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

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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 impaired corneal innervation.

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

Participants: Patients with stage 2 (moderate) or stage 3 (severe) NK in 1 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 treatment, whereas 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 by masked central readers at week 4 (primary efficacy end point) and week 8 (key secondary end point) of controlled treatment. Corneal healing was reassessed 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 end point), 19.6% of vehicle-treated patients achieved corneal healing (<0.5-mm lesion staining) versus 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 end point), 43.1% of vehicle-treated patients achieved less than 0.5-mm lesion staining versus 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. More than 96% of patients who 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. *Ophthalmology 2018;125:1332-1343 © 2018 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

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With approximately 7000 nerve endings per square millimeter, 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 (NK) with partial or total loss of corneal sensation, leading to visual impairment and potentially permanent blindness. Neurotrophic keratitis, also known as neurotrophic keratopathy, is a rare disease (estimated prevalence, 1.6−4.2 cases per 10 000 persons)[3,4] with various underlying causes (most commonly herpetic infections and ocular or neurologic surgeries) that impair corneal innervation.[5,6] Neurotrophic keratitis diagnosis, prognosis, and treatment (reviewed elsewhere)[3,6] are based on disease severity, which is classified broadly into 3 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 subepithelial (stromal) tissue,

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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 resulting from 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 or 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] Nerve growth factor 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 2 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 1 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 *Escherichia 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] Herein, we report phase II study results of topical rhNGF treatment for moderate-to-severe NK.

Methods

Clinical Trial Design

The REPARO (Latin for "repair") trial was a phase I/II double-masked, randomized, multicenter, vehicle-controlled, parallel-group study that 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, predefined as failure to achieve corneal healing, recurrence of NK after healing, or deterioration (lesion size increase of ≥1 mm, best-corrected distance visual acuity [BCDVA] decrease of >5 Early Treatment Diabetic Retinopathy Study [ETDRS] letters, progression to corneal melting or 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 (available at www.aaojournal.org), 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) disease using published criteria.[7] The main inclusion criteria were evidence of decreased corneal sensitivity within the corneal lesion and 1 or more corneal quadrants outside the lesion; BCDVA score of 75 ETDRS letters or fewer (≥0.2 logarithm of the minimum angle of resolution, ≤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 before study enrollment. The main exclusion criteria were stage 2 or 3 NK affecting both eyes, active ocular infection or inflammation unrelated to NK, or other ocular disease or severe vision loss in the affected eye. For complete inclusion and exclusion criteria, see Appendix 2 (available at www.aaojournal.org).

Efficacy Assessments

The primary efficacy variable was corneal healing, defined as less than 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-or-no binary variable at week 4 (primary end point) and week 8 (prespecified secondary end point). 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 or uncontrolled treatment periods. Post hoc efficacy variables included change in lesion size and the primary end point of corneal healing reassessed more conservatively by masked central readers 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 analog scale (VAS) from 0 to 100 mm (0 = no symptoms; 100 = worst possible discomfort) for each of 7 different symptoms: foreign body sensation, burning or 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, dilated fundus ophthalmoscopy, vital signs, hematologic results, and clinical chemistry results.

