.442) or week 8 (p=0.191).

Figure 5 illustrates exploratory Kaplan-Meier analyses of time-to-event variables for the controlled treatment period. The median time to onset of healing (20% reduction in maximum lesion diameter from baseline), which was 14 days in patients receiving vehicle (95% CI 14–28), compared to 8 days in patients receiving rhNGF 10 μg/ml (95% CI 7–14; p=0.002) and 14 days in patients receiving rhNGF 20 μg/ml (95% CI 7–14; p=0.015). For time to corneal healing (<0.5 mm lesion staining), median time was 56 days (95% CI 42–not estimable) in patients receiving vehicle, compared to 29 days in patients receiving rhNGF 10 μg/ml (95% CI 20–55; p=0.002) and 28 days in patients receiving rhNGF 20 μg/ml (95% CI 19–55; p=0.002).

Follow-up data (not powered for efficacy analyses) are presented using descriptive statistics. Of patients receiving vehicle during 8-week controlled treatment, 23 experienced treatment failure (failure to achieve corneal healing, recurrence of NK after healing, or deterioration) and entered the 56-week follow-up period, which included 8 weeks of uncontrolled rhNGF treatment (see Figure 1). Per a secondary baseline randomization scheme, 10 patients received 10 μg/ml rhNGF, and 13 received 20 μg/ml rhNGF. At the end of uncontrolled treatment, corneal healing (<0.5 mm lesion staining, assessed by the investigator) was achieved in 3/10 (30%) patients receiving 10 μg/ml rhNGF, and 8/13 (61.5%) patients receiving 20 μg/ml rhNGF. Figure 6 (available at www.aaojournal.org) shows Kaplan-Meier plots of time-to-event variables for the 8-week uncontrolled treatment portion of the 56-week follow-up period. Onset of healing was assessed as 20% reduction in maximum lesion diameter from the last measurement of the controlled treatment period. Median time to onset of healing was 14.5 days (range, 7–55) in the 10

μg/ml rhNGF group, and 7 days (range, 7–42) in the 20 μg/ml rhNGF group. Median time to corneal healing (<0.5 mm lesion staining) in the 10 μg/ml rhNGF group was 15 days (range, 14–27) and 21 days (range, 7–42) in the 20 μg/ml rhNGF group.

Of patients who achieved corneal healing (<0.5 mm lesion staining) and completed follow-up, very few experienced recurrence of the PED or corneal ulcer. Of those who healed after controlled treatment and completed 48-week follow-up, recurrence was experienced by 1/20 patients in the vehicle group (4.8%), 1/27 patients in the rhNGF 10 μg/ml group (3.6%), and 1/28 patients in the rhNGF 20 μg/ml group (3.4%). Of patients healed after uncontrolled treatment and completed 56-week follow-up, recurrence was experienced by 0/4 patients in the rhNGF 10 μg/ml group, and 2/6 (33%) patients in the rhNGF 20 μg/ml group.

Safety Outcomes

Table 6 summarizes TAEs during controlled treatment, which occurred in 25 patients: 6 (11.5%) receiving rhNGF 10 μg/ml, 9 (17.3%) receiving rhNGF 20 μg/ml, and 10 (19.2%) receiving vehicle. Two patients receiving rhNGF 10 μg/ml, 9 receiving rhNGF 20 μg/ml, and 4 receiving vehicle experienced AEs leading to discontinuation of study treatment. Additional phase II safety results (TAEs during uncontrolled treatment and follow-up periods) are presented in Appendix 4.

Overall, 17 patients (10.9%) experienced serious AEs (SAEs) during controlled treatment: 3 receiving rhNGF 10 μg/ml, 9 receiving rhNGF 20 μg/ml, and 5 receiving vehicle. No SAEs were considered related to study treatment.

Changes from baseline VAS scores were analyzed by repeated measures ANCOVA (controlled treatment period) or descriptive statistics (follow-up period). Decreases in VAS scores were observed in all groups, indicating improvement in ocular tolerability, but differences between groups were not statistically significant for the controlled treatment period or otherwise noteworthy during follow-up.

Patients whose NK worsened during the study were discontinued (and respective treatments unmasked) per protocol. Of vehicle-treated patients, 12 experienced deterioration (2 at week 4, 4 at week 6, 6 at week 8), vs. 4 receiving rhNGF 10 μg/ml (1 at week 4, 1 at week 6, 2 at week 8) and 4 receiving rhNGF 20 μg/ml (1 at week 4, none at week 6, 3 at week 8).

Eight deaths were reported during the study: 2 during controlled treatment (1 receiving rhNGF 10 μg/ml, 1 receiving rhNGF 20 μg/ml) and 6 during follow-up (4 patients in the rhNGF 10 μg/ml group, and 1 each in the 20 μg/ml and vehicle groups). All events leading to death (detailed in Appendix 4) were considered unrelated to study treatment.

Pharmacokinetics and Immunogenicity

As shown in Figure 7 (available at www.aaojournal.org), only 5 patients (3 receiving rhNGF 10 µg/ml, 2 receiving rhNGF 20 µg/ml) had NGF concentrations above the lower limit of quantification (LLQ) of 32.000 pg/mL at any time point. Consistent with phase I studies of rhNGF,[16, 17] these results likely represent individual fluctuations of endogenous NGF independent of study treatment. No anti-NGF antibodies were detected at any time point during controlled/uncontrolled treatment periods or follow-up.

DISCUSSION

This study demonstrated that topical rhNGF safely and effectively improves corneal epithelial integrity in moderate-to-severe NK, confirming results achieved using mNGF.[12, 13] While previous reports demonstrated clinical effectiveness of mNGF 200 µg/ml,[12, 13] preclinical pharmacology tests demonstrated higher potency of *E. coli*-derived rhNGF vs. mNGF—notably, higher affinity for human TrkA (high-affinity NGF receptor) and ~10-fold potency in inducing proliferation of human TF1 cells expressing TrkA (unpublished data). Thus, rhNGF 20 µg/ml was selected as the equivalent therapeutic dose, and 10 µg/ml (lowest concentration compatible with analytical and manufacturing requirements) for dose-response purposes. Both rhNGF doses demonstrated robust efficacy results of corneal healing after 4–8 weeks of treatment. Healing was maintained through follow-up for over 96% of rhNGF-treated patients.

The use of intense topical lubricants and close follow-up in vehicle-treated patients shows the natural course of NK using this conservative treatment approach. A subset of patients receiving constant lubrication with vehicle for up to 8 weeks demonstrated epithelial regrowth and closure of an NK lesion; however, lubrication alone may have a higher risk of disease progression and persistence of a small corneal lesion (<0.5 mm), which may pose a risk of complications (e.g., superinfection and a relapse to more-severe NK). Since healthy corneas may demonstrate some degree of corneal staining,[20] we compared two different definitions of corneal healing. Our results suggest that the more-conservative measure of corneal healing (0 mm lesion staining and no other persistent staining) is more reliable than the conventional measure (<0.5 mm lesion staining) for evaluating corneal healing. Although both measures produced consistent results, the more-conservative assessment showed more consistent differences between rhNGF and vehicle, allowing more definitive discrimination of treatment effect.

Clinical efficacy of topical rhNGF for treating NK was also supported by improvement on other clinically relevant endpoints, including corneal lesion size, time to corneal healing (or onset of healing) , BCDVA, corneal sensitivity measured by CBA, and reflex tearing (which may also reflect corneal sensitivity not detectable by CBA). Although we did not observe statistically significant differences between both rhNGF doses and vehicle in these variables at every time point, the sample size was based on the dichotomous (yes/no) primary endpoint and not powered to detect small but clinically significant differences in secondary, exploratory, or post hoc variables. To this point, the rhNGF 10 μg/ml group (but not the rhNGF 20 μg/ml group) exhibited statistically significant differences compared to

436 the vehicle group in some secondary endpoints (such as visual acuity and reflex tearing);
437 however, in the same endpoints, differences between rhNGF doses did not reach statistical
438 significance. Thus, it is difficult to draw conclusions on dose responsiveness. Nonetheless,
439 patients receiving rhNGF generally had better trends of improvement for most efficacy
440 endpoints vs. patients receiving vehicle.
441
442 Of note, visual acuity was assessed as secondary efficacy endpoint, even though it does not
443 necessarily reflect NK severity or healing status. For example, in stage 2 NK, absence of the
444 epithelium may have little or no impact on vision, while re-epithelialization in the
445 central/paracentral cornea can cause optical aberrations (and hence reduced vision).
446 Figure 2a illustrates this latter point; it would not be surprising that this patient still had
447 reduced vision after 8 weeks of controlled rhNGF 20 μg/ml treatment, despite achieving
448 corneal healing with 0 mm lesion staining and no other persistent staining.
449
450 No safety concerns arose; most AEs were ocular, mild, transient, and did not require
451 discontinuing or corrective treatments. The predominant TAE was eye pain; others
452 included abnormal sensation in the eye, excess lacrimation, photophobia, eyelid pain and
453 eye/eyelid irritation, which may reflect therapeutic actions of rhNGF and normal healing.
454 Indeed, restoring corneal innervation and sensitivity (which, in turn, will promote corneal
455 healing) can be associated with increased ocular surface symptomatology. No
456 immunogenicity to NGF was detected in this study; furthermore, consistent with phase I
457 results,[16, 17] most patients had undetectable serum NGF and/or no systemic AEs. Taken

458 together, these PK and immunogenicity results suggest unlikely systemic absorption or
459 accumulation of topical ophthalmic rhNGF.
460
461 NK is a challenging disease with a high unmet need for treatments that improve corneal
462 sensitivity (which is crucial for restoring corneal epithelial integrity) and promote healing
463 without surgery or compromising vision. In the present study, topical rhNGF demonstrated
464 favorable benefit/risk ratios for patients with mild-to-moderate NK, confirming that rhNGF
465 is a feasible approach to treating NK. The neuroprotective effects of rhNGF may be also
466 extended to other ophthalmic indications with neurodegenerative components, including
467 glaucoma,[21] macular degeneration,[22] and retinitis pigmentosa.[23]
468

469 ACKNOWLEDGMENTS
470 The sponsor participated in the design and conduct of the study and review of the
471 manuscript.
472

Figure Legends

Figure 1. REPARO phase II study design and overall patient disposition.

