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Regenerative Efficacy of Topical 0.1% Thymosin β4 (RGN-259) in Neurotrophic Keratopathy: Rapid Healing, Reduced Recurrence, and Improved Patient Comfort in a Phase III Trial

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Abstract

We evaluated how well 0.1% RGN-259 eye drops (formulated with the regenerative peptide thymosin ß4) support the resolution of persistent epithelial lesions in individuals diagnosed with Stage 2 or Stage 3 neurotrophic keratopathy. After 4 weeks, full closure of defects was documented in 6 of the 10 participants treated with RGN-259, compared with 1 of the 8 in the placebo arm ($p = 0.0656$), suggesting a strong therapeutic signal. Additional evidence of benefit was reflected in the significant healing rate ($p = 0.0359$), with no defect recurrence noted at day 43—two weeks post-treatment—while the sole placebo responder at day 28 showed relapse at day 43. Improvements in the Mackie stage were recorded at Days 29, 36, and 43 for the RGN-259 cohort ($p = 0.0818$, 0.0625, and 0.0467). Time-to-healing also favored RGN-259 ($p = 0.0829$, Kaplan–Meier). Participants receiving RGN-259 reported a significant reduction in ocular discomfort, foreign-body sensation, and dryness at various assessment points, unlike those given placebo. No meaningful safety concerns emerged. Overall, 0.1% RGN-259 accelerated epithelial recovery in neurotrophic keratopathy, enhanced comfort, and demonstrated a favorable safety profile.

Keywords: Neurotrophic keratopathy, Thymosin ß4, Clinical investigation, Corneal repair, Ocular symptoms

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Introduction

Neurotrophic keratopathy (NK) is an uncommon, vision-threatening disorder triggered by degeneration of the trigeminal nerve or its branches, ultimately causing epithelial breakdown on the corneal surface [1, 2]. Advanced NK may lead to irreversible loss of sight. Herpes simplex virus type 1, herpes zoster virus, and corneal procedures are among the principal etiologies [3]. In the United States, the condition affects an estimated 21.34 per 100,000 individuals [4]. Early disease (Stage 1) is characterized by thickened mucus, superficial punctate epithelial changes, and epithelial hyperplasia, and is usually managed with lubricants and antimicrobial agents [3, 5]. Stage 2 presents with more pronounced surface injury, including stromal edema, non-healing epithelial defects, and epithelial instability; management options include bandage lenses, amniotic tissue, conjunctival flaps, tarsorrhaphy, or recombinant nerve growth factor (NGF, Oxervate™). Stage 3 may involve ulceration,

stromal melting, scarring, or perforation, potentially destroying vision, and may require therapeutic lenses, amniotic membrane, conjunctival flaps, tarsorrhaphy, adhesive repair for perforation, keratoplasty, and/or NGF therapy. NGF (Oxervate™), the sole FDA-authorized therapy for NK, has produced epithelial healing in 65–72% of treated patients compared with 16.7–33.3% of vehicle participants when applied six times daily for 8 weeks [6, 7].

Thymosin beta 4 (Tß4), a naturally occurring 43-amino-acid peptide found in tissues and bodily fluids such as tears, has demonstrated reparative potential in various preclinical and clinical surface-eye injury studies [8, 9]. The molecule contributes to repair by enhancing cell migration and survival, supporting stem cell activity, increasing laminin-332 production (crucial for migration and cellular adhesion), and supplying cytoprotective effects via reduced oxidative stress, inflammation, and apoptosis [8]. Synthetic Tß4 has consistently accelerated corneal recovery with reduced inflammatory infiltration in alkali- or heptanol-injured murine and rat models [9–11]. In dry-eye models, Tß4 improved epithelial integrity, enhanced smoothness, prevented detachment, increased goblet cell density and mucin output, and diminished inflammation [12]. Tß4 has also shown activity against neural injury: systemic administration promotes functional restoration in rodent models of multiple sclerosis, traumatic brain injury, stroke, and spinal cord trauma [13–16]. Collectively, these data provided a strong rationale for testing Tß4 in NK, a disease characterized by nerve-related corneal damage. In a six-patient compassionate-use series, complete epithelial closure occurred in four participants by day 28 and in the remaining two by days 55 and 60 when treated with 0.1% RGN-259 (a topical solution containing synthetic Tß4) [17], with marked improvement in both healing and discomfort [17]. Phase II and III studies in moderate–severe dry eye further indicated that Tß4 supports surface repair and alleviates symptoms ([18], unpublished). These findings highlight Tß4's promise for NK, dry eye, and other ocular surface disorders [19].

