

## Manuscript Details

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

### Abstract

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

<b>Taxonomy</b>	Cornea Ulcers, Neurotrophic Keratopathy, Ulcerative Keratitis
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**Highlights: Précis**

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

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

3

4 **Running Head:** Recombinant human nerve growth factor for neurotrophic keratitis

5

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17

18 **Meeting Presentation:** Portions of this work have been presented at: the 2017 Association

19 for Research in Vision and Ophthalmology Annual Meeting, May 6–11, 2017, Baltimore,

20 Maryland; and the European Society of Ophthalmology 2017 Congress, June 10–13, 2017,

21 Barcelona, Spain.

22

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

28

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

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

36

### 37 **Abbreviations and Acronyms:**

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

- 46 growth factor; SAE = serious adverse event; SE = standard error; TAE = treatment-related  
47 adverse event; VAS = visual analogue scale.

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

49 **only:**

50 Appendix 1 (REPARO study group)

51 Appendix 2 (eligibility criteria)

52 Appendix 3 (prior treatments)

53 Appendix 4 (additional phase II safety results)

54 Figure 4 (change in Schirmer I from baseline)

55 Figure 6 (Kaplan-Meier uncontrolled treatment period)

56 Figure 7 (phase II PK)

57 Table 5 (corneal sensitivity improvement)

58

59 **Abstract (350 words)**

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

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

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

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

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

76 **RESULTS:** At week 4 (primary endpoint), 19.6% of vehicle-treated patients achieved  
77 corneal healing (<0.5 mm lesion staining), vs. 54.9% receiving rhNGF 10 µg/ml (+35.3%;  
78 97.06% confidence interval [CI] 15.88–54.71; P<0.001) and 58.0% receiving rhNGF 20  
79 µg/ml (+38.4%; 97.06% CI 18.96–57.83; P<0.001). At week 8 (key secondary endpoint),  
80 43.1% of vehicle-treated patients achieved <0.5 mm lesion staining, vs. 74.5% receiving  
81 rhNGF 10 µg/ml (+31.4%; 97.06% CI 11.25–51.49; P<0.001) and 74.0% receiving rhNGF

82 20 µg/ml (+30.9%; 97.06% CI 10.60–51.13, P<0.002). Post hoc analysis of corneal healing  
83 by the more conservative measure (0 mm lesion staining and no other persistent staining)  
84 maintained statistically significant differences between rhNGF and vehicle at weeks 4 and  
85 8. Over 96% of patients healed after controlled rhNGF treatment remained recurrence-free  
86 during follow-up. Treatment with rhNGF was well tolerated; adverse effects were mostly  
87 local, mild, and transient.

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

90

91 **INTRODUCTION**

92 With approximately 7000 nerve endings/mm<sup>2</sup>, the cornea is the most densely innervated  
93 tissue in humans.<sup>1</sup> Corneal nerves (deriving from the trigeminal ganglion) help maintain  
94 transparency in this avascular tissue and participate in ocular surface homeostasis by  
95 producing neurotrophins and facilitating sensory-dependent corneal and tearing reflexes.<sup>1</sup>  
96 <sup>2</sup> Trigeminal nerve damage may cause neurotrophic keratitis/keratopathy (NK) with  
97 partial or total loss of corneal sensation, leading to visual impairment and potentially  
98 permanent blindness. NK is a rare disease (estimated prevalence: 1.6–4.2 cases per  
99 10,000)<sup>3,4</sup> with various underlying etiologies (most commonly herpetic infections and  
100 ocular or neurological surgeries) that impair corneal innervation.<sup>5,6</sup> NK diagnosis,  
101 prognosis, and treatment (reviewed elsewhere)<sup>3,6</sup> are based on disease severity, which is  
102 classified broadly into three stages.<sup>7</sup> Briefly, stage 1 (mild) NK exhibits ocular surface  
103 irregularity and reduced vision. Stage 2 (moderate) NK exhibits a nonhealing persistent  
104 epithelial defect (PED), and stage 3 (severe) NK exhibits corneal ulceration involving sub-  
105 epithelial (stromal) tissue, which may progress to corneal perforation. All disease stages  
106 cause some vision loss; however, if untreated, moderate NK progresses to severe disease  
107 with associated risks of profound vision loss due to scarring and corneal perforation.  
108 Conventional therapy for stage 1 aims to prevent epithelial breakdown, generally by  
109 administering preservative-free artificial tears and discontinuing toxic topical medications.  
110 Stage 2/3 therapies aim to facilitate corneal healing and prevent corneal thinning (which  
111 may lead to perforation); these include surgeries and procedures (e.g., tarsorrhaphy,  
112 botulinum-induced ptosis, conjunctival flap, amniotic membrane transplantation) to  
113 restore ocular surface integrity, but potentially sacrificing vision and cosmesis.

