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Topical Recombinant Human Nerve Growth Factor (Cenegermin) for Neurotrophic Keratopathy

A Multicenter Randomized Vehicle-Controlled Pivotal Trial

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Purpose: To evaluate the efficacy and safety of topical cenegermin (recombinant human nerve growth factor) in patients with neurotrophic keratopathy.

Design: Multicenter, randomized, double-masked, vehicle-controlled trial.

Participants: Patients with neurotrophic persistent epithelial defect with or without stromal thinning.

Methods: The NGF0214 trial, conducted among 11 sites in the United States, randomized 48 patients 1:1 to cenegermin 20 μg/ml or vehicle eye drops, 6 drops daily for 8 weeks of masked treatment. Follow-up was 24 weeks. Safety was assessed in all patients who received study drug. Efficacy was assessed by intention to treat.

Main Outcome Measures: The primary end point was healing of the neurotrophic lesion (persistent epithelial defect or corneal ulcer) after 8 weeks of masked treatment. Masked central readers measured neurotrophic lesions in randomized clinical pictures, then assessed healing status conventionally (<0.5 mm of fluorescein staining in the greatest dimension of the lesion area) and conservatively (0-mm lesion staining and no other residual staining). Secondary variables included corneal healing at 4 weeks of masked treatment (key secondary end point), overall changes in lesion size, rates of disease progression, and changes in visual acuity and corneal sensitivity from baseline to week 8.

Results: Conventional assessment of corneal healing showed statistically significant differences at week 8: compared to 7 of 24 vehicle-treated patients (29.2%), 16 of 23 cenegermin-treated patients (69.6%) achieved less than 0.5 mm of lesion staining (+40.4%; 95% confidence interval [CI], 14.2%−66.6%; $P = 0.006$). Conservative assessment of corneal healing also reached statistical significance at week 8: compared to 4 of 24 vehicle-treated patients (16.7%), 15 of 23 cenegermin-treated patients (65.2%) achieved 0 mm of lesion staining and no other residual staining (+48.6%; 95% CI, 24.0%−73.1%; $P < 0.001$). Moreover, the conservative measure of corneal healing showed statistical significance at week 4 (key secondary end point). Compared to vehicle, cenegermin-treated patients showed statistically significant reductions in lesion size and disease progression rates during masked treatment. Cenegermin was well tolerated; adverse effects were mostly local, mild, and transient.

Conclusions: Cenegermin treatment showed higher rates of corneal healing than vehicle in neurotrophic keratopathy associated with nonhealing corneal defects. *Ophthalmology 2020;127:14-26 © 2019 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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Ocular surface integrity relies on the corneal nerves, which not only enable sensory-mediated reflexes (such as blinking and tearing), but also mediate production of trophic factors that critically help maintain corneal epithelium and the nerves themselves.[1] Corneal nerve damage may disrupt this homeostasis and lead to neurotrophic keratopathy (also known as neurotrophic keratitis or neurotrophic keratoconjunctivitis), a degenerative disease with an estimated prevalence of 1.6 to 4.2 cases per 10 000 persons.[1,2] Various conditions (such as ocular herpetic infection, ocular or neurologic surgery, trauma, diabetes, and dry eye disease) are associated with neurotrophic keratopathy[3,4]; however, correlations between underlying etiologies and neurotrophic keratopathy severity or clinical outcomes (such as visual acuity and corneal sensitivity) remain unclear.[5]

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Clinical interventions for neurotrophic keratopathy (reviewed elsewhere)[2,4,6] vary widely and generally are based on disease severity. Until recently, neurotrophic keratopathy interventions have been limited to nonstandardized treatments, such as preservative-free artificial tears, serum drops, and therapeutic lenses. Surgical interventions (e.g., tarsorrhaphy, amniotic membrane transplantation, conjunctival flap, and corneal transplantation) generally are reserved for refractory cases but tend to be invasive and themselves may compromise vision.

Cenegermin 0.002% ophthalmic solution (Oxervate; Dompé Farmaceutici SpA, Milan, Italy) recently received approval from the European Commission, the United States Food and Drug Administration, and other health authorities for the treatment of neurotrophic keratopathy. Developed based on studies with murine nerve growth factor (NGF) that showed promise in treating corneal neurotrophic ulcers,[7,8] cenegermin is a recombinant human NGF (rhNGF) produced in *Escherichia coli*. Phase 1 randomized, double-masked, vehicle-controlled studies showed topical cenegermin treatment to be safe in healthy volunteers[9] and in patients with neurotrophic keratopathy.[10] The approved indication for cenegermin was based on the results of NGF0212/REPARO (Latin for "repair"), a phase 2, randomized, double-masked, vehicle-controlled, dose-ranging study conducted in Europe and reported previously,[11] and NGF0214, a pivotal trial conducted in the United States. Both trials demonstrated the efficacy and safety of cenegermin in promoting corneal healing in patients with neurotrophic keratopathy associated with persistent epithelial defect (with or without stromal thinning). However, in REPARO, effects on other clinically relevant end points (such as corneal sensitivity, visual acuity, and disease progression) remained unclear. We sought to define further the role of cenegermin on these and other clinical metrics in the NGF0214 study. Herein we report the results of this pivotal trial.

Methods

Clinical Trial Design

NGF0214 was a multicenter, double-masked, randomized, vehicle-controlled, parallel-group, pivotal trial that evaluated the efficacy and safety of cenegermin 20-μg/ml eye drops in patients with neurotrophic keratopathy. Study sites, investigators, and co-investigators are listed in Appendix 1 (available at www.aaojournal.org). Figure 1 depicts the overall design of the trial, which was registered at ClinicalTrials.gov (identifier, NCT02227147).

Patients

The study enrolled adult patients (≥18 years of age) with neurotrophic keratopathy in one or both eyes. For patients with bilateral neurotrophic keratopathy, the worse affected eye at baseline was designated the study eye. The main inclusion criterion was neurotrophic keratopathy classified as stage 2 (persistent epithelial defect) or stage 3 (corneal ulcer) according to published criteria[12] and refractory to 1 or more conventional nonsurgical treatments. The other main inclusion criteria were decreased corneal sensitivity within the corneal lesion and in at least 1 corneal quadrant outside the lesion; best-corrected distance visual acuity (BCDVA) score of 75 Early Treatment Diabetic Retinopathy Study (ETDRS) letters or fewer (≥+0.2 logarithm of the minimum angle of resolution, ≤20/32 Snellen, or ≤0.625 decimal fraction) in the study eye; and no objective clinical evidence of improvement of the neurotrophic lesion within 2 weeks preceding study enrollment. Patients with stage 3 neurotrophic keratopathy that featured severe thinning (beyond two thirds of the cornea) and impending perforation were not included in the study. The other main exclusion criteria were active ocular infection or inflammation unrelated to neurotrophic keratopathy and other ocular disease or severe vision loss in the affected eye(s). Unless it was the underlying etiology of neurotrophic keratopathy, ocular surgery (including laser or refractive surgical procedures) was not allowed within 3 months before study enrollment. Ocular surgery also was not allowed during the study treatment period, and elective ocular surgery procedures could not be planned for the follow-up period. For complete inclusion and exclusion criteria, see Appendix 2 (available at www.aaojournal.org).

Treatment

The test product (cenegermin 20 μg/ml) or reference product (vehicle), each containing methionine as an antioxidant excipient, was provided by the sponsor and was self-administered by patients at a dosage of 1 eye drop (approximately 35 μl) 6 times daily. The duration of masked treatment was 8 weeks. Of patients randomized to vehicle treatment, those who did not achieve corneal healing (<0.5 mm of fluorescein staining in the greatest dimension of the lesion area) by the end of masked treatment were eligible (at the investigator's discretion) to receive cenegermin during an 8-week open-label treatment period. If any patients healed under masked or open-label cenegermin treatment and then experienced disease recurrence at any point during follow-up, they could receive open-label recurrence treatment with cenegermin for 8 weeks at the investigator's discretion. Any patient could receive a maximum of 2 8-week cenegermin treatment courses before continuing follow-up for 24 weeks.