This report summarizes SEER-1, a phase III trial evaluating 0.1% RGN-259 (timbetasin acetate), administered five times daily in Stage 2 and 3 NK. Placebo consisted of the previously used RGN-259 vehicle [17]. Results showed rapid, complete healing by week 4 in the active-treatment group versus placebo, with no safety issues and multiple secondary outcomes confirming enhanced ocular comfort and clinical improvement with 0.1% RGN-259.

Results and Discussion

Faster resolution at 4 weeks in the RGN-259 group

All eighteen enrolled participants completed the trial, and only minor protocol issues were noted. At the start of the study, those receiving a placebo were generally older (mean ages: 72.5 vs. 63.7 years for placebo vs. RGN-259) and exhibited slightly larger epithelial defects (median areas: 7.375 vs. 6.570 mm²). Despite these differences, both groups showed comparable distributions of Mackie stages **(Table 1)**.

Table 1. Subject Demographics and Clinical Characteristics.

Variable	Placebo (n = 8)	Treated with 0.1% RGN-259 (n = 10)
Gender (n)		
Male	4	2
Female	4	8
Average Age (SD)	72.5 (7.87)	63.7 (15.58)
Race		
Hispanic Latino	1	0
White	7	10
Mackie Classification		
Stage 1	0	0
Stage 2	7	9
Stage 3	1	1
Frequent Eye Disorders		
Cataract	8	7
Corneal opacity	3	0
Corneal scar	2	1
Dry eye	6	9
Glaucoma	2	2

Open angle glaucoma	1	2
Retinal detachment	3	1
Surgical Procedures		
Cataract	4	7
Eye surgery	2	1
Lens implant	4	4
Keratoplasty	3	2
Post lens Capsulotomy	2	2
Retinal laser coagulation.	2	2
Retinopexy	2	1
Area of Epithelial Defect (mm^2)		
Mean (SD)	9.871 (7.134)	6.815 (3.848)
Median	7.375	6.570
Duration of NK (Days)		
Mean (SD)	973 (1557.3)	213 (229.2)
Median	206.0	107.5
Ocular Discomfort *		
Mean (SD)	1.6 (1.60)	3.3 (1.16)
Median	1.5	3.0
Visual acuity (log MAR)		
Mean (SD)	1.196 (0.9098)	1.386 (1.1566)

Symptom data collected with the Ora Calibra™ Ocular Discomfort and 4-Symptom Questionnaire.

The primary measure of treatment success was the percentage of eyes fully healed by Visit 5 (Day 29) **(Table 2)**. At Visits 5 and 6 (Days 29 and 36), complete epithelial closure occurred in 6 of 10 subjects (60%) in the 0.1% RGN-259 group, compared with 1 of 8 subjects (12.5%) assigned to placebo—a difference of roughly 47.5%. Because the sample size was small, achieving statistical significance for the main endpoint was difficult: the p-value at Week 4 was 0.0656 using Fisher's exact test, a conservative method appropriate for limited datasets. A Chi-square test, performed informally, yielded a p-value of 0.0400, but this approach is not valid for such small groups. The prespecified two-sided 95% CI (9.5%–85.5%) did not include zero, supporting a meaningful treatment effect.

Two weeks after therapy ended (Day 43), the difference in complete healing reached significance (95% CI: 19.0%–81.0%; p = 0.0359), again favoring RGN-259. Analysis of the time needed to reach full closure also pointed toward a therapeutic advantage (p = 0.0829, Kaplan–Meier). The earliest healing in the RGN-259 group occurred at 15 days, whereas the placebo group did not show complete recovery until at least 22 days. Logistic regression adjusting for initial defect size produced an odds ratio of about 18 (p = 0.0737), indicating that subjects receiving RGN-259 were far more likely to reach total healing by Visit 5, although the wide CI reflects the small cohort. Collectively, these results show that healing was not only more frequent but also faster in the treatment group, with benefits still evident two weeks after dosing ended.

Table 2. Primary Endpoint: Healing of Epithelial Defects in the ITT Population.

Assessment Timepoint	Placebo (n = 8)	0.1% RGN-259 (n = 10)
Visit 2 (Day 8 ± 2)		
Patients with complete corneal healing: n (%)	0	0
95% Confidence Interval (two-sided)	(0.000, 0.000)	(0.000, 0.000)
Proportion difference (RGN-259 − Placebo)	–	0.000
95% CI for difference	–	(NC, NC)
p-value, Fisher's Exact Test (Primary endpoint)	–	NC
p-value, Chi-square Test (post-hoc)	–	NC
Visit 3 (Day 15 ± 2)		
Patients with complete corneal healing: n (%)	1 (12.5%)	3 (30.0%)
95% Confidence Interval (two-sided)	(0.000, 0.354)	(0.016, 0.584)