The REPARO phase II study enrolled 156 patients with neurotrophic keratitis (NK) of severity stage 2 (persistent epithelial defect) or stage 3 (corneal ulcer). Patients were randomized 1:1:1 to 10 μg/ml rhNGF, 20 μg/ml rhNGF, or vehicle, and received 8 weeks of controlled treatment and 48 weeks of follow-up. A 56-week follow-up period was intended for vehicle-treated patients who experienced treatment failure (see text for details), and included 8 weeks of uncontrolled treatment with 10 or 20 μg/ml rhNGF (dosage assigned at baseline in a secondary randomization scheme) before continuing follow-up for 48 weeks.

Figure 2. Assessment of corneal lesion size on clinical pictures.

A) Representative images showing the progression of a typical oval, paracentral neurotrophic corneal lesion from baseline through week 8 in a patient treated with rhNGF 20μg/ml. Top row: pictures of the cornea illuminated with diffuse white light. Bottom row: corneal lesion healed at week 8 as assessed by the central reading center on fluorescein staining (green) pictures taken under cobalt-blue light illumination. B) Post hoc analysis of least squares mean (LSMean) percentage change from baseline in maximum dimension of persistent epithelial defect (PED) or corneal ulcer after the 8-week controlled treatment period. Error bars represent standard error (SE). Magnitude change in lesion size was greater in patients in the rhNGF treatment groups compared to the vehicle group (not

reaching statistical significance), with a trend towards significance in rhNGF 20 μg/ml vs. vehicle treatment at week 8 (p=0.102, 95% CI -109.61–9.98).

Figure 3. Secondary efficacy analysis of visual acuity score during controlled treatment.

Least squares mean (LSmean) change from baseline in best corrected distance visual acuity (BCDVA) measured in Early Treatment Diabetic Retinopathy Study (ETDRS) letters was analyzed using an analysis of covariance (ANCOVA) model (treatment + baseline score). Compared to vehicle-treated patients, LSmean change from baseline to week 8 was greater in the rhNGF-treated groups, with the difference reaching statistical significance in between patients receiving vehicle and rhNGF 10 μg/ml (p=0.022) but not rhNGF 20 μg/ml (p=0.213). However, the comparison between rhNGF doses was also not significant (0.305).

Figure 4 (online). Exploratory analysis of change in reflex tearing during controlled treatment.

Least squares mean (LSmean) change from baseline in Schirmer wetting distance (cm) at 5 minutes was analyzed using a mixed effects repeated measures model (treatment + visit + treatment x visit interaction + baseline measurement). Compared to vehicle-treated patients, LSmean change from baseline was greater in the rhNGF-treated groups, with differences reaching statistical significance between rhNGF 10 μg/ml and vehicle groups at week 4 (p=0.047) and week 8 (p=0.010). Comparisons between patients receiving rhNGF 20 μg/ml and vehicle were not significant at week 4 (p=0.234) or week 8 (p=0.201).

However, comparisons between rhNGF doses were also not significant at either week 4 (p=0.442) or week 8 (p=0.191). Error bars represent standard error (SE).

Figure 5. Exploratory analyses of Kaplan-Meier time-to-event variables during controlled treatment. Top panel: Time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from baseline). Lower panel: Time to corneal healing (<0.5 mm lesion staining). See text for details.

Figure 6 (online). Exploratory analyses of Kaplan-Meier time-to-event variables during uncontrolled treatment.

Of patients receiving vehicle during the controlled treatment period, 23 experienced treatment failure and received 8 weeks of uncontrolled treatment with rhNGF 10 μg/ml or 20 μg/ml (see text for details). Top panel: time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from last measurement of the controlled treatment period). All patients showed signs of healing (i.e., none were censored). Lower panel: time to corneal healing (<0.5 mm lesion staining).

Figure 7 (online). REPARO Phase II Pharmacokinetics.

Serum concentration of nerve growth factor (NGF) plotted over time for patients in the REPARO Phase II study. Blood samples were taken prior to the initial daily dose for PK profiling from approximately the first 90 patients receiving recombinant human NGF (rhNGF) or vehicle, at various time points during the 8-week controlled treatment period (day 1, week 1, week 3, week 6, and week 8). The lower limit of quantification (LLQ) was

32.000 pg/mL; concentrations <LLQ are considered as 0 pg/mL for the purpose of these plots. A total of 5 patients had NGF concentrations >LLQ and are depicted in this figure. In the rhNGF 10 μg/ml group (●), 3 patients had measurable serum NGF concentrations: 1 patient at day 0 (6750.7 pg/ml) and at week 8 (728.6 pg/ml), and 2 patients had concentrations >LLQ at all time points. In the rhNGF 20 μg/ml group (◇), 1 had NGF concentrations >LLQ at day 0, week 1, and week 3; 1 patient had NGF concentrations >LLQ at weeks 1 and 3. In the vehicle group (not shown), no patients had detectable serum NGF at any time point tested.