114  
115 Strong evidence supports the treatment of NK with neurotrophic factors.<sup>8</sup> Nerve growth  
116 factor (NGF) has demonstrated important roles in maintaining corneal homeostasis *in*  
117 *vitro*, *ex vivo*, and in animal models.<sup>9, 10</sup> NGF is highly conserved among vertebrates,<sup>11</sup> and  
118 small uncontrolled, open-label studies with murine NGF (mNGF) produced promising  
119 results for the treatment of corneal neurotrophic ulcers.<sup>12, 13</sup> Confirmation of results  
120 obtained with mNGF have been highly anticipated<sup>14</sup>; however, nearly two decades passed  
121 with no approved treatments for NK, and no NGF-based treatments available for any  
122 indication. For NK therapies in general, clinical development has been hindered by the  
123 paucity of adequately sized and rigorously designed studies; indeed, only one randomized  
124 controlled trial of NK patients exists in the published literature to date, and the  
125 investigative treatment (topical fibronectin ophthalmic solution) was not superior to  
126 placebo for healing PEDs.<sup>15</sup> Thus, the natural history of NK is not completely understood,  
127 and approved treatments are not available for use as comparators for further studies. For  
128 NGF in particular, translational development has been mired by its complex tertiary  
129 structure, which complicates the manufacturing of recombinant human NGF (rhNGF)  
130 suitable for clinical use. To this end, we developed an *E. coli*-derived rhNGF formulation for  
131 topical ophthalmic use and demonstrated it to be safe and well tolerated in phase I  
132 randomized, double-masked, vehicle-controlled studies in healthy volunteers<sup>16</sup> and in NK  
133 patients.<sup>17</sup> Here, we report phase II study results of topical rhNGF treatment for moderate-  
134 to-severe NK.

135

136 **METHODS**

137

138 **Clinical Trial Design**

139

140 REPARO (Latin, “repair”) was a phase I/II, double-masked, randomized, multicenter,  
141 vehicle-controlled, parallel group study, which was designed to evaluate the safety and  
142 efficacy of rhNGF eye drops (10 or 20 µg/ml, 6 drops/day for 8 weeks) in patients with  
143 stage 2 or 3 NK. Phase I assessed safety in 18 patients to support proceeding to phase II,  
144 and was conducted, analyzed, and reported separately.<sup>17</sup> Phase II randomized 156 patients  
145 1:1:1 to rhNGF 10 µg/ml, rhNGF 20 µg/ml, or vehicle for an 8-week controlled treatment  
146 period. Follow-up duration (48 or 56 weeks) was determined by baseline group  
147 assignment and corneal healing status during controlled treatment. For vehicle-treated  
148 patients, baseline randomization included the possibility of secondary rhNGF treatment  
149 (10 or 20 µg/ml) in the event of treatment failure during the 8-week controlled treatment  
150 period, pre-defined as failure to achieve corneal healing, recurrence of NK after healing, or  
151 deterioration (lesion size increase of ≥1mm; best corrected distance visual acuity [BCDVA]  
152 decrease of >5 Early Treatment Diabetic Retinopathy Study [ETDRS] letters; progression to  
153 corneal melting/perforation; or onset of infection). This patient subset received 8 weeks of  
154 uncontrolled treatment before continuing follow-up (total follow-up: 56 weeks).

155

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

158

159 **Patients**

160

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

170

171 **Efficacy Assessments**

172

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

180

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

182 5 minutes); time to onset of healing (>20% reduction in maximum diameter of the corneal  
183 lesion from baseline); and time to corneal healing (<0.5 mm lesion staining) during the  
184 controlled/uncontrolled treatment periods. Post hoc efficacy variables included change in  
185 lesion size, and the primary endpoint of corneal healing reassessed more conservatively as  
186 0 mm lesion staining and no other persistent staining outside of the lesion.

187

### 188 **Safety Assessments**

189

190 The primary safety variable was incidence of adverse events (AEs). Ocular tolerability was  
191 recorded by patients on a visual analogue scale (VAS) from 0–100 mm (0 = no symptoms;  
192 100 = worst possible discomfort) for each of 7 different symptoms: foreign body sensation,  
193 burning/stinging, itching, ocular pain, sticky feeling, blurred vision, and photophobia. An  
194 overall VAS score was calculated as the mean of individual symptom scores. Other safety  
195 parameters included visual acuity (BCDVA measured in ETDRS letters), intraocular  
196 pressure (IOP), dilated fundus ophthalmoscopy, vital signs, hematology, and clinical  
197 chemistry.

198

### 199 **Pharmacokinetics and Immunogenicity Assessments**

200

201 Blood samples were collected for pharmacokinetics (PK) profiling and immunogenicity  
202 assessments (anti-NGF antibody shifts from baseline to post-baseline), performed using  
203 enzyme-linked immunosorbent assay (ELISA) as described previously.<sup>16</sup>

204

205 **Masking and Statistical Analysis**

206

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

216

217 Based on the only published randomized controlled trial of NK<sup>15</sup> and uncontrolled studies  
218 of mNGF-treated NK patients,<sup>12, 13</sup> 60% of rhNGF-treated patients were estimated to  
219 achieve <0.5 mm lesion staining at 4 weeks (vs. 30% in vehicle-treated patients). Although  
220 the study's exploratory nature did not warrant adjustment for multiple comparisons, 2-  
221 sided significance of chi-square testing was corrected according to Pocock,<sup>18</sup> yielding a  
222 97.06% confidence interval (CI) for the primary efficacy endpoint of corneal healing.  
223 According to this methodology, phase II required 141 evaluable patients to have 80%  
224 power to detect this difference in the primary efficacy variable, and 156 patients assuming  
225 10–20% dropout. Efficacy analyses were performed on intention-to-treat (ITT)  
226 populations, with missing data imputed using post-baseline last observation carried  
227 forward (LOCF). Also conducted were observed-case and sensitivity analyses (missing