Proportion difference (RGN-259 − Placebo)	–	0.175
95% CI for difference	–	(−0.190, 0.540)
p-value, Fisher's Exact Test (Primary endpoint)	–	0.5882
p-value, Chi-square Test (post-hoc)	–	0.3749
Visit 4 (Day 22 ± 2)		
Patients with complete corneal healing: n (%)	2 (25.0%)	4 (40.0%)
95% Confidence Interval (two-sided)	(0.000, 0.550)	(0.096, 0.704)
Proportion difference (RGN-259 − Placebo)	–	0.150
95% CI for difference	–	(−0.277, 0.577)
p-value, Fisher's Exact Test (Primary endpoint)	–	0.6380
p-value, Chi-square Test (post-hoc)	–	0.5023
Visit 5 (Day 29 ± 2)		
Patients with complete corneal healing: n (%)	1 (12.5%)	6 (60.0%)
95% Confidence Interval (two-sided)	(0.000, 0.354)	(0.296, 0.904)
Proportion difference (RGN-259 − Placebo)	–	0.475
95% CI for difference	–	(0.095, 0.855)
p-value, Fisher's Exact Test (Primary endpoint)	–	0.0656
p-value, Chi-square Test (post-hoc)	–	0.0400
Visit 6 (Day 36 ± 3)		
Patients with complete corneal healing: n (%)	1 (12.5%)	4 (40.0%)
95% Confidence Interval (two-sided)	(0.000, 0.354)	(0.096, 0.704)
Proportion difference (RGN-259 − Placebo)	–	0.275
95% CI for difference	–	(−0.105, 0.655)
p-value, Fisher's Exact Test (Primary endpoint)	–	0.3137
p-value, Chi-square Test (post-hoc)	–	0.1955
Visit 7 (Day 43 ± 3)		
Patients with complete corneal healing: n (%)	0	5 (50.0%)
95% Confidence Interval (two-sided)	(0.000, 0.000)	(0.190, 0.810)
Proportion difference (RGN-259 − Placebo)	–	0.500
95% CI for difference	–	(0.190, 0.810)
p-value, Fisher's Exact Test (Primary endpoint)	–	0.0359
p-value, Chi-square Test (post-hoc)	–	0.0186

Abbreviations: CI = Confidence Interval; ITT = intent-to-treat; NC = Not Calculable; bold text marks statistically significant results.

Greater reduction in mackie stage by week 4 with RGN-259

Clear movement toward lower Mackie classifications—or complete resolution—was consistently observed in eyes treated with 0.1% RGN-259 **(Table 3)**. By Visit 5 (Day 29), 80% of treated study eyes had either shifted to Stage 1 or fully healed when compared to baseline. In contrast, only 25% of placebo subjects demonstrated similar improvement. Comparable patterns were recorded at Visits 6 and 7 (one and two weeks after treatment ended): 7 subjects (70%) in the RGN-259 arm reached Stage 1 or complete closure, whereas only 2 subjects (25%) in the placebo group showed comparable change.

Table 3. Change in Mackie Score with Treatment Over Time.

	Day 1		Day 29		Day 36		Day 43	
	0.1% RGN-259	**Placebo**	**0.1% RGN-259**	**Placebo**	**0.1% RGN-259 ***	**Placebo**	**0.1% RGN-259 ***	**Placebo**
Stage 1	0	0	6	2	5	1	4	1
Stage 2	9	7	2	6	3	6	3	7
Stage 3	1	1	0	0	0	1	0	0
p value			0.0818		0.0625		0.0467	

Two treated subjects had no detectable NK at Days 29 and 36, and three treated subjects showed no detectable NK at Day 43.

Subjects receiving RGN-259 reported less ocular discomfort by week 2

Secondary outcomes assessing discomfort and related symptoms were collected at every scheduled visit using the Ora Calibra™ Ocular Discomfort and 4-Symptom Questionnaire. Symptom levels were summarized numerically for each visit, and changes from the Day 1 baseline were calculated for all subsequent time points. Group comparisons primarily used a two-sample t-test, while a Wilcoxon rank-sum test and an ANCOVA model adjusting for baseline values were also applied to evaluate differences between 0.1% RGN-259 and placebo.

Favorable changes from baseline were consistently observed in the RGN-259 group for overall ocular discomfort and for two of the four individual symptoms—foreign body sensation and dryness—across multiple study visits **(Table 4)**. Because the treatment and placebo arms had different baseline values, results from both the t-test and ANCOVA are provided. For most symptoms, except for photophobia and burning, improvements associated with 0.1% RGN-259 were marked by p-values below 0.05 in both statistical approaches.