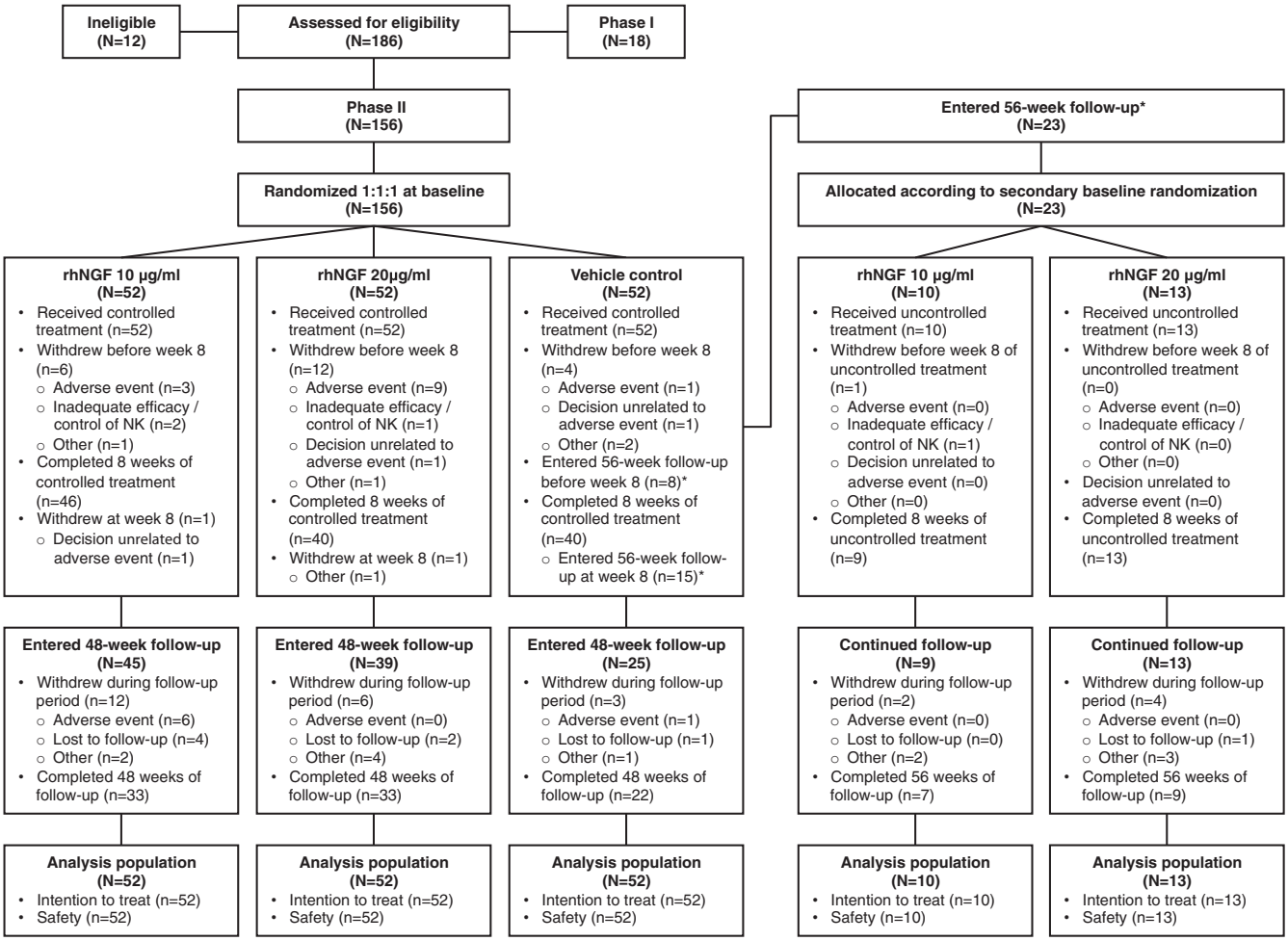

Figure 1. Diagram showing the REPARO phase II study design and overall patient disposition. The REPARO phase II study enrolled 156 patients with neurotrophic keratitis (NK) of severity stage 2 (persistent epithelial defect) or stage 3 (corneal ulcer). Patients were randomized 1:1:1 to 10 µg/ml recombinant human nerve growth factor (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 Clinical Trial Design section 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.

Pharmacokinetics and Immunogenicity Assessments

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

Masking and Statistical Analysis

Patients, investigators, and site or sponsor staff were masked to primary randomized treatment and to the dosage of randomized secondary treatment. Indistinguishable kits for dispensing rhNGF or vehicle were assigned randomly 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 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 less than 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 adjusted to the Pocock threshold ($\alpha = 0.0294$),[18] yielding a 97.06% confidence interval (CI) for the primary efficacy end point 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 a 10% to 20% dropout rate. Efficacy analyses were performed on intention-to-treat populations, with missing data after baseline using the last observation carried forward. Also conducted were observed-case analyses and sensitivity analyses, with missing observations after baseline imputed as failures and by multiple imputation procedures MI and MIANALYZE in SAS software (SAS Institute, Cary, NC).