On enrollment, patients were required to discontinue use of all previous topical ophthalmic medications, bandage contact lenses, or both. During either masked or open-label treatment periods, patients were limited to the study medication (provided by the sponsor) and topical ophthalmic medications allowed by the study protocol (not provided by the sponsor). Allowed medications included preservative-free topical antibiotics or topical antiviral medications prescribed at the investigator's discretion. For patients who achieved corneal healing (<0.5 mm of lesion staining) after either masked or open-label treatment, investigators could prescribe preservative-free artificial tears as needed during follow-up.

If, during the 8-week masked treatment phase, the investigator considered any patient to be at imminent risk of deterioration (lesion size increase of ≥1 mm, progression to corneal melting or perforation, BCDVA decrease of >5 ETDRS letters, or onset of infection), the patient could use preservative-free topical antibiotics, preservative-free topical antivirals (not provided by the sponsor), or both in addition to the assigned study medication.

In patients who achieved corneal healing (<0.5 mm of lesion staining), preservative-free artificial tears (not provided by the sponsor) could be prescribed as needed at the investigator's discretion during the 24-week follow-up period. If any patients randomized to cenegermin treatment did not achieve corneal healing (<0.5 mm of lesion staining) by the end of the 8-week masked treatment, they could receive (at the investigator's discretion) any nonexperimental treatment for neurotrophic keratopathy through the 24-week follow-up period.

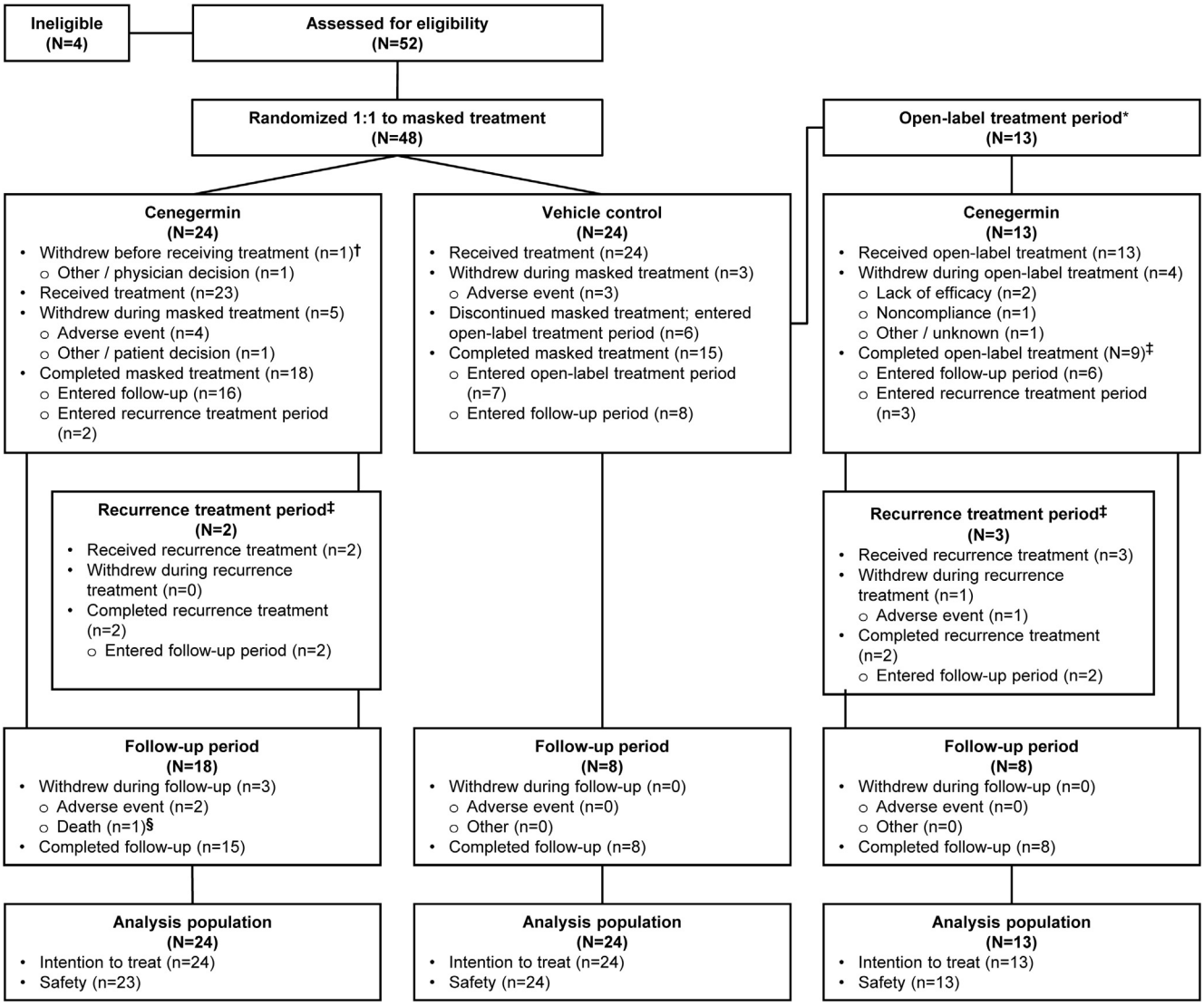

Figure 1. Diagram showing NGF0214 study design and overall patient disposition. The NGF0214 study screened 52 patients and enrolled 48 with neurotrophic keratopathy of severity stage 2 (persistent epithelial defect) or stage 3 (corneal ulcer). Patients were randomized 1:1 to cenegermin (20 μg/ml) or vehicle for an 8-week masked treatment period and 24 weeks of follow-up. *According to the study protocol, patients who did not heal during 8-week masked vehicle treatment were eligible (at the investigator's discretion) to receive cenegermin in an 8-week open-label treatment period before proceeding to follow-up. †One patient gave informed consent and was randomized to cenegermin treatment but withdrew the next day. No study treatment was administered, and no baseline measures for primary efficacy were recorded; thus, this patient was excluded from the last observation carried forward analyses. ‡After 8 weeks of either masked or open-label cenegermin treatment, patients who healed (<0.5 mm of lesion staining) and then experienced a recurrence of persistent epithelial defect or corneal ulcer were eligible (at the investigator's discretion) for one recurrence treatment course of 8 weeks. A maximum of 2 8-week cenegermin treatment courses could be administered to any patient before continuing follow-up for 24 weeks. Of patients originally randomized to vehicle who then completed the open-label treatment period, 4 experienced recurrence; however, 1 patient continued to the end of follow-up without receiving recurrence treatment, so only 3 patients entered the recurrence treatment period. §One patient died 163 days after the last dose of the study drug; the cause of death was unknown and was assessed as unrelated to study treatment.

Efficacy Assessments

The primary efficacy end point was healing of the neurotrophic lesion (persistent epithelial defect or corneal ulcer) after 8 weeks of masked treatment. Corneal healing (defined as <0.5 mm of fluorescein staining in the greatest dimension of the lesion area) was assessed in clinical images as a yes-or-no binary variable by central readers at Cologne Ophthalmological Reading and Image Analysis Center (Cologne, Germany), who were masked to the randomized treatment and image dates. As an additional prespecified primary efficacy end point, corneal healing (yes or no) was assessed by masked central readers according to a more conservative threshold (0 mm of staining in the lesion area and no other persistent staining outside of the lesion area).

A key prespecified secondary end point was corneal healing (yes or no) at week 4 of masked treatment based on masked central readings. Other prespecified secondary end points included percentage change from baseline of lesion size (reading center measurements of greatest dimension of fluorescein staining); change in BCDVA from baseline to week 8, measured at each visit in

ETDRS letters at 4 m (13 feet); change in corneal sensitivity from baseline to week 8, measured in each of 4 corneal quadrants outside the lesion area using the Cochet-Bonnet esthesiometer; Schirmer testing without anesthesia (wetting distance measured in millimeters at 5 minutes); percentage of patients experiencing deterioration (yes or no) from baseline to week 8; and duration of corneal healing in patients who achieved less than 0.5 mm of lesion staining (based on investigator's assessment on slit lamp) after cenegermin treatment.