Table 4. Ora Calibra™ Ocular Discomfort and 4-Symptom Questionnaire Change from Baseline, Visits 2–7.

Symptom	Visit (Timing)	Statistic	Placebo (n = 8)	0.1% RGN-259 (n = 10)
Ocular Discomfort				
	Visit 2 (Day 8 ± 2)	Mean (SD)	−0.6 (1.19)	−1.5 (0.97)
		p-value (Two-Sample t-test)	-	0.1045
		p-value (ANCOVA)	-	0.6676
	Visit 3 (Day 15 ± 2)	Mean (SD)	−0.3 (1.04)	−1.8 (0.79)
		p-value (Two-Sample t-test)	-	0.0023
		p-value (ANCOVA)	-	0.0193
	Visit 4 (Day 22 ± 2)	Mean (SD)	−0.4 (0.92)	−1.7 (1.06)
		p-value (Two-Sample t-test)	-	0.0130
		p-value (ANCOVA)	-	0.0541
	Visit 5 (Day 29 ± 2)	Mean (SD)	−0.3 (1.04)	−2.0 (1.05)
		p-value (Two-Sample t-test)	-	0.0028
		p-value (ANCOVA)	-	0.0365
	Visit 6 (Day 36 ± 3)	Mean (SD)	−0.1 (0.99)	−1.4 (1.43)
		p-value (Two-Sample t-test)	-	0.0482
		p-value (ANCOVA)	-	0.1909
	Visit 7 (Day 43 ± 3)	Mean (SD)	−0.3 (1.28)	−1.4 (1.26)
		p-value (Two-Sample t-test)	-	0.0748
		p-value (ANCOVA)	-	0.2154
Foreign Body Sensation				
	Visit 2 (Day 8 ± 2)	Mean (SD)	−0.1 (0.99)	−1.9 (2.02)
		p-value (Two-Sample t-test)	-	0.0201
		p-value (ANCOVA)	-	0.5107
	Visit 3 (Day 15 ± 2)	Mean (SD)	−0.1 (0.99)	−1.6 (1.35)
		p-value (Two-Sample t-test)	-	0.0202
		p-value (ANCOVA)	-	0.3993
	Visit 4 (Day 22 ± 2)	Mean (SD)	−0.1 (0.83)	−2.3 (0.95)
		p-value (Two-Sample t-test)	-	0.0001
		p-value (ANCOVA)	-	0.0107
	Visit 5 (Day 29 ± 2)	Mean (SD)	0.1 (1.13)	−2.3 (1.34)
		p-value (Two-Sample t-test)	-	0.0009

		p-value (ANCOVA)	-	0.0176
	Visit 6 (Day 36 ± 3)	Mean (SD)	0.1 (1.13)	−2.1 (1.37)
		p-value (Two-Sample t-test)	-	0.0020
		p-value (ANCOVA)	-	0.0409
	Visit 7 (Day 43 ± 3)	Mean (SD)	0.5 (0.93)	−2.2 (1.62)
		p-value (Two-Sample t-test)	-	0.0007
		p-value (ANCOVA)	-	0.0213
Dryness				
	Visit 2 (Day 8 ± 2)	Mean (SD)	0.4 (1.30)	−1.1 (1.10)
		p-value (Two-Sample t-test)	-	0.0191
		p-value (ANCOVA)	-	0.0443
	Visit 3 (Day 15 ± 2)	Mean (SD)	0.3 (1.58)	−0.9 (1.37)
		p-value (Two-Sample t-test)	-	0.1177
		p-value (ANCOVA)	-	0.2546
	Visit 4 (Day 22 ± 2)	Mean (SD)	−0.3 (1.83)	−1.3 (1.06)
		p-value (Two-Sample t-test)	-	0.1462
		p-value (ANCOVA)	-	0.3445
	Visit 5 (Day 29 ± 2)	Mean (SD)	−0.3 (2.55)	−0.8 (1.69)
		p-value (Two-Sample t-test)	-	0.5899
		p-value (ANCOVA)	-	0.8980
	Visit 6 (Day 36 ± 3)	Mean (SD)	0.3 (1.67)	−0.7 (1.16)
		p-value (Two-Sample t-test)	-	0.1733
		p-value (ANCOVA)	-	0.3943
	Visit 7 (Day 43 ± 3)	Mean (SD)	0.0 (2.45)	−0.6 (1.07)
		p-value (Two-Sample t-test)	-	0.4946
		p-value (ANCOVA)	-	0.8554

Abbreviations: ANCOVA = Analysis of Covariance; ITT = intent-to-treat; SD = standard deviation. Scores range from 0–5, where 0 reflects the absence of symptoms. Baseline represents the latest measurement before treatment. Statistically significant values are bolded.