Table 1. Patient Demographics and Baseline Characteristics

Characteristics	Recombinant Human Nerve Growth Factor		Vehicle (N = 52)
	10 μg/ml (N = 52)	*20 μg/ml (N = 52)*	
Age (yrs)			
Mean (SD)	59.0 (17.17)	62.5 (14.01)	60.4 (16.78)
Median (minimum–maximum)	61.5 (20–87)	63.5 (18–95)	60.5 (23–91)
Female gender, no. (%)	30 (57.7)	30 (57.7)	35 (67.3)
Ethnicity, no. (%)			
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, no. (%)			
Asian	1 (1.9)	0	1 (1.9)
Black	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, no. (%)			
Stage 2	21 (40.4)	27 (51.9)	28 (53.8)
Stage 3	31 (59.6)	25 (48.1)	24 (46.2)
Underlying cause, no. (%)			
Diabetes mellitus	3 (5.8)	4 (7.7)	4 (7.7)
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 for 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 resulting from 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

(*Continued*)

Table 1. (Continued.)

Characteristics	Recombinant Human Nerve Growth Factor		Vehicle (N = 52)
	10 μg/ml (N = 52)	20 μg/ml (N = 52)	
Venous sinus thrombosis	1 (1.9)	0	0
Viral conjunctivitis (unspecified)	0	0	1 (1.9)

HSV = herpes simplex virus; N/A = not available (ethnicity and race were not collected in all countries); NK = neurotrophic keratitis; SD = standard deviation.
*Includes herpes simplex, herpes zoster, and recurrent herpetic keratitis.

For binary secondary and exploratory efficacy end points, 2-sided significance was set at $\alpha = 0.05$. Change in BCDVA score from baseline to week 8 was analyzed by an analysis of covariance 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 weeks 4 and 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 also were 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 of Sapienza University of Rome and an independent ethics committee from each country with 1 or more participating sites (Appendix 1, available at www.aaojournal.org). The study complied with the Declaration of Helsinki, relevant parts of the Code of Federal Regulations Title 21, and good clinical practice and good laboratory practice guidelines. Written informed consent was obtained before study-related procedures. Compliance was assessed at each visit and verified by study monitors during onsite visits.

Results

Patients and Treatment

The REPARO investigators (Appendix 1, available at www.aaojournal.org) 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 or more patients. Figure 1 provides an overview of patient disposition (including reasons for withdrawal). Of 186 patients screened from January 2013 through 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 causes included herpetic eye disease (44 patients) and ocular or neurologic surgery (21 patients each). Prior treatments for NK (most commonly artificial tears, gels, or ointments and topical antibiotics) are listed in Appendix 3 (available at www.aaojournal.org).

Efficacy Outcomes

Table 2 summarizes efficacy analyses at weeks 4 and 8 (last observation carried forward). Corneal healing (<0.5-mm lesion staining) was achieved at week 4 (primary end point) in 19.6% of vehicle-treated patients versus 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 end point) was achieved in 43.1% of vehicle-treated patients versus 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 observations after baseline 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 (Fig 2B). At week 4, least squares mean 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 versus vehicle-treated patients, trending toward significance in rhNGF 20 μg/ml versus vehicle at week 8 ($P = 0.102$; 95% CI, −109.61% to 9.98%).

Visual acuity outcomes were assessed as changes from baseline to week 8. As shown in Figure 3, compared with vehicle-treated patients, least squares mean 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 in those receiving rhNGF 20 μg/ml ($P = 0.213$). However, the difference between rhNGF doses was not significant ($P = 0.305$). Best-corrected distance visual acuity assessed as a gain of 15 ETDRS letters (yes or no) from baseline to week 8 produced similar results (Table 4). Compared with 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

Table 2. Primary Efficacy Analysis of Corneal Healing (<0.5-mm Lesion Staining)

Results	Recombinant Human Nerve Growth Factor		Vehicle (N = 52)*
	*10 μg/ml (N = 52)**	*20 μg/ml (N = 52)**	
Healed at week 4, no. (%)	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 (rhNGF 20 μg/ml − rhNGF 10 μg/ml), %	3.1		
97.06% CI	−18.38 to 24.58		
P value	0.754		
Healed at week 8, no. (%)	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 (rhNGF 20 μg/ml − rhNGF 10 μg/ml), %	−0.5		
97.06% CI	−19.46 to 18.44		
P value	0.953		

CI = confidence interval; rhNGF = recombinant human nerve growth factor.
*Number of patients randomized to each treatment.