Safety Assessments

Safety exploratory end points were adverse events, ocular tolerability, intraocular pressure, fundus ophthalmoscopy results, hematologic analysis results, clinical chemistry analysis results, and immunogenicity results. All adverse events reported herein were treatment emergent (i.e., conditions that either arose or worsened in intensity or frequency after initiation of study treatment). An adverse event was considered treatment related if its relationship to the study drug was recorded as possible, probable, or highly probable. If a relationship was missing, the adverse event also was considered to have a possible relationship to study treatment. Serious adverse events were events that were life threatening or resulted in death, initiation or prolongation of hospitalization, persistent or significant incapacity, substantial disruption of activities of daily living, or a congenital anomaly or birth defect. Adverse events were analyzed during each study period and summarized by preferred term and coded system organ class according to the Medical Dictionary for Regulatory Activities, version 19.0. Ocular tolerability was recorded by patients on a visual analog scale and calculated as described previously.[11] Intraocular pressure was measured either by Goldmann applanation tonometry or a handheld applanation tonometer (e.g., Tono-Pen, Reichert Technologies, Depew, NY) after instillation of topical anesthesia. Dilated ophthalmoscopy examinations included the retina, macula, choroid, and optic nerve head. Blood samples were collected for hematologic, clinical chemistry, and immunogenicity assessments (anti-NGF antibodies), which were performed as described previously[9] and forwarded to a central laboratory (Envigo, formerly Harlan Laboratories, Cambridgeshire, United Kingdom) for masked analysis.

Masking and Statistical Analysis

Patients, investigators, and site or sponsor staff were masked to the randomized treatment. Kits for dispensing study treatment (cenegermin or vehicle) were identical in appearance and assigned randomly according to codes generated in SAS statistical software version 9.3 (SAS Institute, Cary, NC) by programmers not directly involved in study analysis. A contract research organization maintained the masked database (Oracle Clinical software version 4.6.4, Oracle Corporation, Redwood Shores, CA) and performed prespecified statistical analyses. The sponsor was not involved in data collection for primary efficacy analyses, and central readers were masked to randomized treatment assignment and duration of treatment (visit number). Immunologic data from the masked central laboratory were provided to statisticians after official unmasking of the study. According to protocol, unmasking was allowed if knowledge of the randomized treatment assignment was required to provide appropriate care for medical emergencies (including deterioration as defined previously). At the end of the 8-week masked treatment period, patients who did not achieve corneal healing (<0.5 mm of lesion staining) were unmasked and eligible for treatment (at the investigator's discretion) with medications allowed by the study protocol: cenegermin-treated patients could receive any nonexperimental treatment for neurotrophic keratopathy, and vehicle-treated patients were eligible for open-label cenegermin treatment (Fig 1). Unmasking for remaining patients was allowed only after database lock (after the last randomized patient completed 4 weeks of follow-up) for final statistical analysis.

Sample size calculations were based on interim results of the phase 1/2 REPARO trial,[10,11] which estimated that 70% of cenegermin-treated patients (vs. 30% of vehicle-treated patients) would achieve the primary efficacy end point of corneal healing (<0.5 mm of lesion staining) after 8 weeks of masked treatment. According to these assumptions and presumed dropout rates between 10% and 20%, the study randomized 48 patients to yield at least 38 evaluable patients to achieve 80% power of detecting a 40% difference in the primary efficacy variable.

There were no planned interim analyses or multiple comparisons for this study; thus, the primary efficacy end point of corneal healing after 8 weeks of masked treatment (assessed as a binary yes-or-no variable by masked central readers as described previously) was analyzed by 2×2 chi-square testing and reported with a 95% confidence interval (CI). Analyses were performed on the intention-to-treat population with missing assessments of corneal healing (yes or no) imputed based on the last observation carried forward. A sensitivity analysis was performed using nonresponder imputation, which considers all missing observations as failures, and subjected to the chi-square test and Fisher exact test (1- and 2-sided). Additional sensitivity analyses included observed-case analysis (removing patients who discontinued before week 4 of masked treatment) and multiple imputation procedures MI and MIANALYZE in SAS software (SAS Institute, Cary, NC). Baseline variables, such as treatment assignment, demographics (e.g., age, gender), and neurotrophic keratopathy history (e.g., baseline lesion size, time since diagnosis), were examined post hoc with multiple logistic regression for potential effects on corneal healing.

Changes from baseline in continuous variables, such as neurotrophic lesion size (greatest dimension of lesion staining in millimeters), visual acuity (BCDVA in ETDRS letters), reflex tearing (Schirmer wetting distance in millimeters per 5 minutes), and corneal sensitivity (Cochet-Bonnet esthesiometry in centimeters), were analyzed as secondary and exploratory end points. Missing data were imputed using last postbaseline measurements carried forward according to the statistical analysis plan.

For changes in corneal lesion size from baseline to week 4 and week 8, reading center measurements of the greatest dimension of fluorescein staining (last postbaseline measurement carried forward) were summarized using descriptive statistics according to the statistical analysis plan. Reading center measurements at week 4 and week 8 were assessed post hoc by an analysis of covariance (ANCOVA) using treatment as a factor and both continuous (e.g., age) and categorical (e.g., gender) baseline variables. The contrast of marginal linear predictions between treatment groups was reported with standard error, 95% CI, and *P* value.

Change in BCDVA from baseline to week 8 was a prespecified analysis by an ANCOVA model using randomized treatment group as a factor and controlling for baseline BCDVA score, time since diagnosis of neurotrophic keratopathy (months), and baseline Schirmer values (millimeters). Changes in corneal lesion size were analyzed post hoc using an ANCOVA with randomized treatment as a factor and baseline lesion size as a continuous covariate. Deterioration (defined as decrease in BCDVA by >5 ETDRS letters, onset of infection, or disease progression) was recorded as a yes-or-no variable on the electronic case report form at each visit of the masked treatment period and then analyzed by chi-square tests according to the statistical analysis plan. Disease progression (defined as an increase in lesion size of ≥1 mm, progression in

lesion depth to corneal melting or perforation, or both), also recorded on electronic case report forms, was assessed post hoc. The overall number of patients experiencing deterioration, disease progression, or both were based on tabulations of electronic case report forms recorded on scheduled visits, then verified post hoc with manual counts (including deterioration events recorded as adverse events during unscheduled visits) and analyzed post hoc by chi-square testing.

Corneal healing after open-label treatment and recurrence treatment was assessed as less than 0.5 mm of lesion staining (according to slit-lamp assessment) and is presented using descriptive statistics. The duration of corneal healing (defined as time to intake of recurrence retreatment) also is presented with descriptive statistics.

Study Oversight

Institutional review board approval was obtained from each participating site (Appendix 1, available at www.aaojournal.org) for the study protocol, amendments, and all study-related documents (including informed consent forms). The study complied with the tenets of the Declaration of Helsinki, International Conference of Harmonization Tripartite Guidelines for Good Clinical Practice, current international and national regulations, the study protocol, and respective standard operating procedures of the participating sites, sponsor, and contract research organization. Written informed consent was obtained before any study-related procedures, and study monitors verified compliance during onsite visits.

Results

Patients and Treatment

Between May 1, 2015, and December 8, 2015, 52 patients from 11 sites (Appendix 1) provided informed consent and were screened for the study; 48 were enrolled and randomized 1:1 to receive active treatment or vehicle. Table 1 summarizes patient demographics and baseline characteristics. Consistent with previous reports,[3–5,8,10,11] the most common underlying etiologies were herpetic eye disease (19 patients, including 2 with multiple etiologies), ocular surgery (8 patients, including 1 with multiple etiologies), and dry eye disease (6 patients). Three enrolled patients demonstrated bilateral neurotrophic keratopathy: 2 randomized to cenegermin (dry eye disease and stem cell deficiency) and 1 randomized to vehicle (unknown etiology). Figure 1 presents an overview of patient disposition, including reasons for withdrawal, recurrence treatment rates, and follow-up.