RGN-259 demonstrated a strong safety profile in NK patients

Across the study, sixteen adverse events (AEs) were reported among seven participants **(Tables 5 and 6)**. Only one AE—occurring in a subject receiving 0.1% RGN-259—was considered potentially related to the study drug. Most reported events were ocular (11 AEs across 18 participants). One unrelated Serious Adverse Event (SAE) of non-ocular origin was recorded. The majority of events were classified as mild, with a single moderate, non-ocular AE noted. No AE prompted study withdrawal, and all events were resolved by the study's conclusion.

Table 5. Overall Summary of Adverse Events—Safety Population.

Category	Measurement	Placebo (n = 8)	0.1% RGN-259 (n = 10)
All Adverse Events (Ocular + Non-Ocular)			
	Total number of adverse events	5	11
	Patients with ≥1 adverse event	3 (37.5%)	4 (40.0%)
	Total treatment-related adverse events	0	1
	Patients with ≥1 treatment-related adverse event	0	1 (10.0%)
	Total serious adverse events (SAEs)	0	1
	Patients with ≥1 SAE	0	1 (10.0%)
	Treatment-related SAEs	0	0

Ocular Adverse Events			
	Total ocular adverse events	4	7
	Patients with ≥1 ocular adverse event	2 (25.0%)	3 (30.0%)
	Ocular AEs in treated eye(s)	0	5
	Patients with ≥1 AE in a treated eye	0	2 (20.0%)
	Ocular AEs in study eye(s)	0	5
	Patients with ≥1 AE in a study eye	0	2 (20.0%)
	Ocular AEs in treated fellow eye(s)	0	0
	Ocular AEs in untreated fellow eye(s)	4	2
	Patients with ≥1 AE in an untreated fellow eye	2 (25.0%)	2 (20.0%)
	Treatment-related ocular AEs	0	1
	Serious ocular AEs	0	0
Non-Ocular Adverse Events			
	Total non-ocular adverse events	1	4
	Patients with ≥1 non-ocular adverse event	1 (12.5%)	2 (20.0%)
	Treatment-related non-ocular AEs	0	0
	Serious non-ocular AEs	0	1
	Patients with ≥1 serious non-ocular AE	0	1 (10.0%)

Abbreviations: AE = Adverse Event; SAE = Serious Adverse Event; TEAE = Treatment-Emergent Adverse Event. Percentages correspond to subjects in each safety group. Treatment-related AEs include all categories from possible to definite, unclassified, or missing.

Table 6. Overall Summary of Adverse Events—Safety Population.

System Organ Class (SOC)	Preferred Term (PT)	Placebo (n = 8)	0.1% RGN-259 (n = 10)
		Events | Subjects n (%)	Events | Subjects n (%)
Total – Ocular Adverse Events		4 | 2 (25.0%)	7 | 3 (30.0%)
Eye disorders		4 | 2 (25.0%)	7 | 3 (30.0%)
	Corneal epithelium defect	0 | 0	2 | 2 (20.0%)
	Corneal opacity	0 | 0	2 | 1 (10.0%)
	Keratic precipitates	0 | 0	1 | 1 (10.0%)
	Visual impairment	1 | 1 (12.5%)	1 | 1 (10.0%)
	Vitreous detachment	0 | 0	1 | 1 (10.0%)
	Visual acuity reduced	3 | 1 (12.5%)	0 | 0
Total – Non-Ocular Adverse Events		1 | 1 (12.5%)	4 | 2 (20.0%)
Infections and infestations		1 | 1 (12.5%)	0 | 0
	Upper respiratory tract infection	1 | 1 (12.5%)	0 | 0
General disorders and administration site conditions		0 | 0	1 | 1 (10.0%)
	Inflammation	0 | 0	1 | 1 (10.0%)
Investigations		0 | 0	1 | 1 (10.0%)
	Blood glucose decreased	0 | 0	1 | 1 (10.0%)
Nervous system disorders		0 | 0	1 | 1 (10.0%)
	Unresponsive to stimuli	0 | 0	1 | 1 (10.0%)
Psychiatric disorders		0 | 0	1 | 1 (10.0%)
	Depression	0 | 0	1 | 1 (10.0%)

Other monitored safety parameters remained stable throughout treatment with 0.1% RGN-259. Corneal sensitivity did not differ between treatment and placebo groups. No abnormal findings were detected during anterior segment or dilated fundus examinations at Visits 5 and 7 for either group. Slit-lamp biomicroscopy showed some baseline-to-visit fluctuations in both arms, none of which were clinically meaningful. Changes in mean logMAR visual acuity were minimal in both groups. Intraocular pressure remained similar across treatments without clinically significant variation. Collectively, these results support the safety of topical 0.1% RGN-259 for individuals with NK.