testing of reflex tearing as an exploratory variable. Compared with vehicle, more patients receiving rhNGF 10 or 20 μg/ml exhibited improvement in corneal sensitivity (measured by CBA) from baseline to weeks 4 and 8, but the differences between treatment groups were not significant (Table S5, available online at www.aaojournal.org). Figure S4 (available at www.aaojournal.org) shows results of Schirmer tests of reflex tearing. Least squares mean change from baseline was greater in the rhNGF-treated groups compared with 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 also were 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 days), compared with 8 days in patients receiving rhNGF 10 μg/ml (95% CI, 7–14 days; $P = 0.002$) and 14 days in patients receiving rhNGF 20 μg/ml (95% CI, 7–14 days; $P = 0.015$). For time to corneal healing (<0.5-mm lesion staining), median time was 56 days (95% CI, 42 days–not estimable) in patients receiving vehicle, compared with 29 days in patients receiving rhNGF 10 μg/ml (95% CI, 20–55 days; $P = 0.002$) and 28 days in patients receiving rhNGF 20 μg/ml (95% CI, 19–55 days; $P = 0.002$).

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

Table 3. Post Hoc Efficacy Analysis of Corneal Healing (0-mm Lesion Staining, No Other Persistent Staining)

Results	Recombinant Human Nerve Growth Factor		Vehicle (N = 52)*
	*10 μg/ml (N = 52)**	*20 μg/ml (N = 52)**	
Healed at week 4, no. (%)	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 (rhNGF 20 μg/ml − rhNGF 10 μg/ml), %	9.0		
97.06% CI	−12.55 to 30.51		
P value	0.366		
Healed at week 8, no. (%)	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 (rhNGF 20 μg/ml − rhNGF 10 μg/ml), %	9.3		
97.06% CI	−10.96 to 29.47		
P value	0.321		

CI = confidence interval; rhNGF = recombinant human nerve growth factor.
*Number of patients randomized to each treatment.