Efficacy Outcomes

Figure 2 summarizes the primary efficacy analysis of corneal healing after 8 weeks of masked treatment, assessed by the reading center (last observation carried forward). Also shown is the key secondary analysis of corneal healing at week 4, based on reading center assessments. One patient randomized to cenegermin did not receive any baseline or postbaseline reading center assessments to carry forward and was excluded from these efficacy analyses.

The conventional definition of corneal healing (<0.5 mm of lesion staining) showed statistically significant differences between treatment groups at week 8 (Fig 2A), with healing in 7 of 24 vehicle-treated patients (29.2%) compared with 16 of 23 cenegermin-treated patients (69.6%) achieving healing (+40.4%; 95% CI, 14.2%−66.6%; $P = 0.006$). The more conservative definition of corneal healing (0 mm of lesion staining and no other residual staining) yielded statistically significant differences between treatments at both time points (Fig 2B). At week 4, corneal healing (0 mm of lesion staining and no other residual staining) was achieved in 5 of 24 patients receiving vehicle (20.8%), compared with 13 of 23 cenegermin-treated patients (56.5%) who achieved healing (+35.7%; 95% CI, 9.7%−61.7%; $P = 0.012$). At week 8, 4 of 24 vehicle-treated patients (16.7%) achieved 0 mm of lesion staining and no other residual staining, compared 15 of 23 cenegermin-treated patients (65.2%) who achieved corneal healing (+48.6%; 95% CI, 24.0%−73.1%; $P < 0.001$).

Table 2 summarizes sensitivity analyses of the primary efficacy data using nonresponder imputation (all missing observations imputed as failures) and analyses by 1- and 2-sided Fisher exact tests in addition to chi-square testing. The results of nonresponder imputation produced the same conclusions as last observation carried forward imputation (Fig 2), which supports the robustness of the primary efficacy analyses at week 8 and key secondary analyses at week 4 using conventional and conservative definitions of corneal healing. Observed-case and multiple imputation analyses produced similar results (not shown).

To explore potential effects of baseline demographics and disease characteristics, we performed multiple logistic regression on the binary outcome of corneal healing (both conventional and conservative definitions) at week 4 and week 8 of masked treatment (last observation carried forward). Potential explanatory variables (in addition to the randomized treatment) included demographic factors (age, gender), disease characteristics (baseline lesion size, neurotrophic keratopathy severity, time since diagnosis), and underlying etiologies (Table 1). Randomized treatment (cenegermin vs. vehicle) and baseline lesion size (greatest dimension of fluorescein staining) were variables with significant predictive values in logistic regression models and are summarized in Table 3 with respective odds ratios (ORs), CIs, and P values. Other potential variables in our study population, such as underlying etiology (e.g., herpetic vs. nonherpetic etiologies) and interactions between treatment and demographic variables, did not reach statistical significance in logistic regression (data not shown).

Consistent with the primary efficacy analyses (Fig 2), the treatment variable (cenegermin vs. vehicle) showed a positive correlation overall with the outcome of corneal healing (OR, >1). For the conventional definition of corneal healing (<0.5 mm of lesion staining), the treatment variable did not reach statistical significance at week 4 (OR, 3.13; 95% CI, 0.83−11.8; $P = 0.091$), but was significant at week 8 (OR, 7.31; 95% CI, 1.86−28.8; $P = 0.004$). For the conservative definition of corneal healing (0 mm of lesion staining and no other persistent staining), the treatment variable was a significant predictor of corneal healing at both week 4 ($P = 0.006$) and week 8 ($P = 0.001$).

Conversely, baseline lesion size (greatest diameter of lesion staining in millimeters) showed a negative association (OR, <1) with corneal healing (i.e., larger baseline lesion measurements predicted lower probability of corneal healing), independently of randomized treatment. However, in our study population, this association was statistically significant at week 4 only; by the end of treatment (week 8), randomized treatment assignment (cenegermin vs. vehicle) was the only significant predictor of corneal healing.

Based on our study population, there were no significant associations among patient demographics, neurotrophic keratopathy stage at baseline, or time since diagnosis. Although underlying etiologies did not show significant associations with healing outcome in logistic regression, the rarity of some etiologies in our patient population (such as corneal dystrophy, diabetes, and ocular surface injuries) preclude the ability to make conclusions on potential associations with clinical outcomes.

Table 1. Baseline Demographics and Characteristics

Characteristics	Cenegermin (N = 24)	Vehicle (N = 24)	Overall (N = 48)
Age (yrs)			
Mean (SD)	65.9 (13.85)	64.5 (14.15)	65.2 (13.87)
Median (minimum–maximum)	66.5 (33–94)	65.0 (35–92)	65.5 (33–94)
Age group (yrs), no. (%)			
<50	2 (8.33)	3 (12.5)	5 (10.4)
50–64	9 (37.5)	8 (33.3)	17 (35.4)
65–74	7 (29.2)	7 (29.2)	14 (29.2)
75–84	4 (16.7)	4 (16.7)	8 (16.7)
85+	2 (8.33)	2 (8.33)	4 (8.33)
Age 65 or older, no. (%)	13 (54.2)	13 (54.2)	26 (54.2)
Female, no. (%)	14 (58.3)	15 (62.5)	29 (60.4)
Ethnicity, no. (%)			
Hispanic, Latino, or Spanish	0	1 (4.17)	1 (2.08)
Not Hispanic, Latino, or Spanish	20 (83.3)	19 (79.2)	39 (81.3)
N/A	4 (16.7)	4 (16.7)	8 (16.7)
Race, no. (%)			
Asian	1 (4.17)	0 (0.0)	1 (2.08)
Black	3 (12.5)	2 (8.33)	5 (10.4)
Native Hawaiian or other Pacific Islander	0 (0.0)	1 (4.17)	1 (2.08)
White	20 (83.3)	20 (83.3)	40 (83.3)
Other	0	1 (4.17)	1 (2.08)
Primary neurotrophic keratopathy diagnosis, no. (%)			
Stage 2	15 (62.5)	18 (75.0)	33 (68.8)
Stage 3	9 (37.5)	6 (25.0)	15 (31.3)
Bilateral neurotrophic keratopathy, no. (%)	2 (8.3)	1 (4.17)	3 (6.25)
Neurotrophic lesion size at baseline (maximum diameter; mm)*			
Mean (SD)	3.51 (1.98)	3.02 (1.88)	3.26 (1.93)
Median (minimum–maximum)	3.10 (0.53–8.23)	2.99 (0.23–6.10)	3.10 (0.23–8.23)
Time since diagnosis of neurotrophic keratopathy (mos)			
Mean (SD)	31.1 (108.34)	33.0 (73.83)	32.1 (91.72)
Median (minimum–maximum)	4.0 (0–535)	13.0 (0–366)	6.5 (0–535)
Time since diagnosis of neurotrophic keratopathy stage 2/3 (mos)			
Mean (SD)	7.5 (14.51)	7.9 (8.59)	7.7 (11.80)
Median (minimum–maximum)	3.0 (0–71)	3.5 (0–28)	3.0 (0–71)
Underlying neurotrophic keratopathy etiology, no. (%)			
Corneal dystrophy	1 (4.17)	1 (4.17)	2 (4.17)
Diabetes mellitus	0	1 (4.17)	1 (2.08)
Dry eye disease	3 (12.50)	3 (12.50)	6 (12.50)
Herpetic eye disease	9 (37.50)	8 (33.33)	17 (35.42)
Herpes simplex	5 (20.83)	4 (16.67)	9 (18.75)
Herpes zoster	2 (8.33)	4 (16.67)	6 (12.50)
Herpetic keratitis/unspecified	2 (8.33)	0	2 (4.17)
Multifactorial	2 (8.33)	0	2 (4.17)
Herpetic eye disease (herpes simplex); ocular surgery (penetrating keratoplasty)	1 (4.17)	0	1 (2.08)
Herpetic eye disease (herpes zoster); diabetes mellitus	1 (4.17)	0	1 (2.08)
Neurosurgical procedure	1 (4.17)	1 (4.17)	2 (4.17)
Trigeminal ablation	1 (4.17)	0	1 (2.08)
Unspecified	0	1 (4.17)	1 (2.08)
Ocular surface injury/inflammation	2 (8.33)	1 (4.17)	3 (6.25)
Chemical burn/multiple transplant surgeries	1 (4.17)	0	1 (2.08)
Unspecified	1 (4.17)	1 (4.17)	2 (4.17)
Ocular surgery or procedure	3 (12.50)	4 (16.67)	7 (14.58)
Anterior and posterior surgeries	0	1 (4.17)	1 (2.08)
Cataract surgery	1 (4.17)	0	1 (2.08)
Conjunctival lesion excision	0	1 (4.17)	1 (2.08)
Unspecified	2 (8.33)	2 (8.33)	4 (8.33)
Other	2 (8.33)	5 (20.83)	7 (14.58)
Infectious keratitis (unspecified)	1 (4.17)	0	1 (2.08)
Radiation (unspecified)	0	1 (4.17)	1 (2.08)
Stem cell deficiency	1 (4.17)	0	1 (2.08)
Stevens-Johnson syndrome	0	1 (4.17)	1 (2.08)

(*Continued*)

Table 1. (Continued.)