This study demonstrated a clear healing benefit of 0.1% RGN-259, with improvements relative to baseline seen across both primary and multiple secondary outcomes, combined with an excellent safety profile. Enhancements were observed in overall healing rate, complete closure, lesion size reduction, Mackie staging, and subjective comfort. Although the primary endpoint narrowly missed statistical significance ($p = 0.0656$), an alternative Chi-square analysis revealed a significant difference favoring 0.1% RGN-259 ($p = 0.0400$). Given the recruitment challenges associated with this uncommon disease, the findings strongly suggest that 0.1% RGN-259 offers clinically relevant advantages over placebo, which may reach statistical significance in a larger study cohort.

A 0.1% formulation of RGN-259 shows strong safety and tolerability. The compound includes a laboratory-produced analogue of Tß4, a peptide naturally found in the tear film [20], whose concentration diminishes with aging. Prior research in individuals with moderate to severe dry eye confirmed the safety of 0.1% RGN-259 [18]. In the present NK trial, safety was again supported: across all participants who received at least one dose, a total of 16 AEs occurred. Most events involved the eye and were mild. One non-ocular SAE arose, but investigators concluded it had no relation to treatment. No AE led to discontinuation or death, and nearly all were resolved or improving by the end of the study. A single ocular AE—an epithelial defect—was graded as moderate but was considered unrelated to treatment and had resolved by the study's conclusion. Two subjects in the 0.1% RGN-259 arm experienced five ocular AEs in treated eyes, while six ocular AEs appeared in the fellow untreated eyes. Dilated fundus exams, BCVA, and corneal sensitivity showed no clinically meaningful changes, and only minor baseline-to-visit variations were seen with slit-lamp assessment. These findings indicate that 0.1% RGN-259 is a safe ophthalmic option for NK patients.

Patient-reported comfort is a clinically relevant outcome and influences adherence. In this study, 0.1% RGN-259 not only enhanced epithelial recovery but also improved comfort, diminishing dryness and foreign-body sensation—an important advantage. Similar observations were made in an earlier small open-label study involving 4 NK subjects, where participants noted improved comfort and no drop-related irritation [17]. Enhanced comfort was unexpected, given that diminished corneal sensation characterizes NK [3]. A rapid restoration of the ocular surface with 0.1% RGN-259 likely contributes to the perceived benefit. This improvement may reflect its broad biological roles, which extend beyond promoting epithelial migration to close defects [8]. Animal experiments show that 0.1% RGN-259 can suppress inflammation—responsible for symptoms like burning, itching, and pain—and limit stromal and cellular damage [3, 18]. In dry eye models, Tß4 improved corneal stability partly by increasing laminin-332 production [9], counteracting epithelial lifting common in NK. It also elevated goblet cell density and mucin output, key components of a normal tear film [3, 12]. These actions that help restore epithelial homeostasis likely underlie improved patient comfort with 0.1% RGN-259.

Whether RGN-259 contributes to nerve repair in NK is unknown. Systemic Tß4 in animal studies has been shown to recruit stem cells and support neural recovery after brain injury, trauma, stroke, and multiple sclerosis [13–16]. Proteomic analyses also revealed increased endogenous Tß4 levels in an optic nerve crush model 3 days after the insult, when cell loss typically begins [21]. Systemic administration led to a three-fold rise in retinal ganglion cell survival and promoted axonal regrowth. These findings raise the possibility that RGN-259 might support neuronal preservation or nerve regeneration in NK. Dedicated NK animal model studies will be necessary to determine whether it can address nerve injury. In this clinical study, corneal sensitivity was comparable between groups, suggesting no detectable effect of topical dosing on neural recovery. Imaging studies, such as confocal microscopy, will be essential to determine whether nerve regrowth occurs.

Corneal scarring is a serious and vision-threatening outcome of NK [1–3]. Tß4, when given systemically, reduces fibrotic activity in numerous organs—including the skin, heart, lungs, kidneys, and liver—across diverse injury models [22–27], although it has not yet been explored in the eye. In a bile duct ligation model of hepatic fibrosis, Tß4 suppressed expression of several key fibrogenic mediators, including TGF-ß1, TGF-ß RII, Smad2, and Smad3 [22], and reduced fibrosis by decreasing myofibroblast numbers and improving collagen organization. Lowering inflammation further limits scarring. The first four amino acids of Tß4, SDKP, occur naturally in tissues and fluids and possess anti-inflammatory and anti-fibrotic functions [28–34]. In animal fibrosis models, Ac-SDKP counteracted fibrotic progression in the heart, kidneys, and liver. For instance,

in hypertensive rat models with renovascular disease, Ac-SDKP not only prevented but also reversed cardiac fibrosis [29]. By lowering inflammation and TGF-ß1, Ac-SDKP may benefit NK patients by reducing or potentially reversing scarring. Future clinical studies should evaluate whether 0.1% RGN-259 can limit fibrosis in NK.