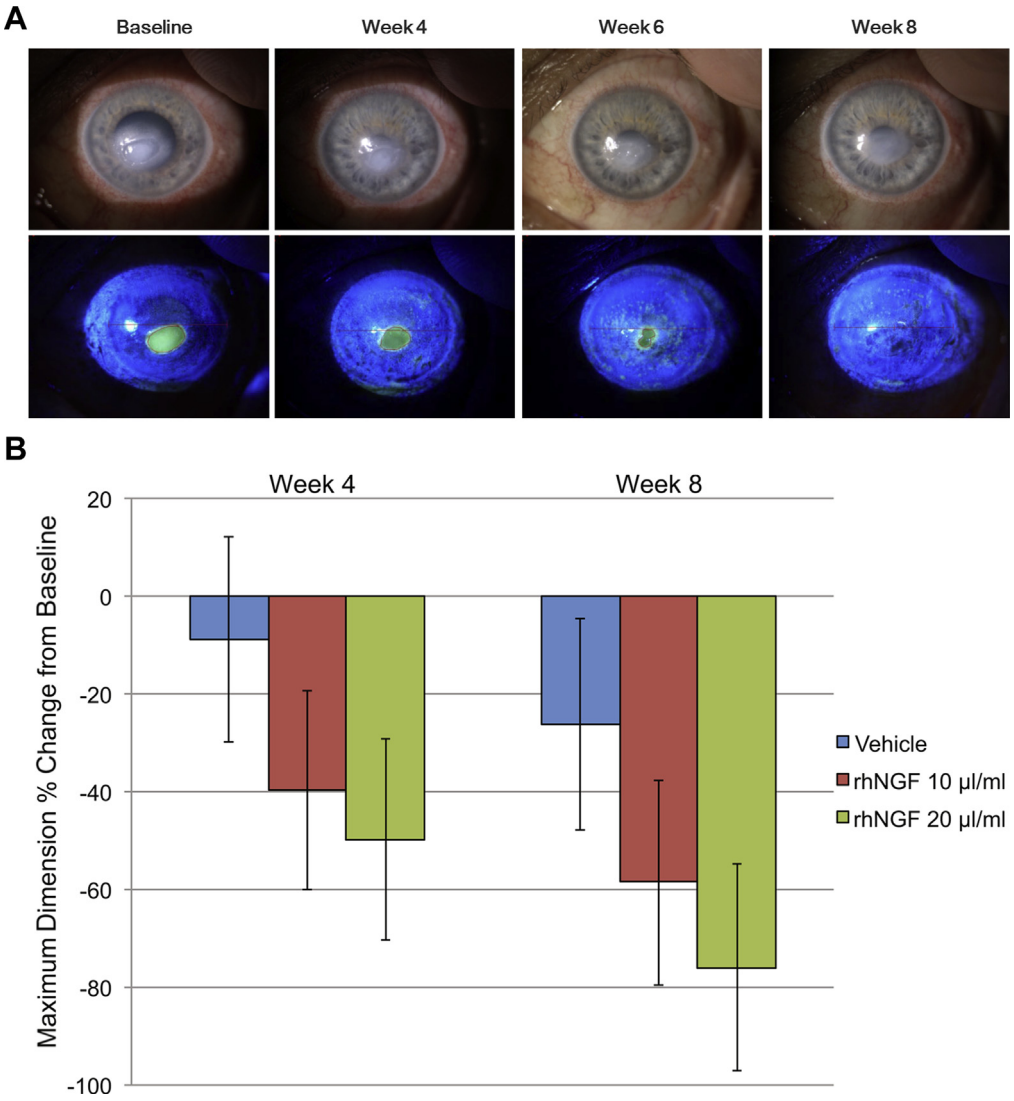

Figure 2. Images showing assessment of corneal lesion size on clinical pictures. **A**, Representative images showing the progression of a typical oval, paracentral, neurotrophic corneal lesion from baseline through week 8 in a patient treated with 20 μg/ml recombinant human nerve growth factor (rhNGF). **Top row**, Photographs 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*) photographs obtained under cobalt-blue light illumination. **B**, Bar graph showing post hoc analysis of least squares mean percentage change from baseline in maximum dimension of persistent epithelial defect or corneal ulcer after the 8-week controlled treatment period. Error bars represent standard error. Magnitude change in lesion size was greater in patients in the rhNGF treatment groups compared with the vehicle group (not reaching statistical significance), with a trend toward significance in 20 μg/ml rhNGF versus vehicle treatment at week 8 ($P = 0.102$; 95% confidence interval, −109.61 to 9.98).

(Fig 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 of 10 patients (30%) receiving 10 μg/ml rhNGF and in 8 of 13 patients (61.5%) receiving 20 μg/ml rhNGF. Figure S6 (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 days) in the 10-μg/ml rhNGF group and 7 days (range, 7−42 days) 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 days) and 21 days (range, 7−42 days) 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 of 20 patients in the vehicle group (4.8%), 1 of 27 patients in the rhNGF 10-μg/ml group (3.6%), and 1 of 28 patients in the rhNGF 20-μg/ml group (3.4%). Of patients who healed after uncontrolled treatment and completed 56 weeks of follow-up, recurrence was experienced by 0 of 4 patients in the rhNGF 10-μg/ml group and 2 of 6 patients (33%) in the rhNGF 20-μg/ml group.

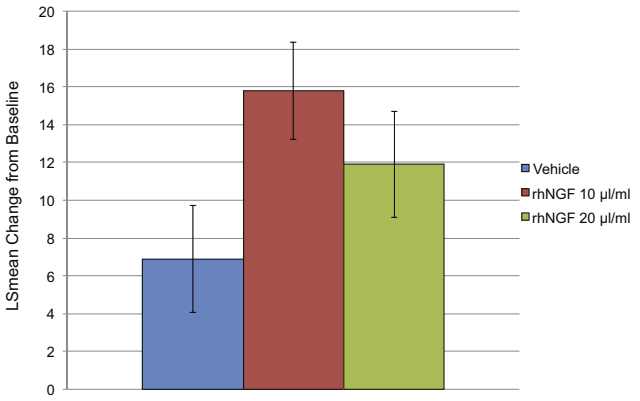

Figure 3. Bar graph showing secondary efficacy analysis of visual acuity score during controlled treatment. Least squares mean (LSmean) change from baseline in best-corrected distance visual acuity measured in Early Treatment Diabetic Retinopathy Study letters was analyzed using an analysis of covariance model (treatment + baseline score). Compared with vehicle-treated patients, LSmean change from baseline to week 8 was greater in the rhNGF-treated groups, with the difference reaching statistical significance between patients receiving vehicle and those receiving 10 µg/ml recombinant human nerve growth factor (rhNGF; $P = 0.022$), but not 20 µg/ml rhNGF ($P = 0.213$). However, the comparison between rhNGF doses also was not significant ($P = 0.305$).