Characteristics	Cenegermin (N = 24)	Vehicle (N = 24)	Overall (N = 48)
Trigeminal infiltration (metastatic cancer)	0	1 (4.17)	1 (2.08)
Unknown origin	0	1 (4.17)	1 (2.08)
Unspecified	0	1 (4.17)	1 (2.08)
Topical medication (glaucoma medication)	1 (4.17)	0	1 (2.08)

N/A = not available (ethnicity and race were not reported for all patients); SD = standard deviation.
*One patient randomized to the cenegermin group did not undergo a baseline measurement by the reading center, and so was excluded from the summary of neurotrophic lesion size at baseline.

For changes in corneal lesion size from baseline, reading center measurements at weeks 4 and 8 of masked treatment (last post-baseline measurement carried forward) were analyzed using descriptive statistics according the study protocol and are presented alongside baseline measurements in Figure 3. Lesion measurements in cenegermin-treated patients seemed to be reduced substantially from baseline compared with those of patients receiving vehicle. Therefore, we conducted a post hoc analysis of change in corneal lesion size from baseline using an ANCOVA with treatment (cenegermin or vehicle) as a factor and baseline lesion size as a continuous covariate. Cenegermin-treated patients showed significantly reduced lesion sizes compared with vehicle-treated patients; contrasts of marginal linear predictions (cenegermin vs. vehicle treatment) were −1.146 at week 4 (standard error, 0.431; 95% CI, −2.018 to −0.274; $P = 0.011$) and 1.503 at week 8 (standard error, 0.448; 95% CI, 2.409−0.597; $P = 0.002$). Effects of other baseline variables (age, gender, disease stage, time since diagnosis, and etiology) were not significant in the ANCOVA model.

Visual acuity outcomes at week 8 (secondary efficacy end point) were assessed using BCDVA in ETDRS letters. At baseline, mean BCDVA in patients randomized to cenegermin was 8.3 ETDRS letters (Snellen equivalent, 20/800), compared with 17.6 ETDRS letters (Snellen equivalent, 20/500) in patients randomized to vehicle. By week 8, increases from baseline were observed in both treatment groups (last observation carried forward). Best-corrected distance visual acuity changes from baseline were assessed by an ANCOVA with randomized treatment as a factor and controlling for baseline BCDVA, time since diagnosis of neurotrophic keratopathy (months), and baseline Schirmer test values (millimeters), but did not reach statistical significance ($P = 0.745$). Visual acuity also was assessed as 15-letter gains (yes or no) at week 8 (last observation carried forward), which was achieved in 3 of 23 cenegermin-treated patients (13.0%) versus 4 of 24 vehicle-treated patients (16.7%). The difference between treatment groups was not statistically significant ($P = 0.727$, chi-square test).

Secondary efficacy variables included measurements of corneal sensitivity (measured within the lesion by Cochet-Bonnet esthesiometer) and reflex tearing (assessed using Schirmer testing). Mean Cochet-Bonnet esthesiometer measures at baseline were 0.81 (standard deviation [SD], 1.187) in the cenegermin group versus 0.65 (SD, 0.699) in the vehicle group (n = 24 per group); in patients with available postbaseline observations, Cochet-Bonnet measurements were 2.91 (SD, 2.144) in cenegermin-treated patients (n = 18) versus 1.83 (SD, 1.952) in vehicle-treated patients (n = 15) by week 8. Comparisons between treatment groups did not reach statistical significance through an ANCOVA performed with treatment as a factor and controlling for baseline Cochet-Bonnet esthesiometer measures, Schirmer values, and time since diagnosis of neurotrophic keratopathy ($P = 0.207$).

In Schirmer tear tests at week 8, cenegermin-treated patients (n = 17) showed a least-squares mean increase from baseline of 6.1 mm (95% CI, 1.3−10.9 mm), whereas vehicle-treated patients showed a small decrease (−0.1 mm; 95% CI, −5.3 to 5.0 mm). The treatment difference (+6.3 mm; 95% CI, −1.1 to 13.7 mm) was analyzed using an ANCOVA with treatment as factor and controlling for baseline Schirmer test results and time since diagnosis of neurotrophic keratopathy (months), as well as by Wilcoxon rank-sum test results. Results suggested a trend favoring

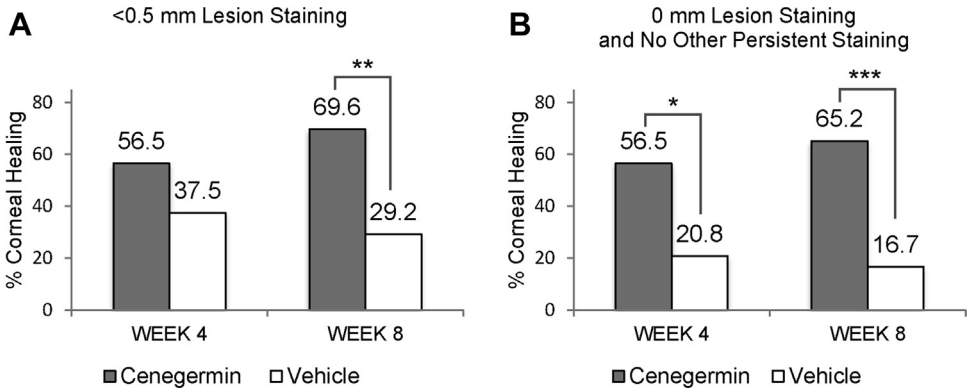

Figure 2. Bar graphs showing primary efficacy analysis of corneal healing during masked treatment. Percentage of patients achieving corneal healing with cenegermin treatment (gray) or vehicle (white). Corneal healing was defined (**A**) conventionally as less than 0.5 mm of lesion staining or (**B**) more conservatively as 0 mm of lesion staining and no other persistent staining. Patients were randomized to treatment with cenegermin (n = 24) or vehicle (n = 24), and corneal healing was assessed as a yes-or-no variable at week 4 and week 8 (missing assessments imputed by last observation carried forward). See text for details. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ (2×2 chi-square analysis).

Table 2. Sensitivity Analysis of Corneal Healing (Nonresponder Imputation)

	Week 4		Week 8	
	Cenegermin (N = 24)	*Vehicle (N = 24)*	*Cenegermin (N = 24)*	*Vehicle (N = 24)*
<0.5-mm maximum diameter of lesion staining, no. (%)	13/24 (54.2)	9/24 (37.5)	15/24 (62.5)	6/24 (25.0)
Difference (cenegermin minus vehicle), %	16.7		37.5	
95% confidence interval	−11.1 to 44.5		11.5–63.5	
P value				
2×2 chi-square test	0.247		0.006	
Fisher exact test, 1 sided	0.193		0.006	
Fisher exact test, 2 sided	0.385		0.009	
0 mm of lesion staining and no other residual staining, no. (%)	13/24 (54.2)	5/24 (20.8)	14/24 (58.3)	3/24 (12.5)
Difference (cenegermin minus vehicle), %	33.3		45.8	
95% confidence interval	7.62–59.1		22.1–69.6	
P value				
2×2 chi-square test	0.017		< 0.001	
Fisher exact test, 1 sided	0.018		< 0.001	
Fisher exact test, 2 sided	0.036		0.002	

Missing values were imputed as failures.

cenegermin treatment at week 8 that approached statistical significance both in the ANCOVA model ($P = 0.09$) and the rank-sum test ($P = 0.07$).