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

230

231 For binary secondary and exploratory efficacy endpoints, two-sided significance was set at  
232 0.05. Change in BCDVA score from baseline to week 8 was analyzed by an analysis of  
233 covariance (ANCOVA) model using treatment group and baseline BCDVA score. Mixed  
234 effects repeated measures models using treatment, visit, and baseline measurements were  
235 used to assess changes in lesion size (maximum dimension) and reflex tearing (Schirmer  
236 test wetting distance) from baseline to week 4 and week 8. The time to onset of healing  
237 (>20% reduction in maximum diameter of the corneal lesion from baseline) and corneal  
238 healing (<0.5 mm maximum diameter of fluorescein staining) were analyzed using Kaplan-  
239 Meier methods and the log-rank test (for the controlled treatment period) and descriptive  
240 statistics (for the uncontrolled treatment period). Data collected during follow-up were  
241 also analyzed using descriptive statistics.

242

### 243 **Study Oversight**

244

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

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

253

254 **RESULTS**

255

256 **Patients and Treatment**

257

258 REPARO investigators (Appendix 1) represented 39 sites in 9 European countries  
259 (Belgium, France, Germany, Hungary, Italy, Poland, Portugal, Spain, and the United  
260 Kingdom); 32 sites in 6 countries enrolled  $\geq 1$  patient. Figure 1 provides an overview of  
261 patient disposition (including reasons for withdrawal). Of 186 patients screened January  
262 2013–May 2015, 174 were enrolled—18 in phase I,<sup>17</sup> and 156 in phase II. Patient  
263 demographics and baseline characteristics were well balanced in the REPARO phase II  
264 study, with no clinically notable differences between treatment groups (Table 1).  
265 Consistent with published literature,<sup>5, 6, 13, 19</sup> common underlying etiologies included  
266 herpetic eye disease (44 patients) and ocular or neurological surgery (21 patients each).  
267 Prior treatments for NK (most commonly artificial tears/gels/ointments and topical  
268 antibiotics) are shown in Appendix 3 (available at [www.aaojournal.org](http://www.aaojournal.org)).

269

270 **Efficacy Outcomes**

271

272 Table 2 summarizes efficacy analyses at weeks 4 and 8. Corneal healing ( $< 0.5$  mm lesion  
273 staining) was achieved at week 4 (primary endpoint) in 19.6% of vehicle-treated patients  
274 vs. 54.9% receiving rhNGF 10  $\mu\text{g}/\text{ml}$  (+35.3%; 97.06% CI 15.88–54.71;  $P < 0.001$ ) and  
275 58.0% receiving rhNGF 20  $\mu\text{g}/\text{ml}$  (+38.4%; 97.06% CI 18.96–57.83;  $P < 0.001$ ). Corneal  
276 healing at week 8 (key secondary endpoint) was achieved in 43.1% of vehicle-treated

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

286

287 Figure 2A shows representative images of corneal fluorescein staining at baseline through  
288 week 8. Lesion size changes from baseline (determined by the reading center) were  
289 analyzed post hoc for clinically significant differences between treatments (Figure 2B). At  
290 week 4, least squares mean (LSmean) lesion size change from baseline was -49.8% with  
291 rhNGF 20 µg/ml, -39.5% with rhNGF 10 µg/ml, and -8.9% with vehicle. At week 8, lesion  
292 size change was -76.0% with rhNGF 20 µg/ml, -58.4% with rhNGF 10 µg/ml, and -26.2%  
293 with vehicle. Overall, rhNGF-treated patients exhibited greater (but statistically  
294 nonsignificant) lesion size reductions from baseline vs. vehicle-treated patients, trending  
295 towards significance in rhNGF 20 µg/ml vs. vehicle at week 8 (p=0.102, 95% CI -109.61–  
296 9.98).

297

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

300 letters) from baseline to week 8 was significantly different in patients receiving rhNGF 10  
301  $\mu\text{g}/\text{ml}$  ( $p=0.022$ ) but not those receiving rhNGF 20  $\mu\text{g}/\text{ml}$  ( $p=0.213$ ). However, the  
302 difference between rhNGF doses was not significant ( $p=0.305$ ). BCDVA assessed as gain of  
303 15 ETDRS letters (yes/no) from baseline to week 8 produced similar results (Table 4).  
304 Compared to vehicle, 15-letter gains were achieved by more patients receiving rhNGF 10  
305  $\mu\text{g}/\text{ml}$  (+27.5%; 95% CI: 8.33–46.67;  $p=0.008$ ) and rhNGF 20  $\mu\text{g}/\text{ml}$  (+19%; 95% CI: 0.91–  
306 38.83;  $p=0.068$ ), with no statistically significant difference between rhNGF doses  
307 ( $p=0.421$ ).