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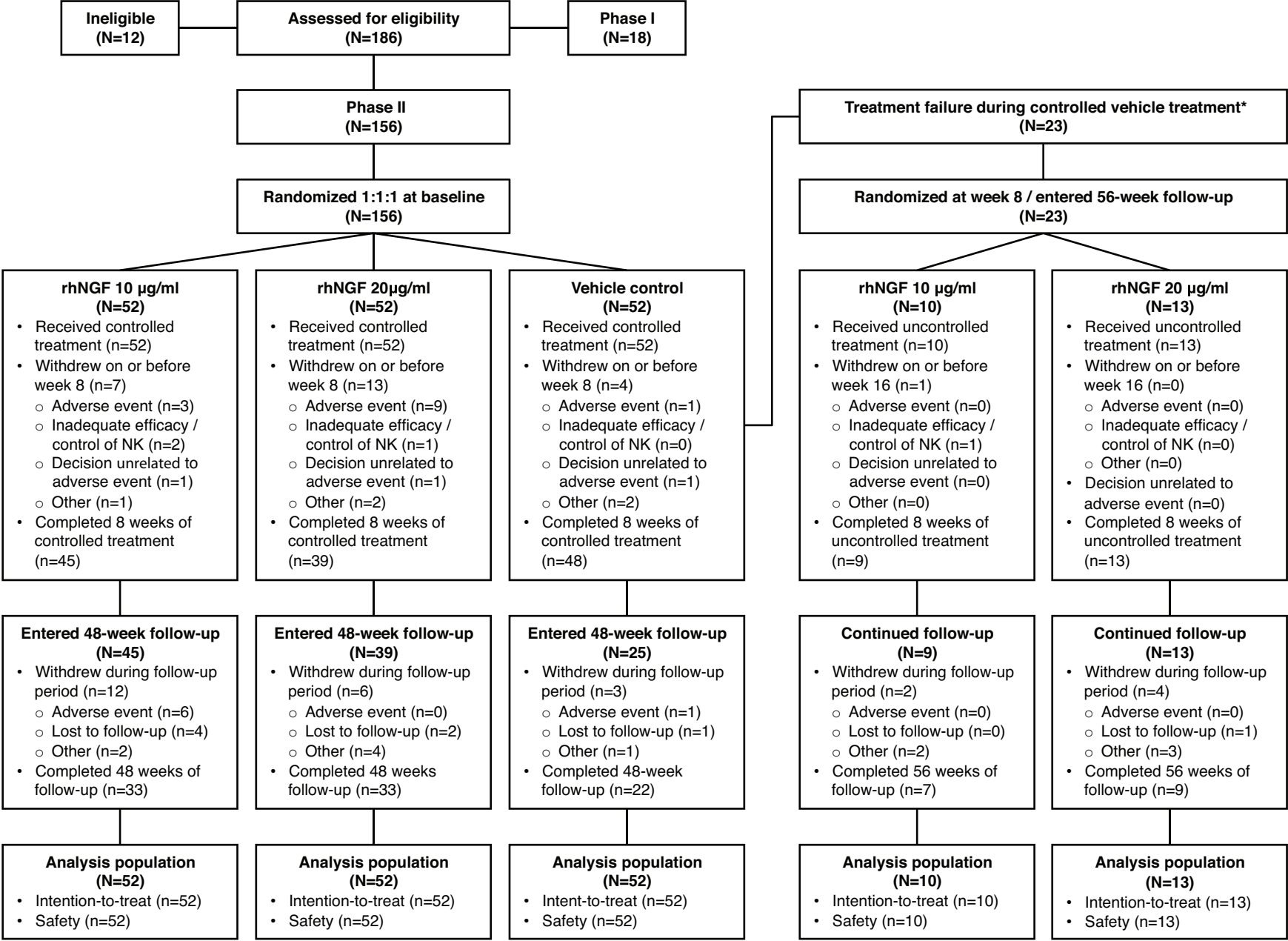
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Phase I (N=18)
Phase II (N=156)
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• Received controlled treatment (n=52)
• Withdrew on or before week 8 (n=7)
○ Adverse event (n=3)
○ Inadequate efficacy / control of NK (n=2)
○ Decision unrelated to adverse event (n=1)
○ Other (n=1)
• Completed 8 weeks of controlled treatment (n=45)
rhNGF 20µg/ml (N=52)
• Received controlled treatment (n=52)
• Withdrew on or before week 8 (n=13)
○ Adverse event (n=9)
○ Inadequate efficacy / control of NK (n=1)
○ Decision unrelated to adverse event (n=1)
○ Other (n=2)
• Completed 8 weeks of controlled treatment (n=39)
Vehicle control (N=52)
• Received controlled treatment (n=52)
• Withdrew on or before week 8 (n=4)
○ Adverse event (n=1)
○ Inadequate efficacy / control of NK (n=0)
○ Decision unrelated to adverse event (n=1)
○ Other (n=2)
• Completed 8 weeks of controlled treatment (n=48)
Entered 48-week follow-up (N=45)
• Withdrew during follow-up period (n=12)
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• Completed 48 weeks of follow-up (n=33)
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• Withdrew during follow-up period (n=3)
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• Completed 48-week follow-up (n=22)
Analysis population (N=52)
• Intention-to-treat (n=52)
• Safety (n=52)
Analysis population (N=52)
• Intention-to-treat (n=52)
• Safety (n=52)
Analysis population (N=52)
• Intent-to-treat (n=52)
• Safety (n=52)
Treatment failure during controlled vehicle treatment* (N=23)
Randomized at week 8 / entered 56-week follow-up (N=23)
rhNGF 10 µg/ml (N=10)
• Received uncontrolled treatment (n=10)
• Withdrew on or before week 16 (n=1)
○ Adverse event (n=0)
○ Inadequate efficacy / control of NK (n=1)
○ Decision unrelated to adverse event (n=0)
○ Other (n=0)
• Completed 8 weeks of uncontrolled treatment (n=9)
rhNGF 20 µg/ml (N=13)
• Received uncontrolled treatment (n=13)
• Withdrew on or before week 16 (n=0)
○ Adverse event (n=0)
○ Inadequate efficacy / control of NK (n=0)
○ Other (n=0)
• Decision unrelated to adverse event (n=0)
• Completed 8 weeks of uncontrolled treatment (n=13)
Continued follow-up (N=9)
• Withdrew during follow-up period (n=2)
○ Adverse event (n=0)
○ Lost to follow-up (n=0)
○ Other (n=2)
• Completed 56 weeks of follow-up (n=7)
Continued follow-up (N=13)
• Withdrew during follow-up period (n=4)
○ Adverse event (n=0)
○ Lost to follow-up (n=1)
○ Other (n=3)
• Completed 56 weeks of follow-up (n=9)
Analysis population (N=10)
• Intention-to-treat (n=10)
• Safety (n=10)
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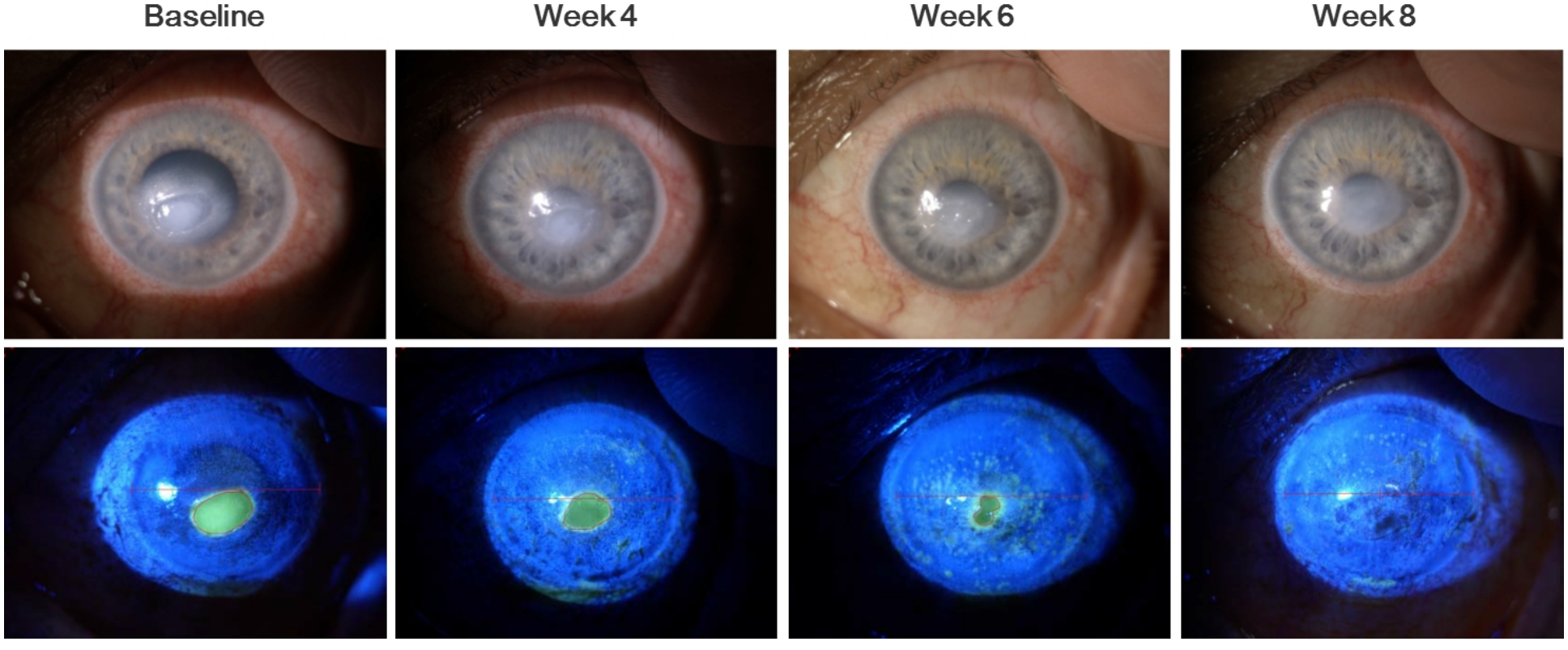
• Intention-to-treat (n=13)
• Safety (n=13)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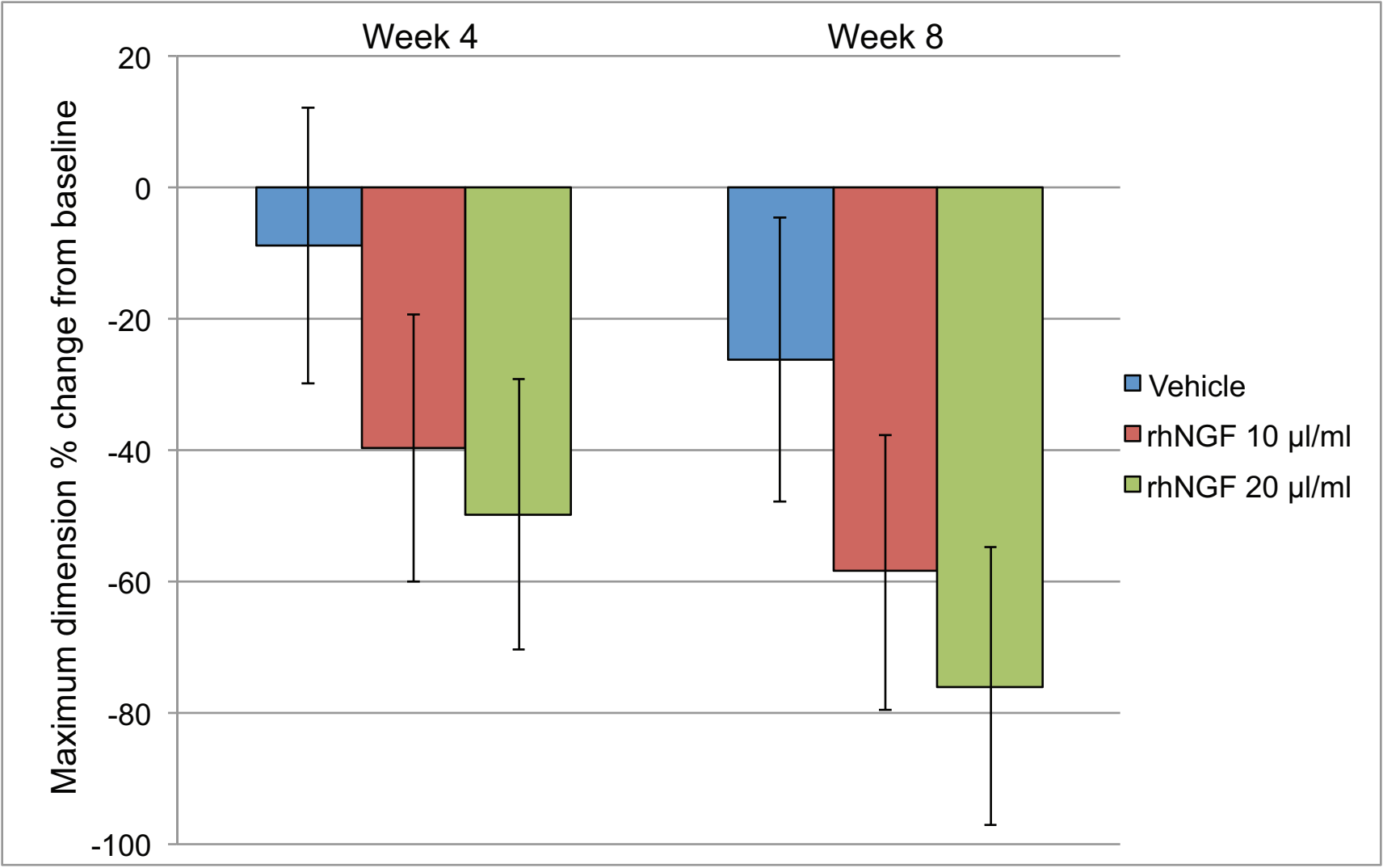
Week 6
Week 8