Safety Outcomes

Table 6 summarizes treatment-related AEs 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 (treatment-related AEs during uncontrolled treatment and follow-up periods) are presented in Appendix 4 (available at www.aaojournal.org). Overall, 17 patients (10.9%) experienced serious AEs during controlled treatment: 3 receiving rhNGF 10 µg/ml, 9 receiving rhNGF 20 µg/ml, and 5 receiving vehicle. No serious AEs were considered related to study treatment.

Changes from baseline VAS scores were analyzed by repeated-measures analysis of covariance (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 patients at week 4, 4 patients at week 6, and 6 patients at week 8), versus 4 receiving rhNGF 10 µg/ml (1 patient at week 4, 1 patient at week 6, and 2 patients at week 8) and 4 receiving rhNGF 20 µg/ml (1 patient at week 4, no patients at week 6, and 3 patients at week 8).

Eight deaths were reported during the study: 2 during controlled treatment (1 receiving rhNGF 10 µg/ml and 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 (Appendix 4, available at www.aaojournal.org) were considered unrelated to study treatment.

Pharmacokinetics and Immunogenicity

As shown in Figure S7 (available at www.aaojournal.org), only 5 patients (3 receiving rhNGF 10 µg/ml and 2 receiving rhNGF 20 µg/ml) demonstrated serum NGF concentrations more than the lower limit of quantification of 32.000 pg/ml at any time point tested. 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 or uncontrolled treatment periods or follow-up.

Discussion

This study demonstrated that topical rhNGF safely and effectively improves corneal epithelial integrity in moderate

Table 4. Secondary Efficacy Analysis of Patients Achieving 15-Letter Gains in Best-Corrected Distance Visual Acuity

Results	Recombinant Human Nerve Growth Factor		Vehicle (N = 52)*
	*10 µg/ml (N = 52)**	*20 µg/ml (N = 52)**	
15-letter gain in BCDVA at week 4, no. (%)	18/49 (36.7)	14/41 (34.1)	9/43 (20.9)
Difference (rhNGF − vehicle), %	15.8	13.2	
95% CI	−2.36 to 33.97	−5.72 to 32.15	
P value	0.097	0.175	
Difference (rhNGF 20 µg/ml − rhNGF 10 µg/ml), %	−2.6		
95% CI	−22.41 to 17.23		
P value	0.798		
15-letter gain in BCDVA at week 8, no. (%)	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 to 38.83	
P value	0.008	0.068	
Difference (rhNGF 20 µg/ml − rhNGF 10 µg/ml), %	−8.5		
95% CI	−29.21 to 12.14		
P value	0.421		

BCDVA = best-corrected distance visual acuity; CI = confidence interval; rhNGF = recombinant human nerve growth factor.
Patients without a yes-or-no response available at week 4 and week 8 are not considered in this table. The significance level is 0.05.
*Number of patients randomized to each treatment.