308  
309 Corneal sensitivity during the controlled treatment period was measured directly in the  
310 corneal lesion and outside quadrants using the CBA as secondary efficacy variable, and  
311 indirectly by Schirmer testing of reflex tearing as an exploratory variable. Compared to  
312 vehicle, more patients receiving rhNGF 10 or 20  $\mu\text{g}/\text{ml}$  exhibited improvement in corneal  
313 sensitivity (cm) from baseline to weeks 4 and 8, but the differences between treatment  
314 groups were not significant (Table 5, available online at [www.aaojournal.org](http://www.aaojournal.org)). Figure 4  
315 (available at [www.aaojournal.org](http://www.aaojournal.org)) shows results of Schirmer tests of reflex tearing. LSmean  
316 change from baseline was greater in the rhNGF-treated groups compared to those receiving  
317 vehicle, with differences reaching statistical significance between rhNGF 10  $\mu\text{g}/\text{ml}$  and  
318 vehicle groups at week 4 ( $p=0.047$ ) and week 8 ( $p=0.010$ ). Comparisons between patients  
319 receiving rhNGF 20  $\mu\text{g}/\text{ml}$  and vehicle were not significant at week 4 ( $p=0.234$ ) or week 8  
320 ( $p=0.201$ ). However, comparisons between rhNGF doses were also not significant at either  
321 week 4 ( $p=0.442$ ) or week 8 ( $p=0.191$ ).

322

323 Figure 5 illustrates exploratory Kaplan-Meier analyses of time-to-event variables for the  
324 controlled treatment period. The median time to onset of healing (20% reduction in  
325 maximum lesion diameter from baseline), which was 14 days in patients receiving vehicle  
326 (95% CI 14–28), compared to 8 days in patients receiving rhNGF 10 µg/ml (95% CI 7–14;  
327 p=0.002) and 14 days in patients receiving rhNGF 20 µg/ml (95% CI 7–14; p=0.015). For  
328 time to corneal healing (<0.5 mm lesion staining), median time was 56 days (95% CI 42–  
329 not estimable) in patients receiving vehicle, compared to 29 days in patients receiving  
330 rhNGF 10 µg/ml (95% CI 20–55; p=0.002) and 28 days in patients receiving rhNGF 20  
331 µg/ml (95% CI 19–55; p=0.002).

332

333 Follow-up data (not powered for efficacy analyses) are presented using descriptive  
334 statistics. Of patients receiving vehicle during 8-week controlled treatment, 23 experienced  
335 treatment failure (failure to achieve corneal healing, recurrence of NK after healing, or  
336 deterioration) and entered the 56-week follow-up period, which included 8 weeks of  
337 uncontrolled rhNGF treatment (see Figure 1). Per a secondary baseline randomization  
338 scheme, 10 patients received 10 µg/ml rhNGF, and 13 received 20 µg/ml rhNGF. At the end  
339 of uncontrolled treatment, corneal healing (<0.5 mm lesion staining, assessed by the  
340 investigator) was achieved in 3/10 (30%) patients receiving 10 µg/ml rhNGF, and 8/13  
341 (61.5%) patients receiving 20 µg/ml rhNGF. Figure 6 (available at [www.aaojournal.org](http://www.aaojournal.org))  
342 shows Kaplan-Meier plots of time-to-event variables for the 8-week uncontrolled  
343 treatment portion of the 56-week follow-up period. Onset of healing was assessed as 20%  
344 reduction in maximum lesion diameter from the last measurement of the controlled  
345 treatment period. Median time to onset of healing was 14.5 days (range, 7–55) in the 10

346  $\mu\text{g/ml}$  rhNGF group, and 7 days (range, 7–42) in the 20  $\mu\text{g/ml}$  rhNGF group. Median time to  
347 corneal healing ( $<0.5$  mm lesion staining) in the 10  $\mu\text{g/ml}$  rhNGF group was 15 days (range,  
348 14–27) and 21 days (range, 7–42) in the 20  $\mu\text{g/ml}$  rhNGF group.

349

350 Of patients who achieved corneal healing ( $<0.5$  mm lesion staining) and completed follow-  
351 up, very few experienced recurrence of the PED or corneal ulcer. Of those who healed after  
352 controlled treatment and completed 48-week follow-up, recurrence was experienced by  
353 1/20 patients in the vehicle group (4.8%), 1/27 patients in the rhNGF 10  $\mu\text{g/ml}$  group  
354 (3.6%), and 1/28 patients in the rhNGF 20  $\mu\text{g/ml}$  group (3.4%). Of patients healed after  
355 uncontrolled treatment and completed 56-week follow-up, recurrence was experienced by  
356 0/4 patients in the rhNGF 10  $\mu\text{g/ml}$  group, and 2/6 (33%) patients in the rhNGF 20  $\mu\text{g/ml}$   
357 group.

358

### 359 **Safety Outcomes**

360

361 Table 6 summarizes TAEs during controlled treatment, which occurred in 25 patients: 6  
362 (11.5%) receiving rhNGF 10  $\mu\text{g/ml}$ , 9 (17.3%) receiving rhNGF 20  $\mu\text{g/ml}$ , and 10 (19.2%)  
363 receiving vehicle. Two patients receiving rhNGF 10  $\mu\text{g/ml}$ , 9 receiving rhNGF 20  $\mu\text{g/ml}$ , and  
364 4 receiving vehicle experienced AEs leading to discontinuation of study treatment.

365 Additional phase II safety results (TAEs during uncontrolled treatment and follow-up  
366 periods) are presented in Appendix 4.

367

368 Overall, 17 patients (10.9%) experienced serious AEs (SAEs) during controlled treatment:  
369 3 receiving rhNGF 10 µg/ml, 9 receiving rhNGF 20 µg/ml, and 5 receiving vehicle. No SAEs  
370 were considered related to study treatment.

371  
372 Changes from baseline VAS scores were analyzed by repeated measures ANCOVA  
373 (controlled treatment period) or descriptive statistics (follow-up period). Decreases in VAS  
374 scores were observed in all groups, indicating improvement in ocular tolerability, but  
375 differences between groups were not statistically significant for the controlled treatment  
376 period or otherwise noteworthy during follow-up.