Week 4
Week 8
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-20
-40
-60
-80
-100
Maximum dimension % change from baseline
Vehicle
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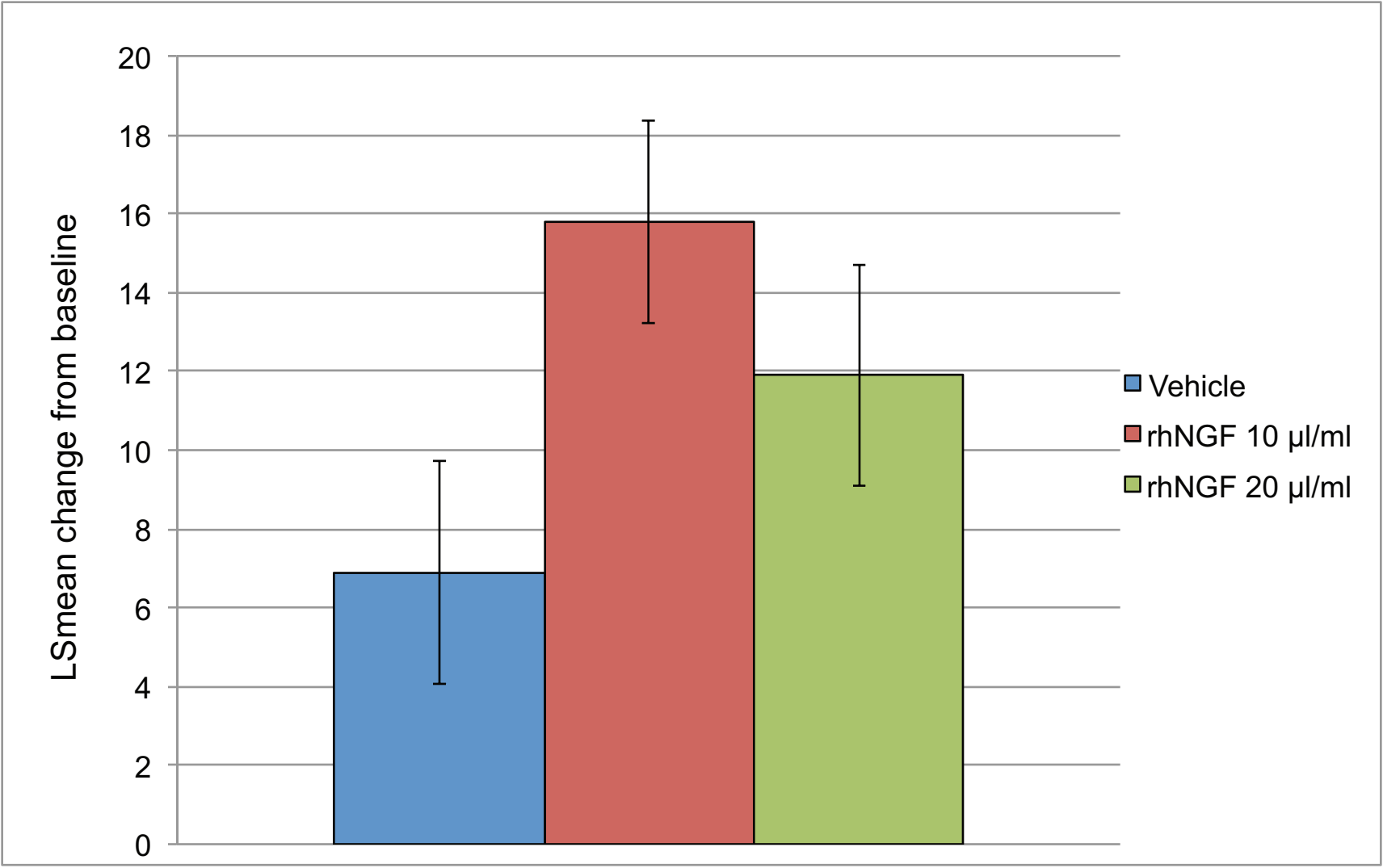
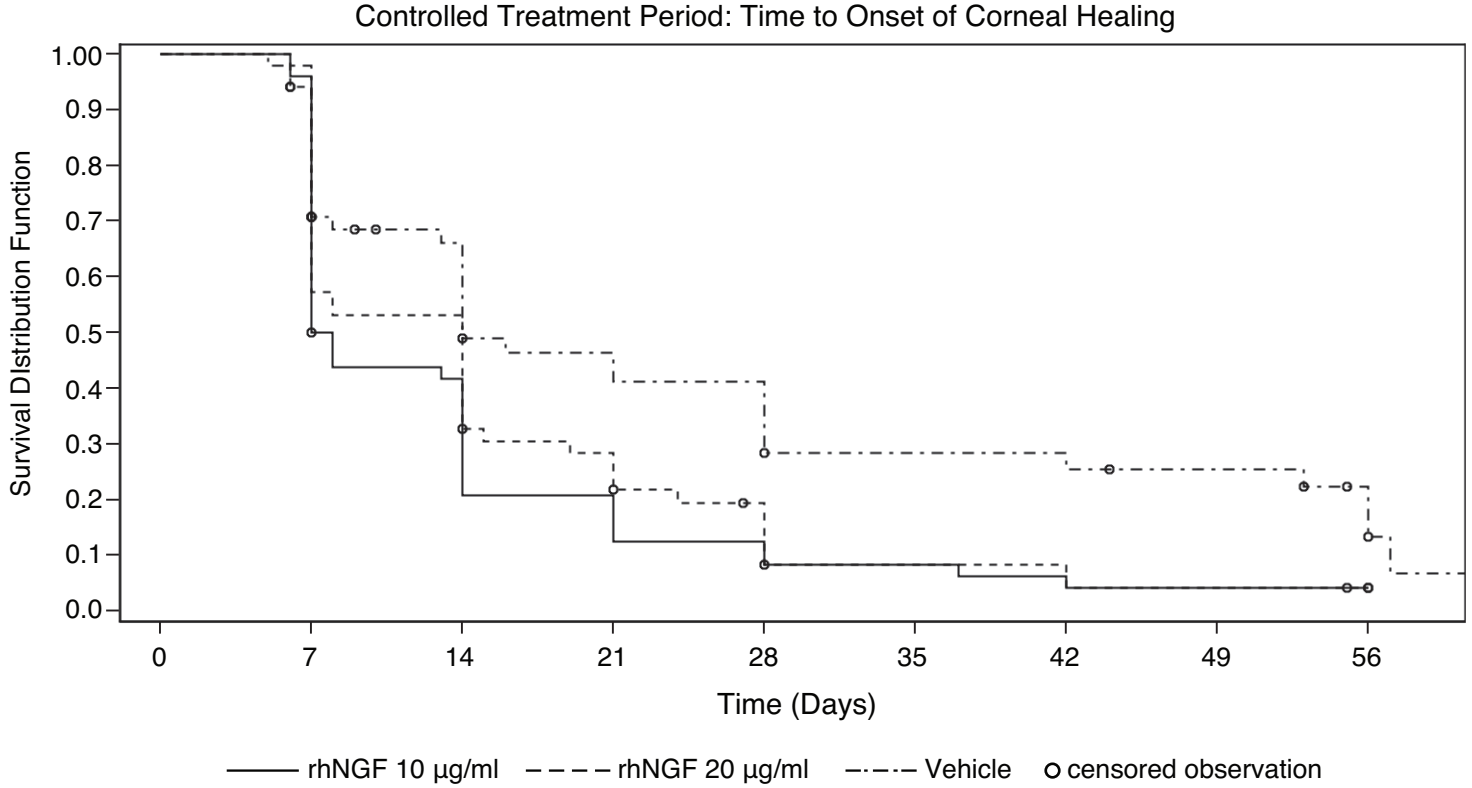
rhNGF 20 µl/ml