The percentage of patients experiencing deterioration from baseline to week 8 was assessed as a secondary end point and is presented in Table 4. Deterioration was defined as a decrease in BCDVA by more than 5 ETDRS letters, onset of infection, disease progression (increase in lesion size of ≥1 mm, progression in lesion depth to corneal melting or perforation, or both), or a combination thereof. During the masked treatment period, lower rates of deterioration occurred in cenegermin-treated patients compared with vehicle-treated patients. Overall, 6 events classified as deterioration occurred in 6 of 23 patients receiving cenegermin (26.1%), compared with 19 events in 12 of 24 patients receiving vehicle (50%); the treatment difference (23.9%) suggests fewer deterioration events in cenegermin-treated patients, but statistical significance was not achieved in post hoc chi-square analysis ($P = 0.092$; 95% CI, 0.5078671–0.0296063). Events assessed only as disease progression (increase in lesion size of ≥1 mm, progression in lesion depth to corneal melting or perforation, or both) also were analyzed post hoc. Overall, 5 of 23 patients (21.7%) receiving cenegermin experienced 5 events of disease progression, whereas 12 of 24 patients receiving vehicle (50%) experienced 19 events of disease progression. The treatment difference (28.3%) reached statistical significance in post hoc analysis ($P = 0.044$; 95% CI, 0.5442013 to −0.0210161). After week 4 of masked treatment, 4 events of deterioration or disease progression occurred in 3 patients receiving vehicle. In contrast, no progression or deterioration events occurred in cenegermin-treated patients.

Per protocol, if patients healed after either masked or open-label cenegermin treatment (<0.5 mm of lesion staining) and then experienced recurrence of persistent epithelial defect or corneal ulcer during follow-up, they were eligible for an additional 8-week

Table 3. Logistic Regression Analysis of Corneal Healing

Explanatory Variables	Week 4 (N = 47)*		Week 8 (N = 47)*	
	Treatment†	*Lesion Size*‡	*Treatment*†	*Lesion Size*‡
Outcome variable				
<0.5-mm maximum diameter of lesion staining				
Odds ratio	3.13	0.61	7.31	0.74
95% confidence interval	0.83–11.8	0.42–0.89	1.86–28.8	0.52–1.05
P value				
Explanatory variable	0.091	0.010	0.004	0.089
Overall model	0.0076		0.0041	
0-mm lesion staining and no other residual staining				
Odds ratio	8.59	0.59	13.0	0.72
95% confidence interval	1.86–39.8	0.39–0.90	2.83–60.0	0.49–1.06
P value				
Explanatory variable	0.006	0.014	0.001	0.094
Overall model	0.0008		0.0005	

*All randomized patients with evaluable observations in the study (last observation carried forward). One patient (randomized to cenegermin) withdrew before receiving treatment and did not have a baseline reading center measurement, and so was excluded from analysis.
†Randomized treatment received during masked treatment (cenegermin = 1; vehicle = 0).
‡Lesion size at baseline (maximum diameter in millimeters), reading center measurement.

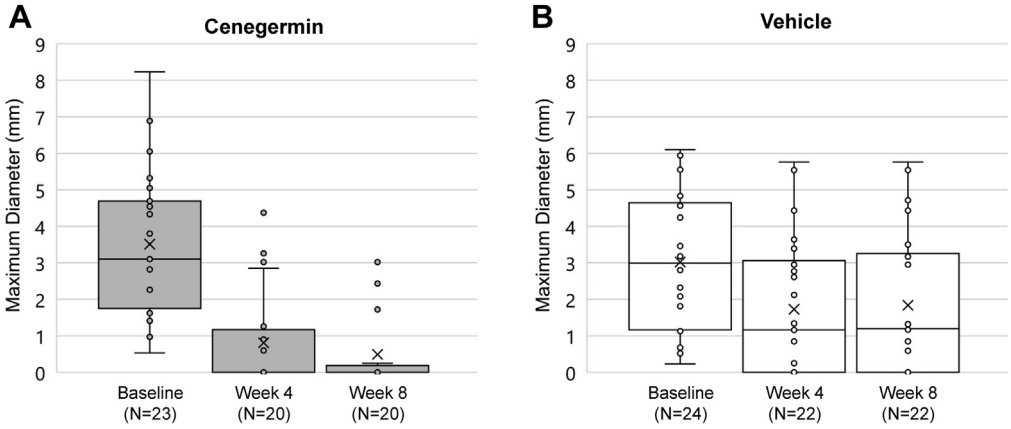

Figure 3. Box-and-whisker plots showing corneal lesion size change from baseline to week 8. Patients were randomized to 8 weeks of masked treatment with (**A**) cenegermin or (**B**) vehicle. Reading center measurements of corneal lesions (greatest diameter of fluorescein staining in millimeters) are represented in a boxplot at each time point. Missing values at weeks 4 and 8 were imputed as last postbaseline measurements carried forward. In the cenegermin group, 1 patient (who withdrew shortly after randomization) did not receive study treatment or a baseline reading center measurement, and thus was excluded from the baseline summary. Five patients (3 randomized to cenegermin and 2 randomized to vehicle) did not demonstrate any postbaseline values to carry forward and were excluded from summaries at weeks 4 and 8. Cenegermin-treated patients showed reduced lesion sizes overall compared with vehicle-treated patients, reaching statistical significance in an analysis of covariance using treatment as a factor and baseline measurements as covariates (see text for details). The box represents interquartile range (IQR); the midline represents the median value; the cross represents the mean value; the whiskers represent the local minimum and maximum; and the circle represents the individual data point. Circles beyond the whiskers are outliers according to the Tukey standard (1.5 times the IQR).

recurrence treatment period with cenegermin (Fig 1). Corneal healing after recurrence treatment was assessed by the investigator as less than 0.5 mm of lesion staining and is presented using descriptive statistics. Of the 16 patients who achieved less than 0.5 mm of lesion staining after masked cenegermin treatment, 2 (12.5%) underwent re-treatment for recurrence: 1 patient on the day after completing masked treatment and 1 patient 39 days after the last visit of the masked treatment period. Both patients (100%) healed after recurrence treatment and maintained less than 0.5 mm of lesion staining through the end of follow-up. Of 13 patients (originally randomized to vehicle) who received open-label cenegermin treatment, 9 (69.2%) achieved less than 0.5 mm of lesion staining. Of these patients, 4 of 9 (44.4%) experienced recurrence. One patient experienced recurrence 104 days after the last visit of the open-label cenegermin treatment period but was not retreated with cenegermin (investigator decision) and did not achieve less than 0.5 mm of lesion staining by the end of follow-up. The remaining 3 patients received cenegermin retreatment for recurrence. One patient (33.3%) began cenegermin re-treatment 7 days after the last visit of open-label treatment but

Table 4. Secondary Efficacy Analysis of Deterioration and Disease Progression during Masked Treatment

	Deterioration*		Disease Progression†	
	Cenegermin (N = 24)	*Vehicle (N = 24)*	*Cenegermin (N = 24)*	*Vehicle (N = 24)*
Day 4, no. (%)‡	1/23 (4.3)	N/A	1/23 (4.3)	N/A
Week 1, no. (%)	2/22 (9.1)	4/24 (16.7)	2/22 (9.1)	4/24 (16.7)
Week 2, no. (%)	0/21 (0.0)	5/23 (21.7)	0/21 (0.0)	5/23 (21.7)
Week 3, no. (%)	1/20 (5.0)	5/20 (25.0)	1/20 (5.0)	5/20 (25.0)
Week 4, no. (%)	2/20 (10.0)	1/17 (5.9)	1/20 (5.0)	1/17 (5.9)
Week 6, no. (%)	0/19 (0.0)	2/16 (12.5)	0/19 (0.0)	2/16 (12.5)
Week 8, no. (%)	0/18 (0.0)	2/15 (13.3)	0/18 (0.0)	2/15 (13.3)
Total events, no.§	6	19	5	19
Total patients, no. (%)‖	6/23 (26.1)	12/24 (50.0)	5/23 (21.7)	12/24 (50.0)
Difference (cenegermin minus vehicle), %	−23.9		−28.3	
95% confidence interval	−0.5078671 to 0.0296063		−0.5442013 to −0.0210161	
P value (chi-square test)	0.092		0.044	