377  
378 Patients whose NK worsened during the study were discontinued (and respective  
379 treatments unmasked) per protocol. Of vehicle-treated patients, 12 experienced  
380 deterioration (2 at week 4, 4 at week 6, 6 at week 8), vs. 4 receiving rhNGF 10 µg/ml (1 at  
381 week 4, 1 at week 6, 2 at week 8) and 4 receiving rhNGF 20 µg/ml (1 at week 4, none at  
382 week 6, 3 at week 8).

383  
384 Eight deaths were reported during the study: 2 during controlled treatment (1 receiving  
385 rhNGF 10 µg/ml, 1 receiving rhNGF 20 µg/ml) and 6 during follow-up (4 patients in the  
386 rhNGF 10 µg/ml group, and 1 each in the 20 µg/ml and vehicle groups). All events leading  
387 to death (detailed in Appendix 4) were considered unrelated to study treatment.

388

389



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

426  
427 Clinical efficacy of topical rhNGF for treating NK was also supported by improvement on  
428 other clinically relevant endpoints, including corneal lesion size, time to corneal healing (or  
429 onset of healing) , BCDVA, corneal sensitivity measured by CBA, and reflex tearing (which  
430 may also reflect corneal sensitivity not detectable by CBA). Although we did not observe  
431 statistically significant differences between both rhNGF doses and vehicle in these  
432 variables at every time point, the sample size was based on the dichotomous (yes/no)  
433 primary endpoint and not powered to detect small but clinically significant differences in  
434 secondary, exploratory, or post hoc variables. To this point, the rhNGF 10 µg/ml group (but  
435 not the rhNGF 20 µg/ml group) exhibited statistically significant differences compared to

436 the vehicle group in some secondary endpoints (such as visual acuity and reflex tearing);  
437 however, in the same endpoints, differences between rhNGF doses did not reach statistical  
438 significance. Thus, it is difficult to draw conclusions on dose responsiveness. Nonetheless,  
439 patients receiving rhNGF generally had better trends of improvement for most efficacy  
440 endpoints vs. patients receiving vehicle.

441

442 Of note, visual acuity was assessed as secondary efficacy endpoint, even though it does not  
443 necessarily reflect NK severity or healing status. For example, in stage 2 NK, absence of the  
444 epithelium may have little or no impact on vision, while re-epithelialization in the  
445 central/paracentral cornea can cause optical aberrations (and hence reduced vision).

446 Figure 2a illustrates this latter point; it would not be surprising that this patient still had  
447 reduced vision after 8 weeks of controlled rhNGF 20 µg/ml treatment, despite achieving  
448 corneal healing with 0 mm lesion staining and no other persistent staining.

449

450 No safety concerns arose; most AEs were ocular, mild, transient, and did not require  
451 discontinuing or corrective treatments. The predominant TAE was eye pain; others  
452 included abnormal sensation in the eye, excess lacrimation, photophobia, eyelid pain and  
453 eye/eyelid irritation, which may reflect therapeutic actions of rhNGF and normal healing.  
454 Indeed, restoring corneal innervation and sensitivity (which, in turn, will promote corneal  
455 healing) can be associated with increased ocular surface symptomatology. No  
456 immunogenicity to NGF was detected in this study; furthermore, consistent with phase I  
457 results,<sup>16,17</sup> most patients had undetectable serum NGF and/or no systemic AEs. Taken

458 together, these PK and immunogenicity results suggest unlikely systemic absorption or  
459 accumulation of topical ophthalmic rhNGF.

460

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

468

#### 469 **ACKNOWLEDGMENTS**

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

472

473 **Figure Legends**

474

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

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

484

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

486 A) Representative images showing the progression of a typical oval, paracentral  
487 neurotrophic corneal lesion from baseline through week 8 in a patient treated with rhNGF  
488 20µg/ml. Top row: pictures of the cornea illuminated with diffuse white light. Bottom row:  
489 corneal lesion healed at week 8 as assessed by the central reading center on fluorescein  
490 staining (green) pictures taken under cobalt-blue light illumination. B) Post hoc analysis of  
491 least squares mean (LSMean) percentage change from baseline in maximum dimension of  
492 persistent epithelial defect (PED) or corneal ulcer after the 8-week controlled treatment  
493 period. Error bars represent standard error (SE). Magnitude change in lesion size was  
494 greater in patients in the rhNGF treatment groups compared to the vehicle group (not

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

497

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

499 Least squares mean (LSmean) change from baseline in best corrected distance visual acuity  
500 (BCDVA) measured in Early Treatment Diabetic Retinopathy Study (ETDRS) letters was  
501 analyzed using an analysis of covariance (ANCOVA) model (treatment + baseline score).  
502 Compared to vehicle-treated patients, LSmean change from baseline to week 8 was greater  
503 in the rhNGF-treated groups, with the difference reaching statistical significance in  
504 between patients receiving vehicle and rhNGF 10 µg/ml (p=0.022) but not rhNGF 20 µg/ml  
505 (p=0.213). However, the comparison between rhNGF doses was also not significant  
506 (0.305).