LSmean change from baseline
20
18
16
14
12
10
8
6
4
2
0
Vehicle
rhNGF 10 µl/ml
rhNGF 20 µl/ml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0.7
0.6
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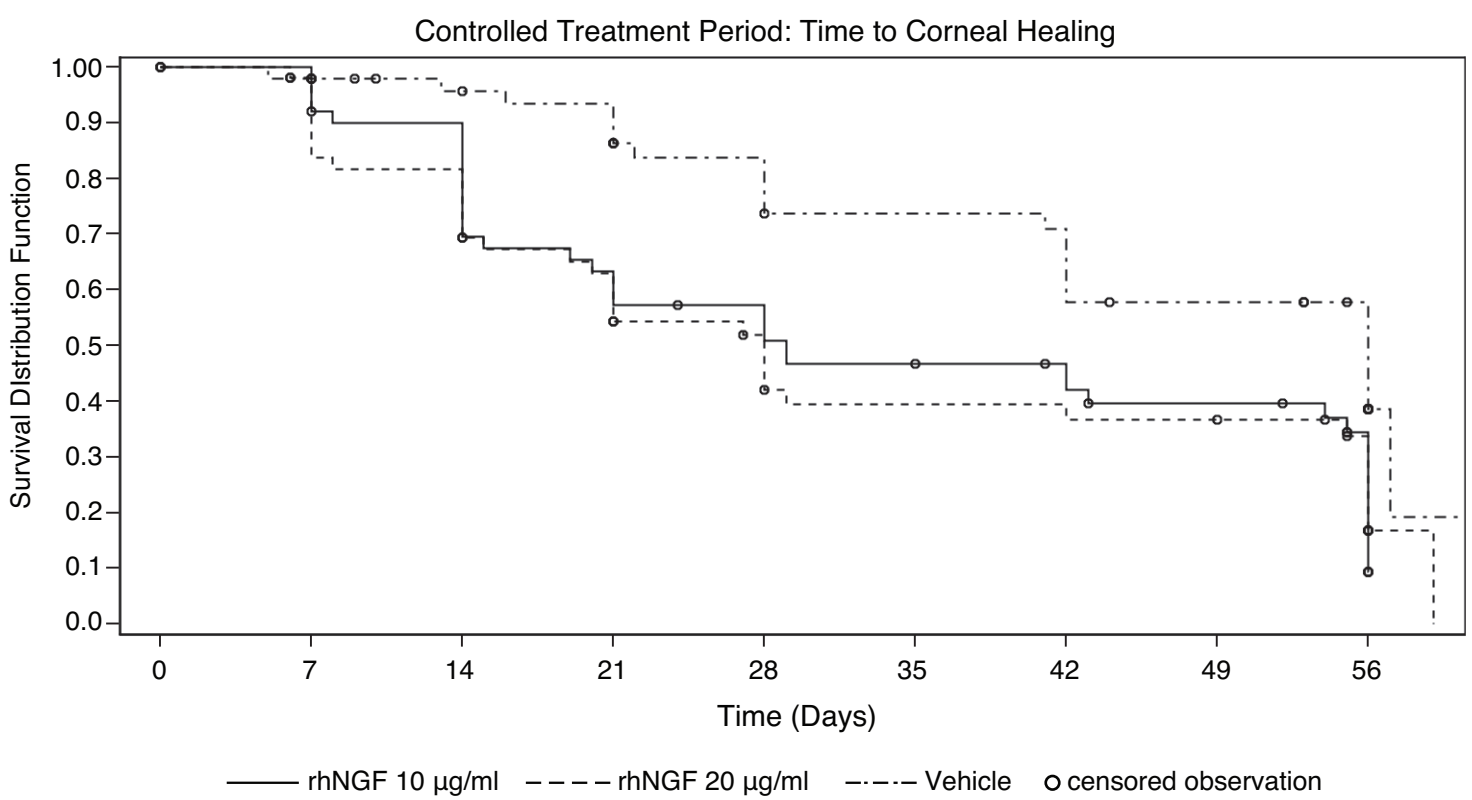
7
14
21
28
35
42
49
56
Time (Days)
rhNGF 10 µg/ml
rhNGF 20 µg/ml
Vehicle
censored observation


Controlled Treatment Period: Time to Corneal Healing
Survival Distribution Function
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0.9
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42
49
56
Time (Days)
rhNGF 10 µg/ml
rhNGF 20 µg/ml
Vehicle
censored observation

Table 1. Patient demographics and baseline characteristics.

Characteristics	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle (N=52)
Age (years)			
Mean (SD)	59.0 (17.17)	62.5 (14.01)	60.4 (16.78)
Median (min, max)	61.5 (20, 87)	63.5 (18, 95)	60.5 (23, 91)
Female, n (%)	30 (57.7)	30 (57.7)	35 (67.3)
Ethnicity, n (%)			
Hispanic, Latino, or Spanish	6 (11.5)	9 (17.3)	5 (9.6)
N/A	4 (7.7)	1 (1.9)	6 (11.5)
Race, n (%)			
Asian	1 (1.9)	0	1 (1.9)
Black or African American	0	0	1 (1.9)
White	46 (88.5)	51 (98.1)	45 (86.5)
N/A	5 (9.6)	1 (1.9)	5 (9.6)
Primary NK diagnosis, n (%)			
Stage 2	21 (40.4)	27 (51.9)	28 (53.8)
Stage 3	31 (59.6)	25 (48.1)	24 (46.2)
Diabetes mellitus	3 (5.8)	4 (7.7)	4 (7.7)
Underlying etiology, n (%)			
Dry eye disease	6 (11.5)	6 (11.5)	5 (9.6)
Herpetic eye disease*	15 (28.8)	11 (21.2)	18 (34.6)
Neurosurgical procedure			
Acoustic neuroma	2 (3.8)	1 (1.9)	3 (5.8)
Auditive neurosurgery	0	1 (1.9)	0
Cerebellar metastasis	0	1 (1.9)	0
Cerebral epidermoid cyst aspiration	0	1 (1.9)	0
Craniotomy due to glioma	1 (1.9)	0	0
Facial nerve reconstruction	1 (1.9)	0	0
Meningioma excision	0	1 (1.9)	1 (1.9)
Schwannoma	1 (1.9)	1 (1.9)	3 (5.8)
Unspecified	1 (1.9)	2 (3.8)	0
Nonviral infection			
Amoebic keratitis	0	2 (3.8)	0
Unspecified	1 (1.9)	0	1 (1.9)
Ocular surface injury / inflammation			
Chemical burn	4 (7.7)	2 (3.8)	3 (5.8)
Unspecified	1 (1.9)	3 (5.8)	2 (3.8)
Ocular surgery or procedure			
Cataract surgery/scleral buckle/vitrectomy	1 (1.9)	1 (1.9)	1 (1.9)
Corneal transplantation	0	0	1 (1.9)
Keratoplasty	2 (3.8)	0	0
Maxillofacial surgery (eyelid suture)	1 (1.9)	0	0
Strontium brachytherapy, mitomycin drops	0	0	1 (1.9)
Unspecified	5 (9.6)	4 (7.7)	4 (7.7)
Other			
Atopic dermatitis	1 (1.9)	0	0
Corneal hypoesthesia	0	1 (1.9)	0
Facial palsy due to measles	1 (1.9)	0	0
Goldenhar syndrome	0	0	1 (1.9)
Graves-Basedow disease	0	1 (1.9)	0
Lagophthalmos	0	0	1 (1.9)
Miller-Fisher syndrome	1 (1.9)	0	0
Multifactorial (HSV, keratoplasty, burn, diabetes)	0	1 (1.9)	0
Neurovascular encephalopathy	0	1 (1.9)	0

Paraneoplastic neuropathy (lung cancer)	0	1 (1.9)	0
Pemphigoid	0	1 (1.9)	1 (1.9)
Polyneuropathy, traumatic erosion	0	1 (1.9)	0
Stroke	1 (1.9)	2 (3.8)	0
Systemic medication	1 (1.9)	0	0
Topical medication (glaucoma medication)	0	1 (1.9)	1 (1.9)
Unknown origin	1 (1.9)	1 (1.9)	0
Venous sinus thrombosis	1 (1.9)	0	0
Viral conjunctivitis (unspecified)	0	0	1 (1.9)

Abbreviations: HSV = herpes simplex virus; min= minimum; max = maximum; N/A: not available (ethnicity and race were not collected in all countries); rhNGF = recombinant human nerve growth factor; SD = standard deviation

*Includes herpes simplex, herpes zoster, and recurrent herpetic keratitis

Table 2. Primary efficacy analysis of corneal healing (<0.5 mm lesion staining).

	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle (N=52)
Healed at week 4, n (%)	28/51 (54.9)	29/50 (58.0)	10/51 (19.6)
Difference (rhNGF - vehicle), %	35.3	38.4	
97.06% CI	15.88, 54.71	18.96, 57.83	
p-value	<0.001	<0.001	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	3.1		
97.06% CI	-18.38, 24.58		
p-value	0.754		
Healed at week 8, n (%)	38/51 (74.5)	37/50 (74.0)	22/51 (43.1)
Difference (rhNGF - vehicle), %	31.4	30.9	
97.06% CI	11.25, 51.49	10.60, 51.13	
p-value	0.001	0.002	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	-0.5		
97.06% CI	-19.46, 18.44		
p-value	0.953		

Abbreviations: CI = confidence interval; µg = microgram; ml = milliliter; N = number of patients randomized to each treatment; rhNGF = recombinant human nerve growth factor

Table 3. Post hoc efficacy analysis of corneal healing (0 mm lesion staining, no other persistent staining).