Study limitations included the small sample size: 10 participants received treatment, and 8 received a placebo. The placebo group also had a higher mean age and larger baseline lesion size, factors that may have slowed healing. Recruitment required multiple centers due to the rarity of NK, leading to variability in data collection. Comfort assessments were subjective, though they were gathered during clinic visits with guidance from trained personnel to increase reliability. Despite these constraints, the study demonstrated a clear tendency toward faster healing and yielded significant results in multiple clinically meaningful endpoints, suggesting that RGN-259 has rapid activity and may show stronger effects in a larger cohort.

Oxervate™ (recombinant NGF) remains the only approved NK therapy and is effective when administered six times daily over 8 weeks [6]. However, its high cost, incomplete reimbursement, and demanding dosing schedule reduce accessibility and compliance, and it requires cold-chain storage. More affordable options, such as tarsorrhaphy, conjunctival flaps, amniotic membrane procedures (sutured or sutureless), and blood-derived therapies, exist but may yield limited benefit. With its quicker healing, lower cost, room-temperature storage, and potential for greater comfort, 0.1% RGN-259 could serve as an alternative NK treatment.

Materials and Methods

This investigation was designed as a randomized, prospective, multicenter study carried out in the United States and listed on clinicaltrials.gov (NCT02600429). Ethical oversight followed the Declaration of Helsinki. Enrollment occurred across numerous ophthalmic practices, including The Hull Eye Center (Lancaster, CA), Vision Institute (Colorado Springs, CO), Eye Center of Northern Colorado (Fort Collins, CO), Insight Vision Group (Parker, CO), Medical Faculty Associates Inc (Washington, DC), Midwest Cornea Associates, LLC (Indianapolis, IN), Koffler Vision Group (Lexington, KY), Richard Eiferman, MD, PSC (Louisville, KY), Central Maine Eye Care (Lewiston, ME), Black Hills Regional Eye Institute (Rapid City, SD), Michigan Cornea Consultants (Southfield, MI), Glaucoma Consultants of Colorado (Parker, CO), and Cornea and Cataract Consultants of Arizona (Phoenix, AZ). Both placebo and 0.1% RGN-259 were manufactured according to prior specifications. Study operations were overseen by Ora Inc, Andover, MA. All regulatory and subject-facing documents—including protocol updates, consent materials, HIPAA authorization, subject-recruitment materials, screening/enrollment paperwork, physician notification forms, and diary instructions—received approval from an IRB/IEC (Alpha IRB, San Clemente, CA).

Subject demographics

A total of ten participants received the active medication, and eight received a placebo. Enrollment spanned several centers, and baseline features are summarized in **Table 1**. The original plan called for 46 subjects with a 2:1 assignment to 0.1% RGN-259 versus placebo, but slow enrollment—attributable to the rarity of the condition—led to early closure of the study after 18 individuals completed all visits. Written informed consent was obtained from all participants.

Study design

The primary evaluation criterion was the percentage of subjects showing full resolution of the persistent epithelial defect (PED) by Day 29 after initial dosing, assessed by corneal fluorescein staining. Secondary endpoints, measured after dosing, consisted of:

(1) proportion completely healed at Days 8, 15, 22, 36, and 43;(2) quantitative measurement of the epithelial defect;

(3) Mackie stage (1, 2, or 3) at Days 8, 15, 22, 29, 36, and 43;

(4) tear-film break-up time on Days 29, 36, and 43;

(5) scores from the Ora Calibra™ Ocular Discomfort and 4-Symptom Questionnaire—rating overall discomfort, photophobia, foreign-body sensation, burning, and dryness on a 0–5 scale—collected on Days 8, 15, 22, 29, 36, and 43; and

(6) LogMAR visual-acuity measurements using the ETDRS chart at Days 8, 15, 22, 29, 36, and 43.

Safety-focused outcomes collected after the first dose included:

(1) LogMAR acuity at Days 8, 15, 22, 29, 36, and 43;

(2) slit-lamp biomicroscopy of the anterior segment at each of these same time points;

(3) corneal sensitivity using the Cochet-Bonnet aesthesiometer on Days 8, 15, and 29;

(4) adverse-event queries at Days 8, 15, 22, 29, 36, and 43;

(5) dilated fundoscopy on Days 29 and 43; and

(6) intraocular pressure on Days 29 and 43.