*Deterioration was defined as decrease in best-corrected distance visual acuity by >5 Early Treatment Diabetic Retinopathy Study letters, onset of infection, disease progression (see below), or a combination thereof.
†Defined as increase in lesion size of ≥1 mm, progression in lesion depth to corneal melting or perforation, or both.
‡One patient receiving cenegermin treatment experienced an adverse event recorded as "disease progression (worsening of neurotrophic keratitis)" and withdrew on day 4 (unscheduled visit). All other deterioration or disease progression events were recorded on scheduled visits (weeks 1, 2, 3, 4, 6, and 8).
§Includes multiple deterioration or disease progression events experienced by individual patients.
‖Patients experiencing multiple deterioration or disease progression events during masked treatment were counted only once for the purposes of this analysis.

did not achieve corneal healing. The other 2 patients (66.7%) began cenegermin retreatment 35 days and 37 days after the last visit of open-label treatment; both healed and maintained less than 0.5 mm of lesion staining through follow-up.

Safety Outcomes

Table 5 summarizes treatment-related adverse events, which are presented with patients grouped according to original randomized treatment assignment. Overall, the most frequently reported treatment-related adverse events were in the system organ class of eye disorders according to the Medical Dictionary for Regulatory Activities, version 19.0. During the masked treatment period, the most common adverse event was eye pain, reported 5 times by 4 of 47 patients (8.5%) overall: 4 events reported by 3 of 23 cenegermin-treated patients (13.0%) and 1 event reported by 1 of 24 patients (4.2%) treated with vehicle. With the exception of joint swelling (reported by 1 patient in the cenegermin group), all other treatment-related adverse events in the masked treatment period were ocular in nature (paresthesia, in the system organ class nervous system disorders, represented transient tingling in the study eye in 1 cenegermin-treated patient). A total of 8 serious adverse events occurred in 7 of 47 patients (14.9%) during the masked treatment period: 3 of 23 cenegermin-treated patients (13.0%) and 4 of 24 vehicle-treated patients (16.7%). No serious adverse events reported during the masked treatment period was considered related to study treatment.

Of 13 patients originally randomized to vehicle treatment who received cenegermin in the open-label treatment period, 3 patients reported 1 eye disorder each. No serious adverse events were reported during the open-label treatment period.

In the follow-up period documented in Table 5, the cenegermin group includes patients who received recurrence treatment, and the vehicle group includes patients who received cenegermin treatment during open-label and recurrence treatment periods. Three patients originally randomized to cenegermin (2 of whom were retreated for recurrence) reported eye pain, and 1 of these patients (with neurotrophic keratopathy as the cause of herpetic keratitis) also experienced ocular herpes simplex during follow-up. In the vehicle group, 2 patients experienced treatment-related adverse events during follow-up; both received cenegermin during the open-label and recurrence treatment periods. One of these patients experienced disease progression and was withdrawn from the study. The other patient experienced eye pain and worsening of a pre-existing cataract.

During follow-up, serious adverse events occurred in 5 patients (3 in the cenegermin group and 2 in the vehicle group). No serious adverse events reported during follow-up were considered related to study treatment.

No deaths were reported in either the masked or open-label treatment periods. During follow-up, 1 patient originally randomized to cenegermin treatment died of unknown causes; this occurred 163 days after the last dose of study drug and was considered unrelated to study treatment.

Analyses of vital signs, ophthalmic parameters, and laboratory parameters did not reveal any clinically significant patterns in patients treated with cenegermin or vehicle. Consistent with results of the REPARO phase 2 study,[11] no anti-NGF antibodies were detected at any testing time point.

Discussion

In this pivotal trial conducted in the United States, topical cenegermin treatment effectively and safely promoted healing of persistent epithelial defects (with or without stromal thinning) in patients with neurotrophic keratopathy. The dosage (cenegermin 20 μg/ml) was based on the European REPARO phase II study,[11] which compared cenegermin 10 μg/ml, cenegermin 20 μg/ml, and vehicle formulations under the same treatment regimen as the NGF0214 study reported herein. Although the European REPARO phase 2 study did not show statistically significant differences between the 2 cenegermin doses in any prespecified efficacy parameters, their safety profiles were similar; furthermore, the 20-μg/ml formulation exhibited better trends of efficacy in post hoc analyses, including reduction in lesion size and reassessment of the primary efficacy parameter of corneal healing (<0.5 mm of lesion staining) using the more conservative measure (0 mm of lesion staining and no other persistent staining). Therefore, the 20-μg/ml dose was selected for the formulation used in the United States NGF0214 study, which sought to assess prospectively the conservative definition of corneal healing (0 mm of lesion staining and no other persistent staining) as a prespecified efficacy end point and to define further the clinical outcomes of cenegermin treatment in neurotrophic keratopathy with metrics not used in the previous European REPARO study.

It is well documented in the medical and scientific literature that palliative treatments for neurotrophic keratopathy (including tarsorrhaphy, preservative-free lubricants, therapeutic contact lenses, and close monitoring) can promote corneal epithelial regrowth over a neurotrophic lesion.[1] However, the cosmetic impact of some surgical interventions (such as tarsorrhaphy and conjunctival flap) may be undesirable to patients; furthermore, palliative treatments for neurotrophic keratopathy may pose a higher risk of disease recurrence, because these treatments do not address the underlying deficits that drive pathophysiologic features. To this point, more vehicle-treated patients exhibited corneal healing at week 4 compared with week 8 (Fig 2), suggesting temporary epithelial proliferation followed by disease recurrence under palliative vehicle treatment. In contrast, cenegermin treatment consistently demonstrated highest corneal healing rates at week 8, suggesting steady improvement over time. These healing profiles withstood multiple sensitivity analyses (Table 2), supporting the robustness of the primary efficacy data.

In neurotrophic keratopathy patients, corneal healing was defined conventionally as less than 0.5 mm of lesion staining, which is based on the lower limit of reliable slit-lamp assessment. This conventional definition of corneal healing may overlook small neurotrophic lesions at less than the limits of slit-lamp detection and may be more subject to interoperator variability among central readers; thus, we also used a conservative measure of 0 mm of lesion staining and no other residual staining, assessed by central readers. Although both measures of corneal healing showed statistically significant differences in the primary efficacy analysis (Fig 2), the requirement for lack of lesion staining and other persistent staining suggests that it is a clinically more meaningful measure of corneal healing.