	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle (N=52)
Healed at week 4, n (%)	25/51 (49)	29/50 (58)	7/51 (13.7)
Difference (rhNGF - vehicle), %	35.3	44.3	
97.06% CI	16.78, 53.80	25.80, 62.75	
p-value	<0.001	<0.001	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	9.0		
97.06% CI	-12.55, 30.51		
p-value	0.366		
Healed at week 8, n (%)	32/51 (62.7)	36/50 (72.0)	17/51 (33.3)
Difference (rhNGF - vehicle), %	29.4	38.7	
97.06% CI	8.82, 50.01	18.72, 58.62	
p-value	0.003	<0.001	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	9.3		
97.06% CI	-10.96, 29.47		
p-value	0.321		

Abbreviations: CI = confidence interval; µg = microgram; ml = milliliter; N = number of patients randomized to each treatment; rhNGF = recombinant human nerve growth factor

Table 4. Secondary efficacy analysis of patients achieving 15-letter gains in BCDVA

	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle (N=52)
15-letter gain in BCDVA at week 4, n (%)	18/49 (36.7)	14/41 (34.1)	9/43 (20.9)
Difference (rhNGF - vehicle), %	15.8	13.2	
95% CI	-2.36, 33.97	-5.72, 32.15	
p-value	0.097	0.175	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	-2.6		
95% CI	-22.41, 17.23		
p-value	0.798		
15-letter gain in BCDVA at week 8, n (%)	24/48 (50.0)	17/41 (41.5)	9/40 (22.5)
Difference (rhNGF - vehicle), %	27.5	19.0	
95% CI	8.33, 46.67	-0.91, 38.83	
p-value	0.008	0.068	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	-8.5		
95% CI	-29.21, 12.14		
p-value	0.421		

BCDVA = best corrected distance visual acuity; CI = confidence interval; µg = microgram; ml = milliliter; N = number of patients randomized to each treatment; rhNGF = recombinant human nerve growth factor.
Patients without a Yes/No response available at Week 4/Week 8 are not considered in this table. The significance level is 0.05.

Table 5. Secondary efficacy analysis of improved corneal sensitivity during controlled treatment.

	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle (N=52)
Improvement in corneal sensitivity at week 4, n (%)	31/45 (68.9)	22/36 (61.1)	26/41 (63.4)
Difference (rhNGF - vehicle), %	5.5	-2.3	
95% CI	-14.53, 25.48	-24.01, 19.40	
p-value	0.592	0.835	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	-7.8		
95% CI	-28.67, 13.12		
p-value	0.465		
Improvement in corneal sensitivity at week 8, n (%)	33/42 (78.6)	29/38 (76.3)	26/38 (68.4)
Difference (rhNGF - vehicle), %	10.2	7.9	
95% CI	-9.15, 29.45	-12.13, 27.92	
p-value	0.303	0.442	
Difference (rhNGF20 µg/ml - rhNGF10 µg/ml), %	-2.3		
95% CI	-20.61, 16.09		
p-value	0.809		

CI = confidence interval; µg = microgram; ml = milliliter; N = number of patients randomized to each treatment; rhNGF = recombinant human nerve growth factor

Patients without a Yes/No response available at Week 4/Week 8 are not considered in this table. The significance level is 0.05.

Table 6. Summary of treatment-related adverse events* by system organ class and preferred term (controlled treatment period).

Body System **MedDRA Preferred Term§**	**rhNGF 10 µg/ml (N=52)**		**rhNGF 20 µg/ml (N=52)**		**Vehicle (N=52)**	
	N'	n (%)	N'	n (%)	N'	n (%)
Any adverse event	**10**	**6 (11.5)**	**15**	**9 (17.3)**	**20**	**10 (19.2)**
Eye disorders	**7**	**5 (9.6)**	**10**	**7 (13.5)**	**16**	**9 (17.3)**
Eye pain	0	0	4	4 (7.7)	3	2 (3.8)
Blepharitis	1	1 (1.9)	1	1 (1.9)	1	1 (1.9)
Corneal neovascularization	0	0	1	1 (1.9)	1	1 (1.9)
Eye irritation	1	1 (1.9)	0	0	1	1 (1.9)
Eye pruritus	0	0	1	1 (1.9)	1	1 (1.9)
Vision blurred	0	0	0	0	2	2 (3.8)
Abnormal sensation in eye	0	0	0	0	1	1 (1.9)
Asthenopia	0	0	0	0	1	1 (1.9)
Conjunctival hyperemia	0	0	0	0	1	1 (1.9)
Corneal deposits	0	0	1	1 (1.9)	0	0
Corneal epithelium defect	0	0	0	0	1	1 (1.9)
Dry eye	0	0	0	0	1	1 (1.9)
Eye discharge	1	1 (1.9)	0	0	0	0
Eyelid edema	0	0	0	0	1	1 (1.9)
Eyelid pain	2	1 (1.9)	0	0	0	0
Lacrimation increased	1	1 (1.9)	0	0	0	0
Macular fibrosis	0	0	1	1 (1.9)	0	0
Ocular hyperemia	0	0	0	0	1	1 (1.9)
Photophobia	1	1 (1.9)	0	0	0	0
Visual acuity reduced	0	0	1	1 (1.9)	0	0
General disorders and administration site conditions	**1**	**1 (1.9)**	**0**	**0**	**3**	**3 (5.8)**
Disease progression†	1	1 (1.9)	0	0	2	2 (3.8)
Instillation site pain	0	0	0	0	1	1 (1.9)
Nervous system disorders	**2**	**2 (3.8)**	**1**	**1 (1.9)**	**1**	**1 (1.9)**
Headache	1	1 (1.9)	1	1 (1.9)	1	1 (1.9)
Neuralgia	1	1 (1.9)	0	0	0	0
Blood and lymphatic system disorders	**0**	**0**	**1**	**1 (1.9)**	**0**	**0**
Neutropenia	0	0	1	1 (1.9)	0	0
Cardiac disorders	**0**	**0**	**1**	**1 (1.9)**	**0**	**0**
Arrhythmia	0	0	1	1 (1.9)	0	0
Infections and infestations	**0**	**0**	**1**	**1 (1.9)**	**0**	**0**
Corneal abscess	0	0	1	1 (1.9)	0	0
Investigations	**0**	**0**	**1**	**1 (1.9)**	**0**	**0**
Blood pressure increased	0	0	1	1 (1.9)	0	0

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; N = number of patients who received each treatment in the specified study period; N' = number of events reported, n = number of patients; rhNGF = recombinant human nerve growth factor.
Percentages (%) are calculated using the population number in each treatment group (N) as the denominator.
*Treatment-related AEs are those events recorded by the investigator as having a possible, probable, or highly probable relationship to study treatment.
† Disease progression was defined as increase in lesion size ≥ 1mm; decrease in best corrected distance visual acuity (BCDVA) by >5 Early Treatment Diabetic Retinopathy Study (ETDRS) letters; progression in lesion depth to corneal melting or perforation; or onset of infection.

Figure 4. Exploratory efficacy analysis of change in reflex tearing during the controlled treatment period.

Least squares mean (LSmean) change from baseline in Schirmer wetting distance in centimeters (cm) at 5 minutes was analyzed using a mixed effects repeated measures model (treatment + visit + treatment x visit interaction + baseline measurement). Compared to vehicle-treated patients, LSmean change from baseline was greater in the rhNGF-treated groups, with differences reaching statistical significance between rhNGF 10 µg/ml and vehicle groups at week 4 (p=0.047) and week 8 (p=0.010). Comparisons between patients receiving rhNGF 20 µg/ml and vehicle were not significant at week 4 (p=0.234) or week 8 (p=0.201). However, comparisons between rhNGF doses were also not significant at either week 4 (p=0.442) or week 8 (p=0.191). Error bars represent standard error (SE).