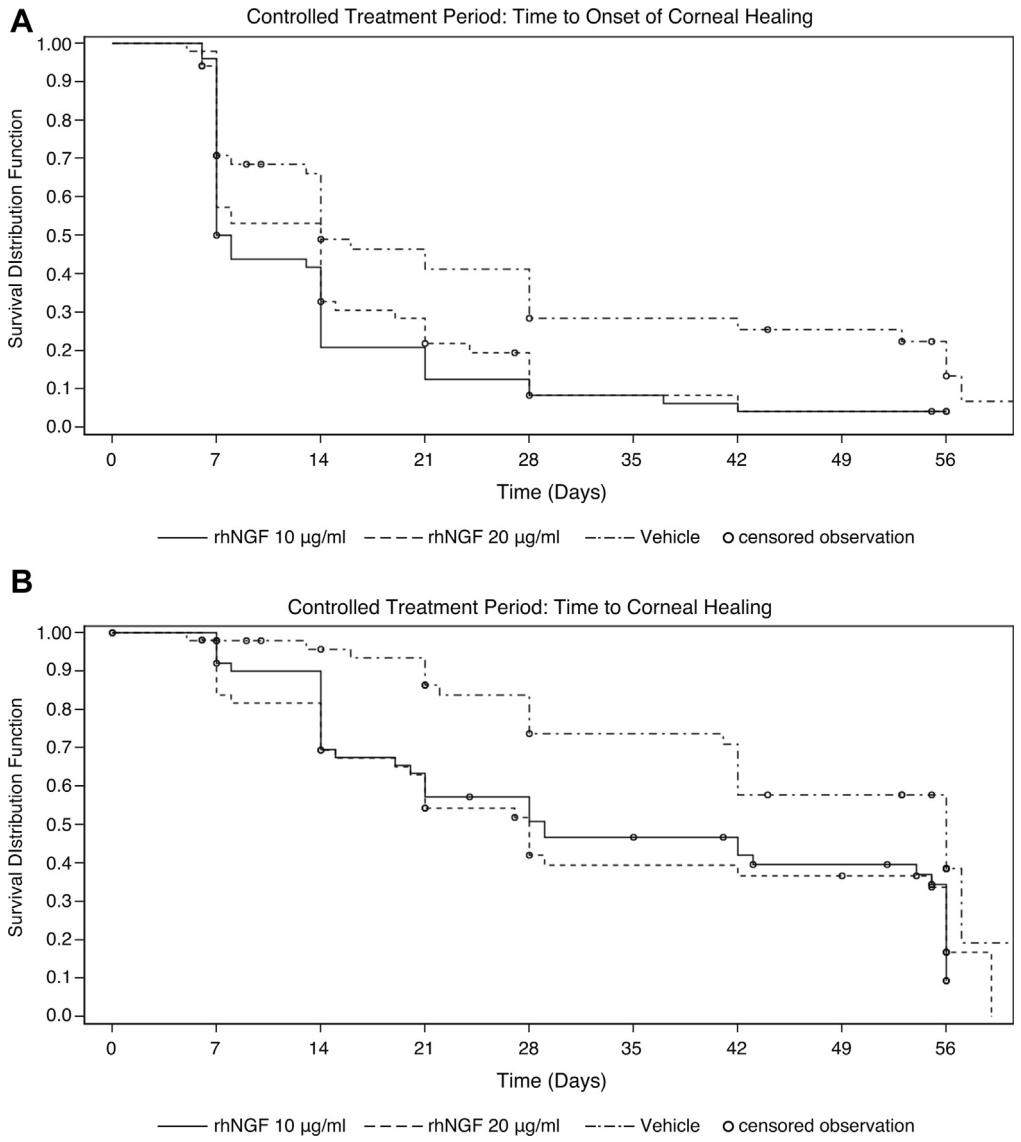

Figure 5. Exploratory analyses of Kaplan-Meier time-to-event variables during controlled treatment. **A**, Median time to onset of healing (>20% reduction in maximum diameter of the corneal lesion from baseline) was 14 days in patients receiving vehicle (95% confidence interval [CI], 14–28 days), versus 8 days in patients receiving rhNGF 10 mg/ml (95% CI, 7–14 days; $P = 0.002$) and 14 days in patients receiving rhNGF 20 mg/ml (95% CI, 7–14 days; $P = 0.015$). **B**, Median time to corneal healing (<0.5-mm lesion staining) was 56 days (95% CI, 42 days–not estimable) in patients receiving vehicle, versus 29 days in patients receiving rhNGF 10 mg/ml (95% CI, 20–55 days; $P = 0.002$) and 28 days in patients receiving rhNGF 20 mg/ml (95% CI, 19–55 days; $P = 0.002$). rhNGF = recombinant human nerve growth factor.

to severe NK, confirming results achieved using mNGF.[12,13] Although previous reports demonstrated clinical effectiveness of mNGF 200 µg/ml,[12,13] preclinical pharmacologic tests demonstrated higher potency of *E. coli*–derived rhNGF versus mNGF: notably, higher affinity for human TrkA (high-affinity NGF receptor) and approximately 10-fold potency in inducing proliferation of human TF1 cells expressing TrkA (Dompé Farmaceutici SpA, unpublished data, 2012). 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 to 8 weeks of treatment. Healing was maintained through follow-up for more than 96% of rhNGF-treated patients.

