Observational study of ceneegermin for the 
treatment of limbal stem cell deficiency 
associated with neurotrophic keratopathy

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Abstract

Background: Neurotrophic keratopathy (NK) and limbal stem cell deficiency (LSCD) have high morbidity and require aggressive management to prevent permanent vision loss. Ceneegermin, a recombinant human nerve growth factor, was approved by the Federal Drug Administration in 2018 for the treatment of NK.

Objectives: To determine the efficacy and safety of ceneegermin in the treatment of LSCD associated with NK.

Design: Prospective cohort study

Methods: Patients diagnosed with LSCD and NK who had failed conventional treatment were enrolled in this prospective open-label study. Patients were treated with ceneegermin for 8 weeks. The primary objective was to determine whether the area of abnormal epithelium decreased following treatment. Corneal sensation, visual acuity (VA), and LSCD severity were also evaluated.

Results: Six eyes of 5 patients were included in the study. Ceneegermin significantly improved the area of abnormal corneal epithelium in 5 of 6 eyes, measuring 73% of total corneal area at the initial visit and 48% at the final visit (P = .036). Corneal sensation improved in all patients, Cochet–Bonnet aesthesiometry measured 14.7 and 26.7 mm at the initial and final visit, respectively (P = .009). VA improved in 4 out of 6 eyes, with mean initial logMAR VA of 1.67 and final logMAR VA of 1.19 (P = .045). Finally, LSCD grading improved using the Aravena scoring system; however, this difference was not statistically significant (P = .14). One patient presented with an epithelial defect at baseline, which resolved following treatment. No patient withdrew from the study due to adverse effects.

Conclusions: Ceneegermin effectively improved the cornea epithelium, VA, and corneal sensation in patients with LSCD and NK who had failed prior treatment. Further studies are necessary to better understand the anatomical changes and to confirm our results with a larger randomized control trial.

Registration: The study was registered at ClinicalTrials.gov with identifier NCT04552730 (https://clinicaltrials.gov/ct2/show/NCT04552730).

Keywords: ceneegermin, limbal stem cell deficiency, neurotrophic keratopathy, oxervate

Introduction

Neurotrophic keratopathy (NK) is a condition associated with decreased or absent corneal sensation leading to impaired viability of corneal epithelial cells and corneal ulceration.1,2 Limbal stem cell deficiency (LSCD) occurs when there is a loss of cornea limbal stem cells or function, causing an inability for the cornea epithelium to maintain a healthy cornea surface.3,4 This can result in abnormal corneal epithelium, epithelial...
defect, cornea vascularization, and cornea opacity. Common causes of NK and LSCD include herpetic keratitis, diabetes mellitus, long-term use of ocular medications, contact lens wear, and chemical injury.\(^5\)

The goal in management of LSCD and NK is optimization of the ocular surface by avoiding toxic chemicals, lubricating the ocular surface, and controlling inflammation. Treatments include preservative-free artificial tears, punctal plugs, autologous serum tears, amniotic membrane transplants, and tarsorrhaphy. Oral doxycycline and topical corticosteroids are sometimes used to reduce ocular surface inflammation. When these techniques are unsuccessful, patients may require more aggressive treatments and surgeries including limbal stem cell transplantation. The goal of these treatment modalities is to protect the cornea and prevent ocular surface instability but do not necessarily treat the underlying disease.

Cenegermin, a recombinant human nerve growth factor, was approved by the Federal Drug Administration (FDA) in 2018 for the treatment of NK.\(^6\)–\(^10\) The goal of this open-label pilot study is to determine the efficacy and safety of cenegermin for the treatment of LSCD in patients with NK.

**Methods**

**Patient enrollment**

A prospective study was performed at the Byers Eye Institute involving 6 eyes from 5 patients diagnosed with LSCD and NK. Study approval was obtained from the Stanford University Institutional Review Board (Protocol 57888). The study was conducted in accordance with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. The study was registered at ClinicalTrials.gov with identifier NCT04552730.