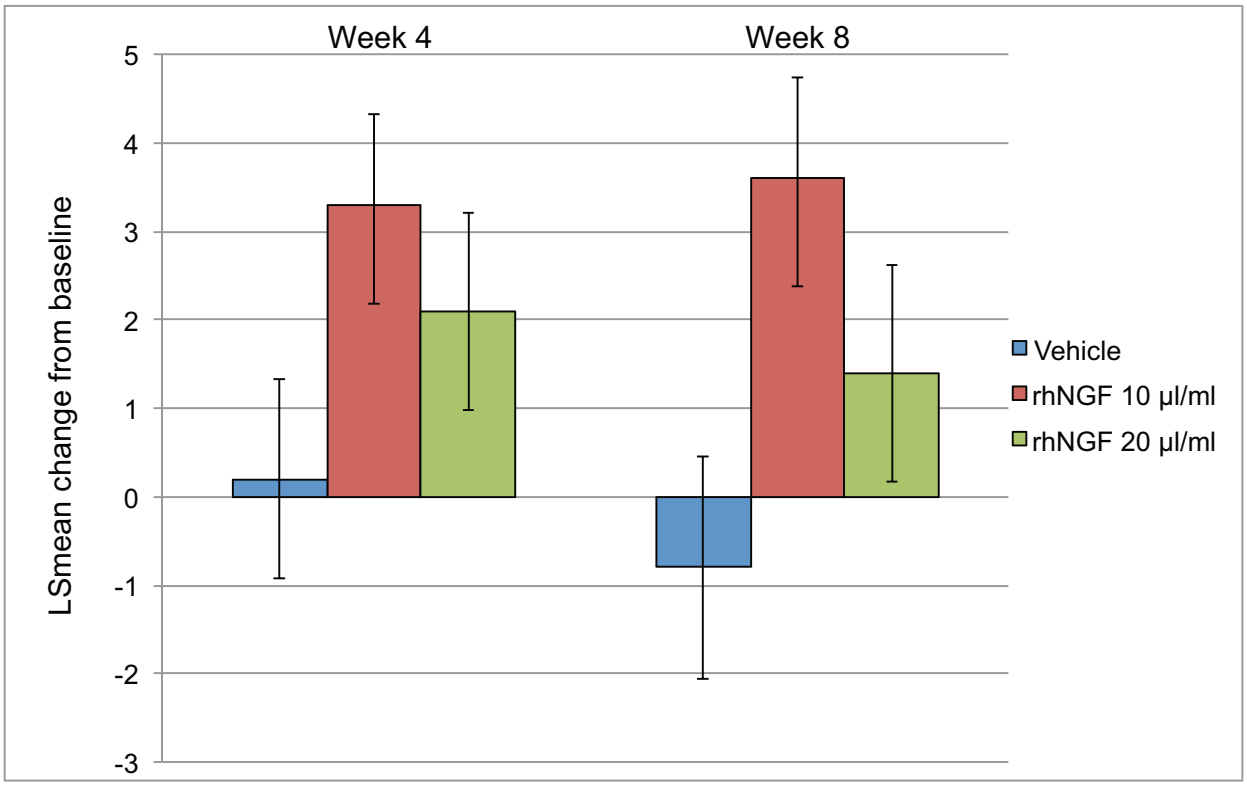


Figure 6. Exploratory analyses of Kaplan-Meier time-to-event variables during uncontrolled treatment.

Of patients receiving vehicle during the controlled treatment period, 23 experienced treatment failure and received 8 weeks of of uncontrolled treatment with rhNGF 10 µg/ml or 20 µg/ml (see text for details). Top panel: time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from last measurement of the controlled treatment period). All patients showed signs of healing (i.e., none were censored). Lower panel: time to corneal healing (<0.5 mm lesion staining).

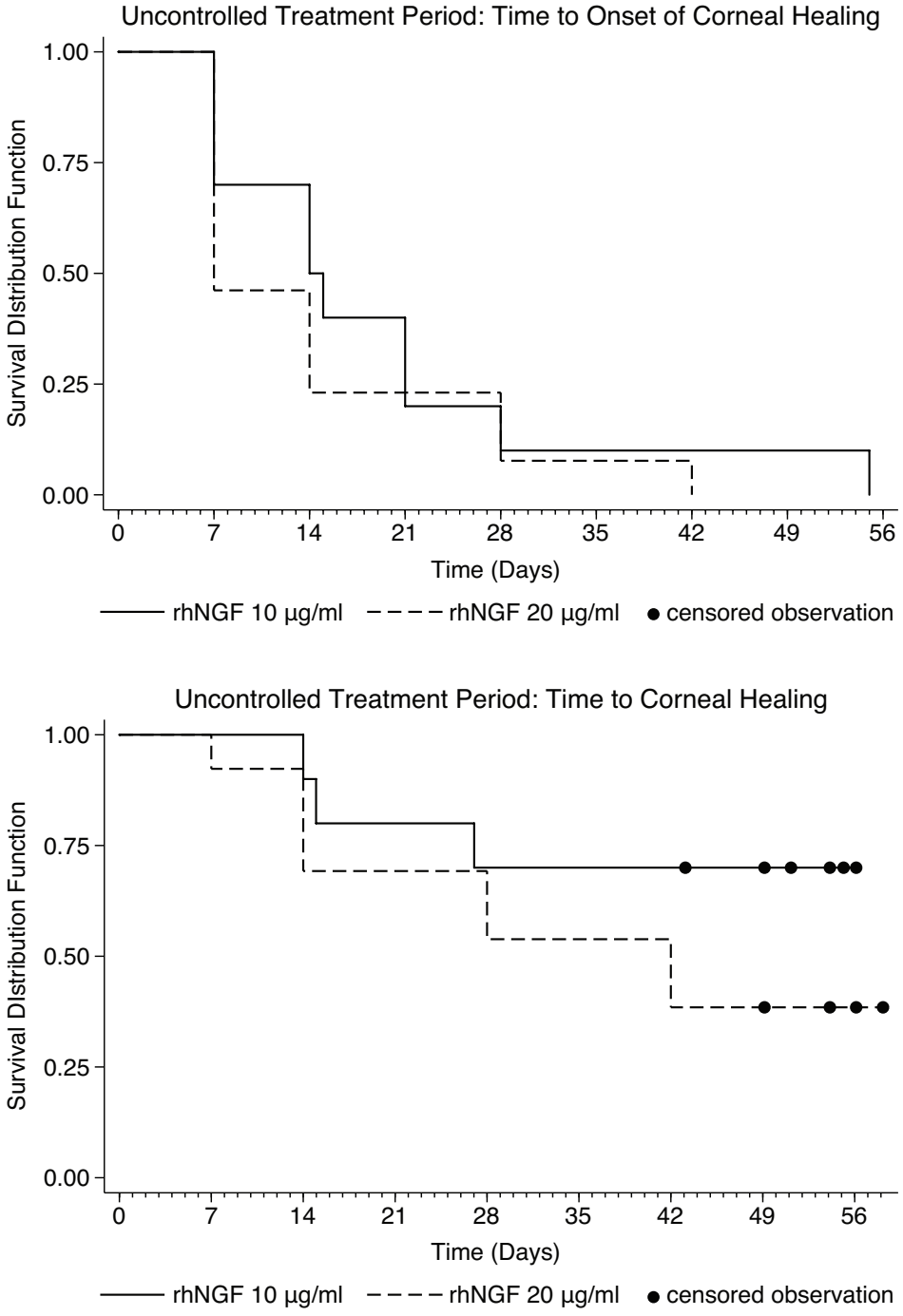
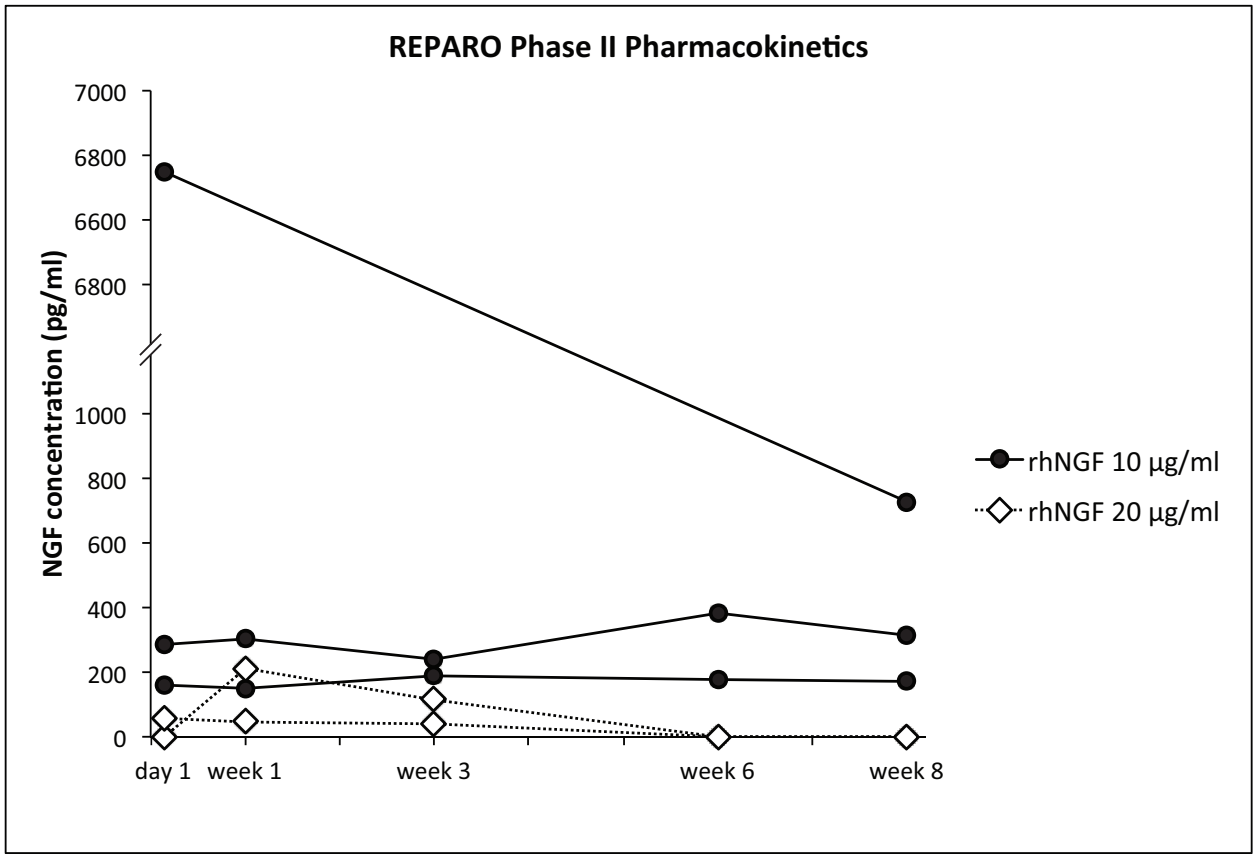


Figure 7. REPARO Phase II Pharmacokinetics.