The use of intense topical lubricants and close follow-up in vehicle-treated patients showed 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). Because healthy corneas may demonstrate some degree of corneal staining,[20] we compared 2 different

Table 6. Summary of Treatment-Related Adverse Events* by System Organ Class and Preferred Term (Controlled Treatment Period)

Body System (MedDRA Preferred Term)	Recombinant Human Nerve Growth Factor				Vehicle (N = 52)†	
	10 μg/ml (N = 52)†		20 μg/ml (N = 52)†			
	No. of Events Reported	No. of Patients (%)	No. of Events Reported	No. of Patients (%)	No. of Events Reported	No. of Patients (%)
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

MedDRA = Medical Dictionary for Regulatory Activities.
Percentages are calculated using the population number in each treatment group as the denominator.
*Treatment-related adverse events are those events recorded by the investigator as having a possible, probable, or highly probable relationship to study treatment.
†Number of patients who received each treatment in the specified study period.
‡Disease progression was defined as increase in lesion size of 1 mm or more, decrease in best-corrected distance visual acuity by more than 5 Early Treatment Diabetic Retinopathy Study letters, progression in lesion depth to corneal melting or perforation, or onset of infection.

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 also was supported by improvement on other clinically relevant end points, including corneal lesion size, time to corneal healing (or onset of healing), BCDVA, corneal sensitivity measured by CBA, and reflex tearing (which also may 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-or-no) primary end point and was 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 with the vehicle group in some secondary end points (such as visual acuity and reflex tearing); however, for the same end points, differences between rhNGF doses did not reach statistical

significance. Thus, it is difficult to draw conclusions on dose responsiveness. Nonetheless, patients receiving rhNGF generally had better trends of improvement for most efficacy end points versus patients receiving vehicle.

Visual acuity was assessed as a secondary efficacy end point, although it does not necessarily reflect NK severity or healing status. For example, in stage 2 NK, absence of the epithelium may have little or no impact on vision, whereas re-epithelialization in the central or paracentral cornea can cause optical aberrations (and hence reduced vision). Figure 2A illustrates this latter point; it would not be surprising that this patient still had reduced vision after 8 weeks of controlled rhNGF 20-μg/ml treatment, despite achieving corneal healing with 0-mm lesion staining and no other persistent staining.

No safety concerns arose; most AEs were ocular, mild, and transient and did not require discontinuing or corrective treatments. The predominant treatment-related AE was eye pain; others included abnormal sensation in the eye, excess lacrimation, photophobia, eyelid pain, and eye or eyelid irritation, which may reflect therapeutic actions of rhNGF and normal healing. Indeed, restoring corneal innervation and sensitivity (which, in turn, will promote corneal healing) can be associated with increased ocular surface symptoms. No immunogenicity to NGF was detected in this study; furthermore, consistent with phase I results,[16,17] most patients showed undetectable serum NGF, no systemic AEs, or both. Taken together, these pharmacokinetic and immunogenicity results suggest unlikely systemic absorption or accumulation of topical ophthalmic rhNGF.

Neurotrophic keratitis is a challenging disease with a high unmet need for treatments that improve corneal sensitivity (which is crucial for restoring corneal epithelial integrity) and promote healing without surgery or compromising vision. In this study, topical rhNGF demonstrated favorable benefit-to-risk ratios for patients with moderate-to-severe NK, confirming that rhNGF is a feasible approach to treating NK. The neuroprotective effects of rhNGF also may be extended to other ophthalmic indications with neurodegenerative components, including glaucoma,[21] macular degeneration,[22] and retinitis pigmentosa.[23]

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F.S.: Consultant and Employee — Dompé Farmaceutici SpA (Milan, Italy)

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HUMAN SUBJECTS: Human subjects were included in this study. The institutional review board of Sapienza University of Rome and an independent ethics committee from each country with 1 or more participating sites approved the study, and informed consent to participate in the study was obtained from all patients. The study was performed in accordance with the tenets of the Declaration of Helsinki, Code of Federal Regulations, and Good Clinical Practice guidelines.

No animal subjects were used in this study.

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Overall responsibility: Bonini, Lambiase, Rama, Sinigaglia, Allegretti, Chao, Mantelli

Abbreviations and Acronyms:
AE = adverse event; **BCDVA** = best-corrected distance visual acuity; **CBA** = Cochet-Bonnet aesthesiometer; **CI** = confidence interval; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **mNGF** = murine nerve growth factor; **NGF** = nerve growth factor; **NK** = neurotrophic keratitis; **PED** = persistent epithelial defect; **rhNGF** = recombinant human nerve growth factor; **SE** = standard error; **VAS** = visual analog scale.

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