Consecutive patients over the age of 18 who presented to the cornea clinic and evaluated by CT from October 1, 2020 to February 28, 2021 and diagnosed with LSCD and NK were asked to participate in the study. Written informed consent was obtained prior to initiation of the study which included use of the medication, inclusion in the study, and publication of the data. NK was confirmed by Cochet–Bonnet aesthesiometry measuring less than 4 cm. These study subjects must have neurotrophic cornea Mackie Classification Stages 1 or 2.\(^11\) LSCD was diagnosed clinically by the presence of abnormal epithelium characterized by a whorl-like epitheliopathy originating from the limbus and extending toward the central cornea.

All patients had failed conventional treatment, such as artificial tears, topical steroids, as well as more aggressive treatments, including punctal plugs, amniotic membrane grafts, and temporary tarsorrhaphy. Exclusion criteria were patients with active ocular infection, anticipated need for bandaged contact lens, amniotic membrane graft, or tarsorrhaphy during the study, patients unable to discontinue contact lens wear, and visual acuity (VA) worse than 20/200 in the better-seeing eye.

**Intervention and follow-up**

After a 2-week observational period demonstrating no improvement with conventional treatment, patients were prescribed cenegermin ophthalmic solution 0.002% (Dompé, Boston, MA) every 2 hours, six times a day for 8 weeks. The primary endpoint was a decrease in size of abnormal epithelium following 8 weeks of treatment compared with baseline. Secondary endpoints included the number of patients with resolution of an epithelial defect (if present), improvement in VA, and an increase in corneal sensation measured by Cochet–Bonnet aesthesiometry at the final visit compared with the screening visit. We attempted to minimize sources of bias in this open-labeled study by collecting objective data and avoiding initiation of a new treatment during the study period.

All patients underwent clinical evaluation at each study visit. Screening visit (Day 0) consisted of an extensive medical history, including a review of previous ocular and systemic medications, and a complete eye exam. VA was documented using a Snellen chart and corneal sensitivity with a Cochet–Bonnet aesthesiometer. A slit lamp exam was performed with measurement of the area of abnormal epithelium and any epithelial defect. The area of abnormal epithelium was calculated by multiplying the vertical and horizontal dimensions. The approximate percentage of cornea involved was determined at the slit lamp with the following equation: Percent area of abnormal epithelium = surface area of abnormal epithelium/total corneal surface area. Patients were examined at baseline prior to starting treatment, at the midway point of treatment at 4 weeks, and at the final visit following 8 weeks of treatment. The examinations and time-points are outlined in Figure 1.
LSCD grading was performed using the clinical scoring system described by Aravena et al. In short, 3 criteria are assessed: degree of limbal involvement (graded as 0–4 points with respect to clock-hours affected), area of corneal surface involvement (graded from 0 to 4 points with respect to percentage affected), and involvement of the visual axis (graded as 0 or 2 points with respect to the central 4 mm of the cornea being affected).3

Data analysis
Data are presented in the form of mean ± 95% standard deviation. A student’s t-test was used to analyze the data points comparing the baseline visit and the final visit. A P value less than .05 was considered statistically significant. All statistical analyses were conducted using Windows Excel (Microsoft).

Results
Six eyes from 5 patients with LSCD and NK were evaluated and enrolled in the study. There were 4 males and 1 female. Average age was 77.2 (range 70–85 years). Patients had various causes for NK; 2 subjects had diabetes mellitus, 1 subject had severe meibomian gland disease, 1 subject had herpes zoster ophthalmicus, and 1 subject had multiple causes, including herpes zoster ophthalmicus with a history of lamellar keratoplasty in the right eye and a history of laser-assisted in situ keratomileusis (LASIK) refractive surgery and long-term use of multiple glaucoma medications in both eyes. Patient demographics and prior treatments are summarized in Table 1.

Evaluation of the primary endpoint demonstrated a significant decrease in the area of abnormal epithelium, from 73 ± 18% at baseline to 48 ± 8% following treatment (P=.036). An improvement was observed in 5 out of 6 eyes. One eye had an epithelial defect at the beginning of the study which resolved with treatment. Representative photographs of Case 2 are displayed in Figure 2.