In this study, we examined potential effects of baseline variables (such as patient demographics and disease parameters) on the clinical outcome of corneal healing. Based

Table 5. Treatment-Related Adverse Events during Masked Treatment, Open-Label Treatment, and Follow-up Periods

System Organ Class*	Cenegermin (N = 23)†		Vehicle (N = 24)†		Overall (N = 47)†	
	No. (%)‡	*Events*§	*No. (%)*	*Events*§	*No. (%)*	*Events*§
Masked treatment period						
Any treatment-related adverse events‖	10 (43.5)	26	8 (33.3)	15	18 (38.3)	41
Eye disorders	9 (39.1)	20	8 (33.3)	13	17 (36.2)	33
Eye pain	3 (13.0)	4	1 (4.2)	1	4 (8.5)	5
Photophobia	1 (4.3)	1	2 (8.3)	2	3 (6.4)	3
Visual acuity reduced	1 (4.3)	1	2 (8.3)	3	3 (6.4)	4
Corneal epithelium defect	2 (8.7)	2	0	0	2 (4.3)	2
Eye irritation	0	0	2 (8.3)	2	2 (4.3)	2
Anterior chamber inflammation	1 (4.3)	1	0	0	1 (2.1)	1
Corneal deposits	1 (4.3)	1	0	0	1 (2.1)	1
Corneal neovascularization	0	0	1 (4.2)	2	1 (2.1)	2
Corneal thinning	0	0	1 (4.2)	1	1 (2.1)	1
Eye discharge	1 (4.3)	1	0	0	1 (2.1)	1
Eye inflammation	1 (4.3)	1	0	0	1 (2.1)	1
Eye pruritus	0	0	1 (4.2)	1	1 (2.1)	1
Eyelid pain	1 (4.3)	2	0	0	1 (2.1)	2
Foreign body sensation	1 (4.3)	1	0	0	1 (2.1)	1
Hyphema	1 (4.3)	1	0	0	1 (2.1)	1
Keratitis	1 (4.3)	1	0	0	1 (2.1)	1
Lacrimation increased	1 (4.3)	1	0	0	1 (2.1)	1
Ocular hyperemia	1 (4.3)	1	0	0	1 (2.1)	1
Posterior capsule opacification	1 (4.3)	1	0	0	1 (2.1)	1
Vision blurred	0	0	1 (4.2)	1	1 (2.1)	1
General disorders and administration site conditions	2 (8.7)	2	2 (8.3)	2	4 (8.5)	4
Disease progression	1 (4.3)	1	1 (4.2)	1	2 (4.3)	2
Foreign body sensation	1 (4.3)	1	1 (4.2)	1	2 (4.3)	2
Investigations	1 (4.3)	1	0	0	1 (2.1)	1
Intraocular pressure increased	1 (4.3)	1	0	0	1 (2.1)	1
Musculoskeletal and connective tissue disorders	1 (4.3)	2	0	0	1 (2.1)	2
Joint swelling	1 (4.3)	1	0	0	1 (2.1)	1
Nervous system disorders	1 (4.3)	1	0	0	1 (2.1)	1
Paresthesia	1 (4.3)	1	0	0	1 (2.1)	1
Open-label treatment period						
Any treatment-related adverse events‡	N/A	N/A	3 (23.1)	3	3 (23.1)	3
Eye disorders			3 (23.1)	3	3 (23.1)	3
Eye pain			1 (7.7)	1	1 (7.7)	1
Eyelid pain			1 (7.7)	1	1 (7.7)	1
Visual acuity reduced			1 (7.7)	1	1 (7.7)	1
Follow-up period¶						
Any treatment-related adverse events‡	3 (13.0)	4	2 (8.3)	3	5 (10.6)	7
Eye disorders	3 (13.0)	3	1 (4.2)	2	4 (8.5)	5
Eye pain	3 (13.0)	3	1 (4.2)	1	4 (8.5)	4
Cataract (worsening)	0	0	1 (4.2)	1	1 (2.1)	1
General disorders and administration site conditions	0	0	1 (4.2)	1	1 (2.1)	1
Disease progression	0	0	1 (4.2)	1	1 (2.1)	1
Infections and infestations	1 (4.3)	1	0	0	1 (2.1)	1

AE = adverse event.
*Medical Dictionary for Regulatory Activities, version 19.0, preferred term. Body system and preferred terms are sorted by descending frequency of event count in the "Overall" column.
†Number of patients who received the randomly assigned treatment in masked treatment period (safety population).
‡Number of patients with treatment-related adverse events counted on a per-patient basis (if a patient reported the same event repeatedly, the event was counted only once). Percentages were calculated using the safety population of each treatment group as the denominator.
§Number of observed events.
‖Treatment-emergent conditions having a relationship to study treatment recorded as possible, probable, highly probable, or missing.
¶Includes any recurrence treatment (see text for details).

on multiple logistic regression modeling in our patient population, the effect of treatment (cenegermin vs. vehicle) was the only significant predictor of healing at week 8, suggesting that a full treatment cycle achieves the therapeutic effect independent of initial lesion size. These results are consistent with the greater healing rates (Fig 2) and overall reductions in corneal lesion size in cenegermin-treated patients versus vehicle-treated patients (Fig 3) and support maintaining cenegermin treatment over the full 8-week course.

Other variables (such as age, disease stage, and time since diagnosis) did not have significant effects on healing status. Underlying etiologies also did not show significant correlations with clinical outcomes; however, certain etiologies (such as diabetes and chemical burns) that may have divergent outcomes in clinical practice are not well represented in our patient population. Thus, it is difficult to form conclusions on the relative efficacy of cenegermin treatment on cases of neurotrophic keratopathy of different etiologies.

In other clinically relevant end points, some statistically significant improvements and favorable trends were observed. For example, fewer cenegermin-treated patients experienced disease progression over the 8-week treatment course compared with vehicle-treated patients. Although few events of disease progression or deterioration occurred overall, it is interesting to note that after week 4 of the masked treatment period, only vehicle-treated patients exhibited signs of disease progression or deterioration. Taken together, these results suggest that cenegermin may prevent disease progression more effectively than vehicle treatment.

In line with the previously reported REPARO phase 2 study,[11] this trial did not yield statistically significant improvements in corneal sensitivity measured by Cochet-Bonnet esthesiometer, yet reflex tearing (which may reflect corneal sensitivity or nerve function not detectable by Cochet-Bonnet esthesiometer) exhibited trends favoring cenegermin treatment. Conclusive data may require larger sample sizes and longer follow-up. Also in line with the REPARO phase 2 study, we assessed BCDVA as a secondary efficacy end point, although visual acuity does not necessarily correlate with neurotrophic keratopathy severity or healing status. For example, persistent epithelial defects may be relatively transparent (and have little to no effect on vision), whereas the haze commonly associated with a healing corneal epithelium may cloud vision temporarily, particularly in the central or paracentral cornea. Therefore, it is not surprising that neither the phase 2 REPARO trial nor the current NGF0214 study showed statistically significant improvements in visual acuity measures.

There were few events of disease recurrence recorded during follow-up. A total of 6 patients experienced recurrence, and 5 of these patients received recurrence treatment with cenegermin. The recurrence rates (and healing rates after cenegermin retreatment) are too small to form any conclusions; logistic regression analyses examining correlations between baseline variables (including etiologies) with recurrence rates were inconclusive (data not shown). However, it is interesting to note that 4 of 5 cenegermin-retreated patients (80%; i.e., patients who received two 8-week courses of cenegermin) achieved corneal healing, which was maintained through the end of the follow-up period.

No obvious safety concerns arose; none of the serious adverse events was considered related to study treatment, and nearly all treatment-related adverse events were ocular in nature (Table 5). The most common treatment-related adverse events (e.g., eye pain, foreign body sensation, and tingling) suggest nociceptor sensitization, which is associated commonly with NGF in preclinical studies.[13] Therefore, most of the ocular adverse events may represent known mechanisms of action of endogenous NGF, which is the basis for the recombinant test product that is identical in amino acid sequence to native human NGF. No immunogenicity to NGF was detected in this study, consistent with previous findings in healthy volunteers[9] and patients with neurotrophic keratopathy.[10,11]

The current study clearly defined favorable benefit-to-risk ratios for topical cenegermin in patients with neurotrophic persistent epithelial defects (with or without stromal thinning). Future studies may decipher the precise pathologic processes modulated by cenegermin—particularly corneal denervation—and the potential therapeutic efficacy of cenegermin in other neurodegenerative diseases. In summary, cenegermin ophthalmic solution represents a safe, novel, and noninvasive pharmacologic treatment for neurotrophic keratopathy and can become part of the treatment algorithm for this often difficult to manage disease with a high need for targeted and effective pharmacotherapies.

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Abbreviations and Acronyms:
ANCOVA = analysis of covariance; **ANOVA** = analysis of variance; **BCDVA** = best-corrected distance visual acuity; **CI** = confidence interval; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **NGF** = nerve growth factor; **OR** = odds ratio; **rhNGF** = recombinant human nerve growth factor; **SD** = standard deviation.

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