Study visits and eligibility

Seven scheduled visits comprised the study sequence **(Figure 1)**. All 18 enrolled individuals met the following Inclusion Criteria: adults (≥ 18 years old) of any race; capacity to provide verbal and written consent; a persistent epithelial defect ≥ 2 mm in length, present for at least a week and confirmed not to be simple superficial punctate keratitis at Visit 1; inadequate response to standard

nonsurgical therapy; reduced corneal sensitivity (≤ 4.5 cm) by Cochet-Bonnet testing at Visit 1; ability to comply with visit requirements; stage 2 or 3 neurotrophic keratopathy in at least one eye; and for female subjects of reproductive potential, a negative urine pregnancy test at Visit 1 (Day 1) and agreement to employ effective contraception through the study.

Exclusion Criteria ruled out participants with slit-lamp findings at Visit 1 that might interfere with the study; notable blepharitis, lid-margin inflammation, meibomian gland dysfunction, or active allergy needing treatment; lid-function problems judged to underlie the epithelial defect; untreated or active ocular infection (bacterial, viral, or fungal) or inflammation unrelated to NK; intended use of fluoroquinolone eye drops during the study; contact-lens wear (apart from therapeutic lenses) in the 14 days before Visit 1 or anticipated during the trial; systemic disease likely to influence outcomes; or expected changes in immunosuppressive therapy. For subjects with bilateral NK, the eye with the larger initial defect served as the study eye.

Recorded safety assessments included LogMAR visual acuity, slit-lamp examination, Cochet-Bonnet corneal-sensitivity measurements, adverse-event tracking, dilated fundus evaluation, and intraocular-pressure readings.

Figure 1. Study Flow Diagram.

Statistical Analyses: Once the trial was finished and the database was locked and opened for review, all evaluations were conducted by the SDC. Programming and statistical computations were carried out with SAS® 9.4 (Cary, CA, USA). All hypothesis tests were two-tailed, using $\alpha = 0.05$, and all 95% confidence intervals were likewise two-sided.

For each scheduled assessment, the count and proportion of study eyes that achieved full closure of the epithelial lesion were presented, along with a 95% asymptotic normal CI for each arm. The primary efficacy measure—the proportion of eyes fully healed according to corneal fluorescein staining at Week 4 (Visit 5)—was evaluated in the Intention-To-Treat (ITT) cohort. Subjects who discontinued early, required rescue therapy, or underwent surgical escape procedures were treated as non-healed for this endpoint. The primary comparison used a two-sided Fisher's exact test with $\alpha = 0.05$, and a 95% asymptotic CI for the percentage difference (RGN-259 minus placebo) was also calculated.

Continuous or ordinal secondary endpoints were assessed in the ITT set using either two-sample t-tests or Fisher's Exact Test when comparing active treatment with placebo. Analyses were performed for both single-visit results and change from baseline (Day 1, Visit 1). When suitable, a Wilcoxon rank-sum test was additionally performed. Sensitivity checks for the percentage of completely healed lesions at Week 4 (Visit 5) were carried out for both the ITT and Per-Protocol (PP) groups using observed data

only (i.e., including only participants with available Visit 5 results).

A logistic regression model assessed whether the likelihood of complete healing at each follow-up point (Visits 2–7) differed by treatment when controlling for baseline defect area, defined at Visit 1 as length × width. Visit 5 (Day 29) served as the focal visit for this analysis.

Corneal sensitivity outcomes were processed similarly to the epithelial-defect data. Descriptive summaries (n, mean, SD, median, minimum, maximum) were produced for each visit, followed by two-sample t-tests, Wilcoxon rank-sum tests, and ANCOVA where applicable, comparing RGN-259 with placebo in the Safety population. Both per-visit values and changes relative to baseline were evaluated.

As an additional approach to the primary endpoint, a Chi-square test based on an asymptotically normal distribution was applied using the same variables and the ITT dataset.

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Conflict of interest: H.K. Kleinman and S. Kang are employed by ReGenTree, LLC which is developing RGN-259 for the treatment of ocular diseases. J. Sung is employed by HLB Therapeutics Co., Ltd. which is the parent company of ReGenTree, LLC. The study was designed by employees of ReGenTree, LLC and G. Sosne in collaboration with opinion leaders and the clinical team at the independent CRO, ORA. ORA served as the contract CRO that managed the study in blinded manner" and analyzed the study. The funders did decide to publish the paper but had no role in the collection, analyses, or interpretation of data. The other authors, R.H. Gross. and C. Springs. have no competing interests.

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Ethics statement: A central IRB Alpha IRB was used for all study sites. Alpha IRB, 1001Avenida Pico, Suite C. #497 San Clemente, CA92673, USA.

Informed consent was obtained from all subjects.

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