507

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

510 Least squares mean (LSmean) change from baseline in Schirmer wetting distance (cm) at 5  
511 minutes was analyzed using a mixed effects repeated measures model (treatment + visit +  
512 treatment x visit interaction + baseline measurement). Compared to vehicle-treated  
513 patients, LSmean change from baseline was greater in the rhNGF-treated groups, with  
514 differences reaching statistical significance between rhNGF 10 µg/ml and vehicle groups at  
515 week 4 (p=0.047) and week 8 (p=0.010). Comparisons between patients receiving rhNGF  
516 20 µg/ml and vehicle were not significant at week 4 (p=0.234) or week 8 (p=0.201).

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

519

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

524

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

527 Of patients receiving vehicle during the controlled treatment period, 23 experienced  
528 treatment failure and received 8 weeks of uncontrolled treatment with rhNGF 10  $\mu\text{g}/\text{ml}$  or  
529 20  $\mu\text{g}/\text{ml}$  (see text for details). Top panel: time to onset of healing (>20% reduction in  
530 maximum diameter of the corneal lesion from last measurement of the controlled  
531 treatment period). All patients showed signs of healing (i.e., none were censored). Lower  
532 panel: time to corneal healing (<0.5 mm lesion staining).

533

534 **Figure 7 (online). REPARO Phase II Pharmacokinetics.**

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

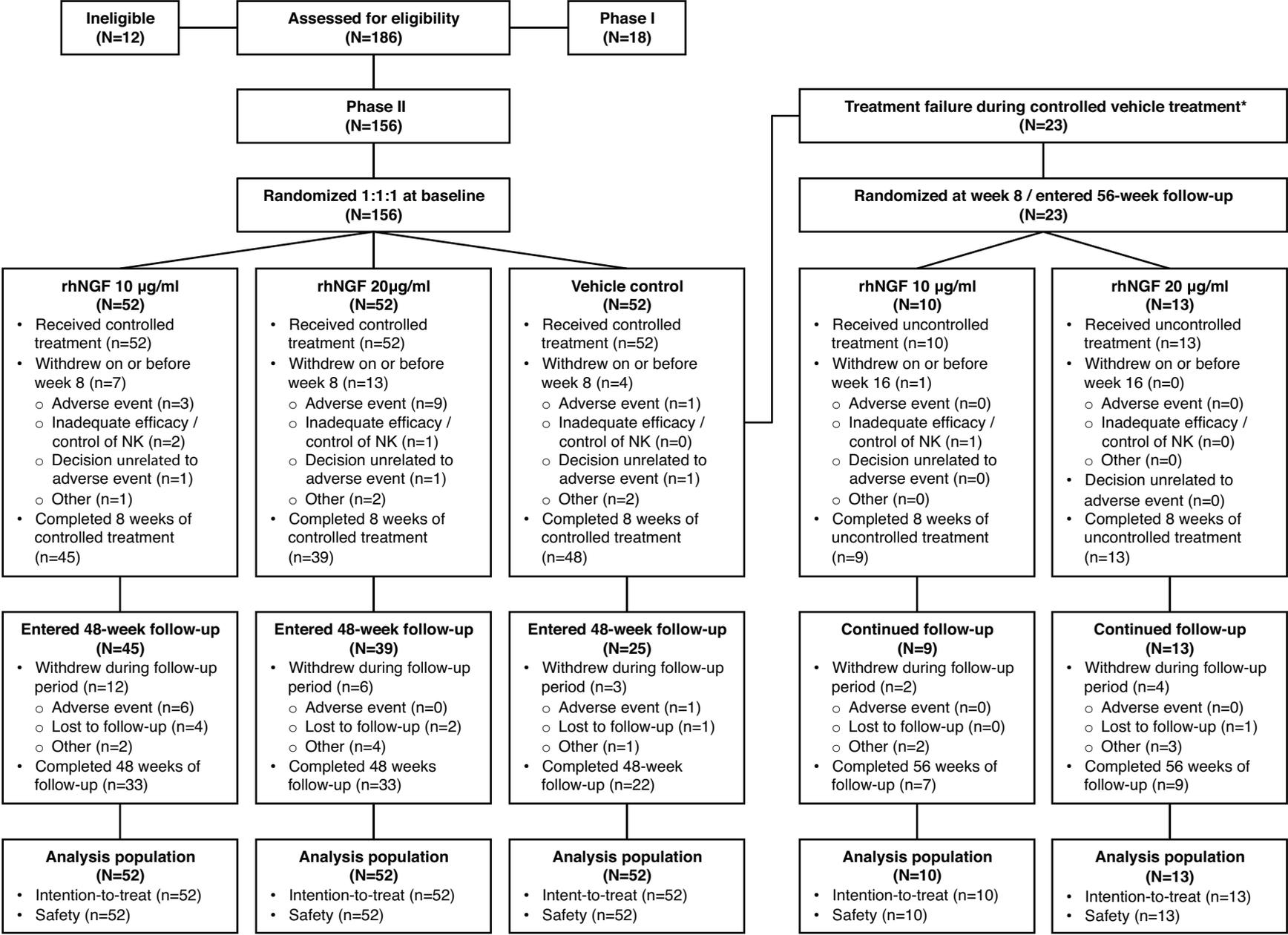
540 32.000 pg/mL; concentrations <LLQ are considered as 0 pg/mL for the purpose of these  
541 plots. A total of 5 patients had NGF concentrations >LLQ and are depicted in this figure. In  
542 the rhNGF 10 µg/ml group (●), 3 patients had measurable serum NGF concentrations: 1  
543 patient at day 0 (6750.7 pg/ml) and at week 8 (728.6 pg/ml), and 2 patients had  
544 concentrations >LLQ at all time points. In the rhNGF 20 µg/ml group (◇), 1 had NGF  
545 concentrations >LLQ at day 0, week 1, and week 3; 1 patient had NGF concentrations >LLQ  
546 at weeks 1 and 3. In the vehicle group (not shown), no patients had detectable serum NGF  
547 at any time point tested.