Serum concentration of nerve growth factor (NGF) plotted over time for patients in the REPARO Phase II study. Blood samples were taken prior to the initial daily dose for PK profiling from approximately the first 90 patients receiving recombinant human NGF (rhNGF) or vehicle, at various time points during the 8-week controlled treatment period (day 1, week 1, week 3, week 6, and week 8). The lower limit of quantification (LLQ) was 32.000 pg/mL; concentrations <LLQ are considered as 0 pg/mL for the purpose of these plots. A total of 5 patients had NGF concentrations >LLQ and are depicted in this figure. In the rhNGF 10 µg/ml group (●), 3 patients had measurable serum NGF concentrations: 1 patient at day 0 (6750.7 pg/ml) and at week 8 (728.6 pg/ml), and 2 patients had concentrations >LLQ at all time points. In the rhNGF 20 µg/ml group (◇), 1 had NGF concentrations >LLQ at day 0, week 1, and week 3; 1 patient had NGF concentrations >LLQ at weeks 1 and 3. In the vehicle group (not shown), no patients had detectable serum NGF at any time point tested.



Appendix 1. REPARO Study Group and Administration

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Appendix 2. REPARO Phase II Study: Complete Eligibility Criteria

Inclusion Criteria

Individuals who met all of the following conditions were eligible for enrollment in this study:

1. Patients 18 years of age or older.
2. Patients with Stage 2 PED or Stage 3 (corneal ulcer) neurotrophic keratitis involving only 1 eye. Patients with contralateral eye affected with Stage 1 NK could be enrolled.
3. PED or corneal ulceration of at least 2 weeks duration refractory to one or more conventional non-surgical treatments for neurotrophic keratitis (eg, preservative-free artificial tears, gels or ointments; discontinuation of preserved topical drops and medications that can decrease corneal sensitivity; therapeutic contact lenses).
4. 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.
5. Best corrected distance visual acuity (BCDVA) score ≤ 75 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, (≥ + 0.2 LogMAR, ≤ 20/32 Snellen or ≤ 0.625 decimal fraction) in the affected eye.
6. No objective clinical evidence of improvement in the PED or corneal ulceration within the 2 weeks prior to study enrolment.
7. Only patients who satisfied all Informed Consent requirements could be included in the study. The patient and/or his/her legal representative must have read, signed and dated the Informed Consent document before any study-related procedures were performed.

The Informed Consent form signed by patients and/or legal representative must have been approved by the IEC/IRB for the current study.

8. Patients must have had the ability and willingness to comply with study procedures.

Patients must have been eligible for the National Health Insurance (where applicable).

Exclusion Criteria

Individuals who met any of the following conditions were excluded from this study:

1. Patients with Stage 2 or 3 NK affecting both eyes.
2. Any active ocular infection (bacterial, viral, fungal or protozoal) or active ocular inflammation not related to NK in the affected eye.
3. 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.
4. 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.
5. Schirmer test without anesthesia ≤ 3 mm/5 minutes in the affected eye.
6. Patients with severe blepharitis and/or severe meibomian gland disease in the affected eye.
7. History of any ocular surgery (including laser or refractive surgical procedures) in the affected eye within the three months before study enrolment. (An exception to the preceding statement was allowed if the ocular surgery was considered to be the cause of the Stage 2 or 3 NK). Ocular surgery in the affected eye was not allowed during the study

treatment period and elective ocular surgery procedures should not have been planned during the duration of the follow-up period.

8. Prior surgical procedure(s) for the treatment of NK (eg, complete tarsorraphy, conjunctival flap, etc) in the affected eye with the exception of amniotic membrane transplantation. Patients previously treated with amniotic membrane transplantation could only be enrolled 2 weeks after the membrane had disappeared within the area of the PED or corneal ulcer or at least 6 weeks after the date of the amniotic membrane transplantation procedure. Patients previously treated with Botox (botulinum toxin) injections used to induce pharmacologic blepharoptosis were eligible for enrolment only if the last injection was given at least 90 days prior to enrolment in the study.
9. Use of therapeutic contact lenses or contact lens wear for refractive correction during the study treatment periods in the eye with NK.
10. 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.
11. Evidence of corneal ulceration involving the posterior third of the corneal stroma, corneal melting or perforation in the affected eye.
12. 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).

13. 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). These treatments were allowed during the study if initiated prior to 30 days before study enrolment provided they remained stable throughout the course of the study treatment periods.
14. Known hypersensitivity to one of the components of the study or procedural medications (eg, fluorescein).
15. History of drug, medication or alcohol abuse or addiction.
16. Use of any investigational agent within 4 weeks of Baseline visit.
17. Participation in another clinical study at the same time as the present study.
18. Females of childbearing potential (those who are not surgically sterilized or post-menopausal for at least 1 year) were excluded from participation in the study if they met any 1 of the following conditions:
    a. were currently pregnant or,
    b. had a positive result on the urine pregnancy test at the Randomization Visit or,
    c. intended to become pregnant during the study treatment period or,
    d. were breast-feeding or,
    e. not willing to use highly effective birth control measures, such as: Hormonal contraceptives –oral, implanted, transdermal, or injected and/or Mechanical barrier methods –spermicide in conjunction with a barrier such as a condom or diaphragm or intra-uterine device (IUD) during the entire course of and 30 days after the study treatment periods.

Appendix 3. REPARO phase II study: prior treatments for neurotrophic keratitis

Treatment, n (%)	rhNGF 10 µg/ml (N=52)	rhNGF 20 µg/ml (N=52)	Vehicle Control (N=52)	Total (N=156)
Artificial tears/gels/ointments	24 (46.2)	29 (55.8)	26 (50.0)	79 (50.6)
Preservative free artificial tears/gels/ointments	27 (51.9)	20 (38.5)	24 (46.2)	71 (45.5)
Topical antibiotics	30 (57.7)	32 (61.5)	33 (63.5)	95 (60.9)
Discontinuation of topical medications	0	2 (3.8)	0	2 (1.3)
Therapeutic contact lens	13 (25.0)	23 (44.2)	11 (21.2)	47 (30.1)
Anti-cholinergic agents	7 (13.5)	2 (3.8)	4 (7.7)	13 (8.3)
Autologous serum eye drops	4 (7.7)	5 (9.6)	5 (9.6)	14 (9.0)
Botulinum a toxin injections	2 (3.8)	1 (1.9)	2 (3.8)	5 (3.2)
Cyanoacrylate glue	2 (3.8)	0	0	2 (1.3)
Collagenase inhibitors	1 (1.9)	0	2 (3.8)	3 (1.9)
Tarsorraphy	2 (3.8)	2 (3.8)	1 (1.9)	5 (3.2)
Conjunctival flap procedure	0	1 (1.9)	0	1 (0.6)
Amniotic membrane transplantation	4 (7.7)	5 (9.6)	3 (5.8)	12 (7.7)
Other	18 (34.6)	20 (38.5)	28 (53.8)	66 (42.3)

Abbreviations: rhNGF = recombinant human nerve growth factor
Percentages are calculated using the population number in each treatment group as the denominator.
Patients may be counted under more than one category.

Appendix 4. REPARO Phase II Study: Additional Safety Results

Treatment-related adverse events (TAEs)

Controlled treatment period

See text.

Uncontrolled treatment period

Overall, 4 patients (17.4%) reported 8 treatment-related AEs during the uncontrolled treatment period in the phase II segment of the study: 2 patients (20.0%) in the rhNGF 10 μg/ml group and 2 patients (15.4%) in the rhNGF 20 μg/ml group. Six of the 8 treatment related AEs were in the class of eye disorders (all in the rhNGF 20 μg/ml group), 1 treatment-related AE was disease progression (rhNGF 10 μg/ml group), and 1 treatment-related AE was increased blood creatinine (rhNGF 10 μg/ml group).

Follow-up period

Overall, 2 patients (1.3%) reported 3 treatment-related AEs during the follow-up period in the phase II segment of the study: 1 patient in the rhNGF 10 μg/ml group experienced a treatment-related AE of vital dye staining cornea present, and 1 patient in the vehicle control group had treatment-related AEs of dry eye and eyelid pain.

Serious adverse events (SAEs) leading to death

Controlled treatment period

Two patients experienced SAEs of malignant neoplasm progression leading to death during the controlled treatment period: One patient receiving rhNGF 10 μg/ml died due to evolution of squamous cell carcinoma, and one patient receiving rhNGF 20 μg/ml group died due to lung cancer progression. Neither death was considered by the investigator to be related to study treatment.

Uncontrolled treatment period

No deaths occurred during the uncontrolled treatment period.

Follow-up period

Six patients experienced SAEs leading to death during the follow-up period: four patients who received rhNGF 10 μg/ml (cardiac failure; myocardial infarction; arrhythmia and dyspnea; and aortic dissection, aortic rupture, and hemorrhagic shock), one patient who received rhNGF 20 μg/ml (respiratory failure), and one patient who received vehicle (respiratory failure). All deaths were considered unrelated to study treatment.