Patients also benefited from the effects of cenegermin with regards to other parameters. A significant improvement in VA was observed, with overall average logMAR of 1.67 ± 0.76 initially and 1.19 ± 0.80 at final evaluation (P=.045). Four of the 6 eyes had improved VA. The VA remained the same in 2 eyes, with limited VA of hand motion (logMAR 2.3) secondary to diabetic retinopathy in 1 patient. No other patients had an ocular comorbidity causing significant vision limitation. Corneal sensation also improved; Cochet–Bonnet aesthesiometry prior to treatment measured 14.7 ± 6 mm and 26.7 ± 12 after treatment (P=.009). When evaluating LSCD using the scale published by Aravena et al.,3 there was improvement with an initial visit grade of 8 ± 2 and a final visit grade of 5 ± 3. However, this difference was not statistically significant (P=.14). These findings are summarized in Table 2.

Discussion
This study evaluates the effect of cenegermin on LSCD in patients with NK. The clinical exam findings of our study demonstrated improvement of limbal stem cell function characterized by decrease in the area of abnormal epithelium and improved VA. We also demonstrated improvement of NK with restoration of corneal sensation and closure of epithelial defect when present. The improvement occurred despite previously failed conventional treatments, including topical anti-inflammatory medications, punctal plugs, autologous serum tears, contact lens, oral doxycycline, amniotic membrane graft, and tarsorrhaphy.

There is significant overlap in the treatment of LSCD and NK. Medical management include
the optimization of the ocular surface and reduction of aggravating factors, as well as controlling inflammation. Surgical options include the use of punctal plugs, amniotic membrane graft, and tarsorrhaphy. Limbal stem cell transplantation has the potential to repopulate stem cells and restore the normal cornea epithelium. To date, there is no medication that will replenish absent or damaged limbal stem cells.

Cenegermin has been shown to benefit patients with ocular surface disease in previous studies. The phase I and phase II studies demonstrated improved corneal healing in patients with cenegermin compared with controls, as well as showing similar efficacy between 2 dosages of medication. Pflugfelder et al. previously showed higher rates of corneal healing after 8 weeks in subjects using cenegermin, including 1 patient with LSCD, compared with vehicle in a randomized controlled trial. Cheung, et al. showed the benefit of adding a bandage contact lens to cenegermin treatment in patients with NK. In this study, 9 eyes from 8 patients had LSCD; corneal sensation improved in all 9 eyes, persistent epithelial defect improved in 1 of 4 eyes, and VA improved in 4 eyes following treatment. In addition to improvement in clinical signs of NK, Mastropasqua et al. showed an anatomical change with an increase in mean nerve density, number of nerve branches, and nerve fiber diameter. Our current study also demonstrated improved ocular surface health and resolution of epithelial defect, similar to studies conducted by Pflugfelder et al. and Cheung et al. Anatomical analysis did not fall within the scope of this project but presents an avenue for future research employing confocal microscopy as well as cytology and immunohistochemistry.

Pain was the most common side effect reported on the clinical trials conducted. Interestingly, Habibi and Lee employed cenegermin for NK in patients who had undergone LASIK. Their study showed initial improvement in dry eye symptoms at 8 weeks followed by worsening vision and symptoms 1 month after cessation prompting them to question if an alternative dosing is required in LASIK patients. In the current study, patient reported proper adherence during all follow-ups and no patients dropped out due to adverse effects. Going forward, follow-up visits after cessation of the medication would clarify whether there is regression of disease despite the initial improvement.

Table 1. Patient demographics, underlying diagnosis leading to ocular surface disease, and prior treatments attempted prior to cenegermin.