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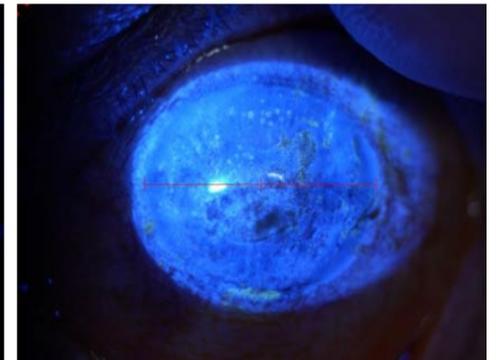
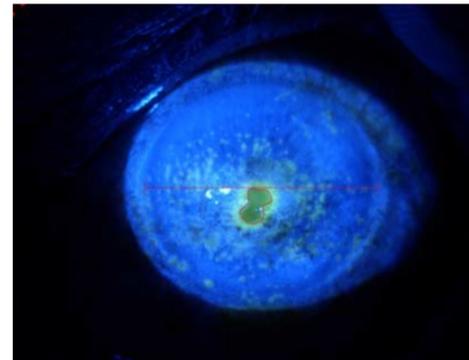
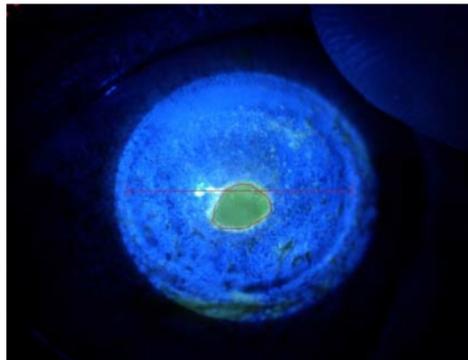
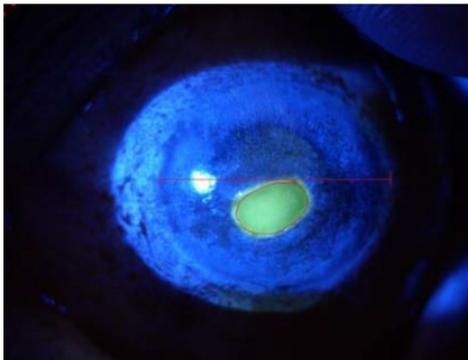