| Case | Sex | Age | Diagnosis                  | Prior treatment                                                                 |
|------|-----|-----|----------------------------|-------------------------------------------------------------------------------|
| 1    | Male| 70  | Diabetes mellitus          | Erythromycin ointment\(^a\), cyclosporine 0.05% drops, Prokera ring, punctal plugs |
| 2    | Male| 80  | HZO                        | Autologous serum tears\(^a\), oral doxycycline\(^a\), sutured AMG, temporary tarsorrhaphy |
| 3    | Male| 84  | Diabetes mellitus          | Erythromycin ointment\(^a\), acetylcysteine drops\(^a\), oral azithromycin\(^a\), cyclosporine 0.05% drops, Prokera ring, punctal plugs |
| 4    | Female| 85 | Meibomian gland disease    | Moxifloxacin drops\(^a\), erythromycin ointment\(^a\), preservative-free dexamethasone 0.1%, Prokera ring, bandaged contact lens |
| 5\(^b\) | Male| 72  | HZO, LASIK, glaucoma, LKP | Oral doxycycline\(^a\), cyclosporine 0.05% drops, lifitegrast drops, punctal cautery, autologous serum tears, PROSE lens |
| 6\(^b\) | Male| 72  | LASIK, glaucoma            | Oral doxycycline\(^a\), cyclosporine 0.05% drops, lifitegrast drops, punctal cautery, autologous serum tears, PROSE lens |

AMG, amniotic membrane graft; HZO, herpes zoster ophthalmicus; LKP, lamellar keratoplasty.

\(^a\)These medications were continued during the cenegermin treatment period.

\(^b\)Cases 5 and 6 both correspond to Subject 5. Case 5 is the right eye and Case 6 is the left eye.
There are a number of limitations to our study. First, the sample size was not calculated, resulting in a small treatment arm that may limit the ability to generalize results to the larger population. Second, because this was not a double-masked study, there is potential of bias of the investigator and study subjects for a positive outcome. Third, the diagnosis of LSCD was based on clinical evaluation and not confirmed by histological examination.

**Table 2.** Comparison of visual acuity, percent abnormal epithelium, Cochet–Bonnet aesthesiometry measurement, and limbal stem cell deficiency (LSCD) grade.

| Subject | Snellen VA (logMAR) | Percent abnormal epithelium | Aesthesiometry (mm) | LSCD grade |
|---------|---------------------|-----------------------------|---------------------|------------|
|         | Initial | Final    | Initial (%) | Final (%) | Initial | Final | Initial | Final |
| 1       | HM (2.3) | HM (2.3) | 60        | 50        | 30        | 35     | 6        | 6     |
| 2       | CF at 3' (1.8) | 20/250 (1.1) | 80        | 40        | 3        | 10     | 10       | 0     |
| 3       | 20/500 (1.4) | 20/70 (0.54) | 90        | 40        | 15       | 30     | 10       | 4     |
| 4       | CF at 2' (1.9) | 20/150 (0.87) | 80        | 60        | 10       | 15     | 8        | 6     |
| 5       | HM (2.3) | CF at 1' (2.0) | 75        | 45        | 10       | 30     | 10       | 8     |
| 6       | 20/40 (0.3) | 20/40 (0.3) | 50        | 50        | 20       | 40     | 6        | 6     |

CF, count fingers; HM, hand motion.
examination without adjunctive modalities, such as confocal microscopy and impression cytology for confirmation of diagnosis. Although the pattern of abnormal epithelium is different in LSCD from NK, there is an overlap which introduces a level of subjectivity to the diagnosis and treatment outcome. The benefit of cenegermin could potentially be due to improvement of LSCD or NK, or a combination of both. Finally, although adjunctive treatment, such as autologous serum tears and oral doxycycline were not initiated during the study, it is possible that these may continue to have an effect on the outcome of the study.

In summary, our study suggests that cenegermin is beneficial in the treatment of LSCD with associated NK. This adds to the list of potential treatment option for refractory keratopathy due to these conditions. Future double-masked randomized control clinical trial with a larger number of study subjects followed for a longer period of time would be helpful in confirming the effectiveness of cenegermin for the treatment of LSCD with NK.

Declarations

Ethics approval and consent to participate
Study approval was obtained from the Stanford University Institutional Review Board (Protocol 57888). The study was registered at ClinicalTrials.gov with identifier NCT04552730. Written informed consent was obtained prior to initiation of the study which included use of the medication and inclusion in the study.

Consent for publication
Written informed consent was obtained for publication of the data.

Author contributions
Alejandro Arboleda: Data curation; Formal analysis; Software; Validation; Writing – original draft; Writing – review & editing.
Christopher N. Ta: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

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Availability of data and materials
Data can be made available upon request.

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Supplemental material
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