Baseline

Week 4

Week 6

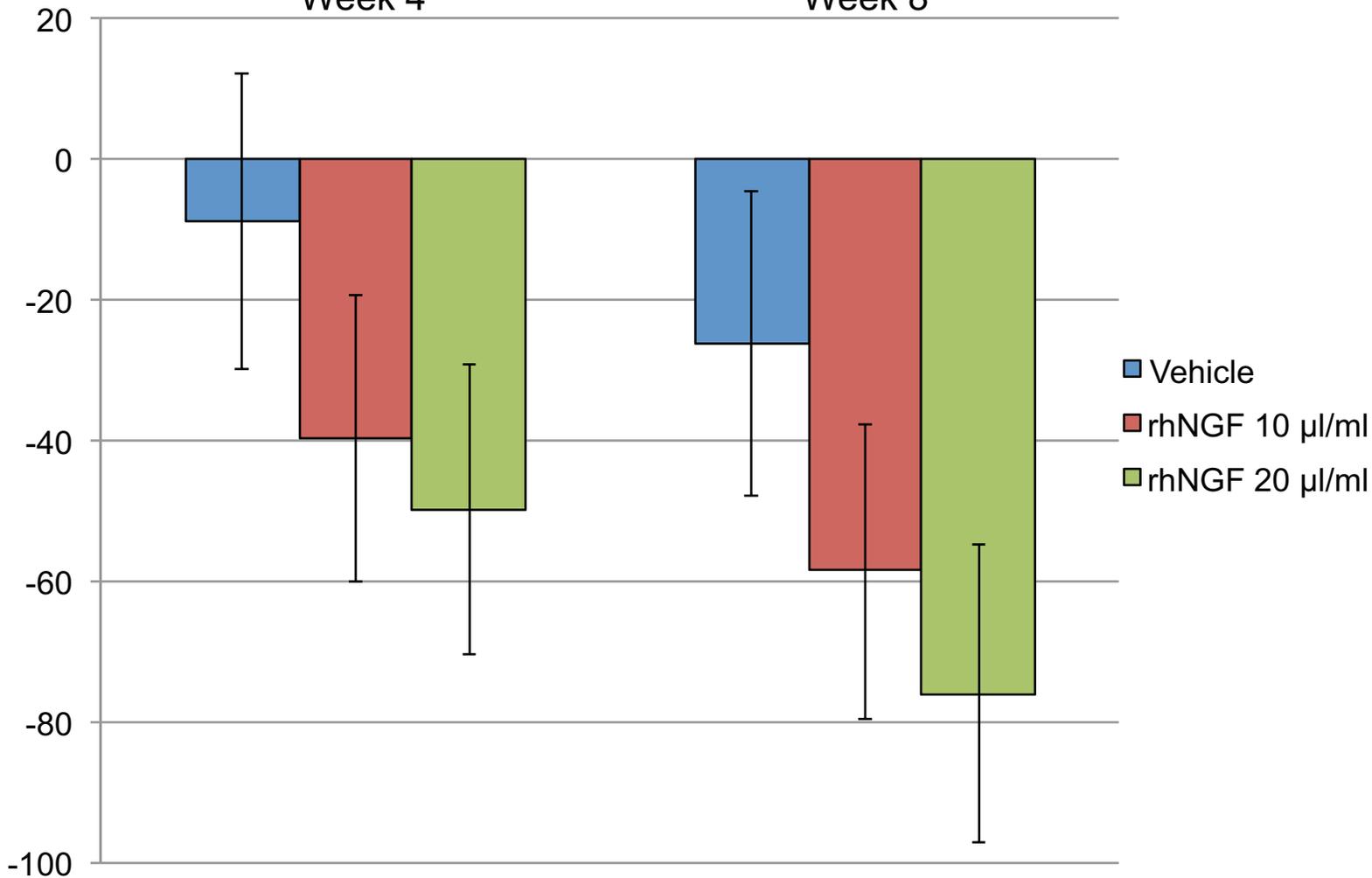
Week 8



Week 4

Week 8

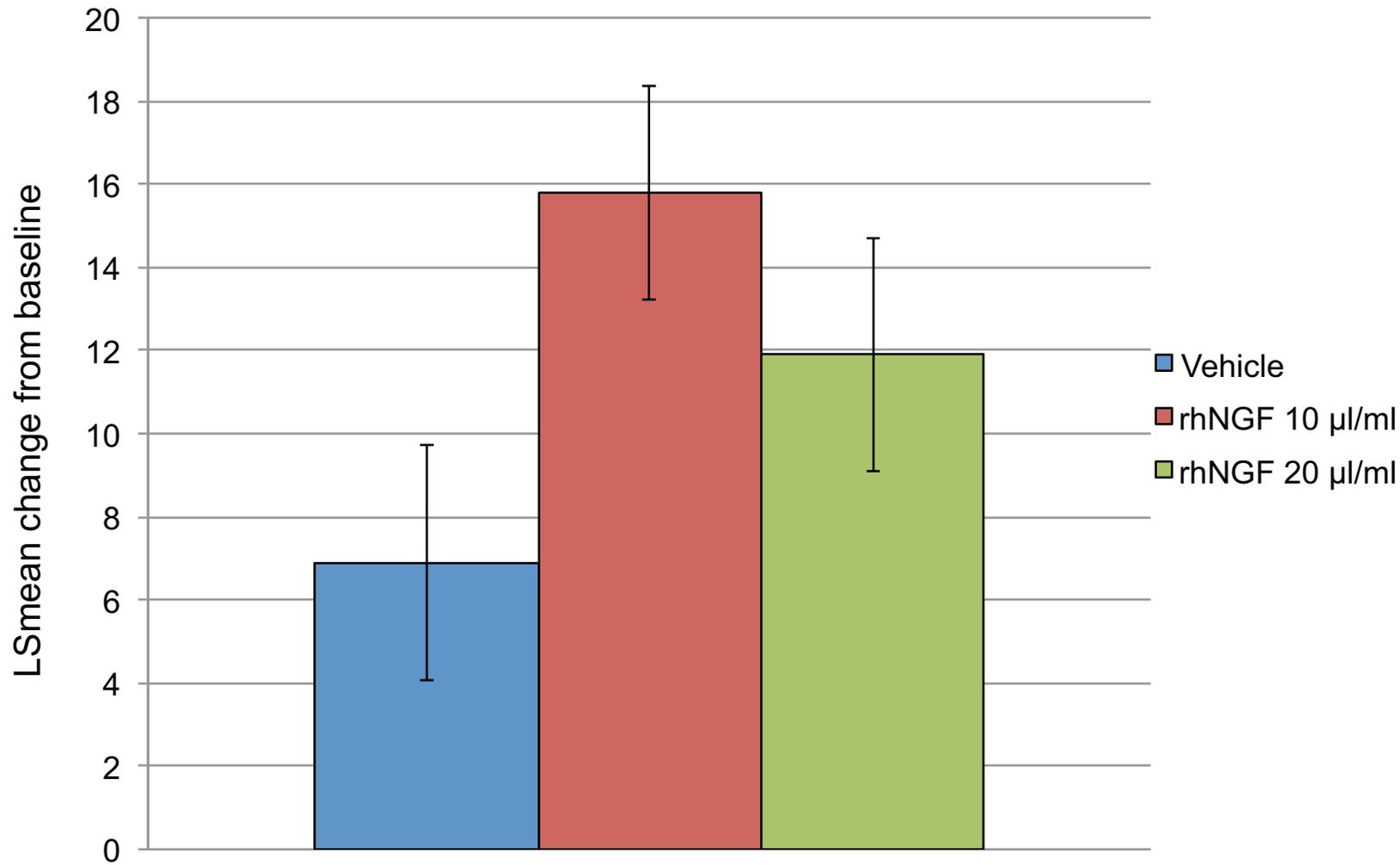
Maximum dimension % change from baseline



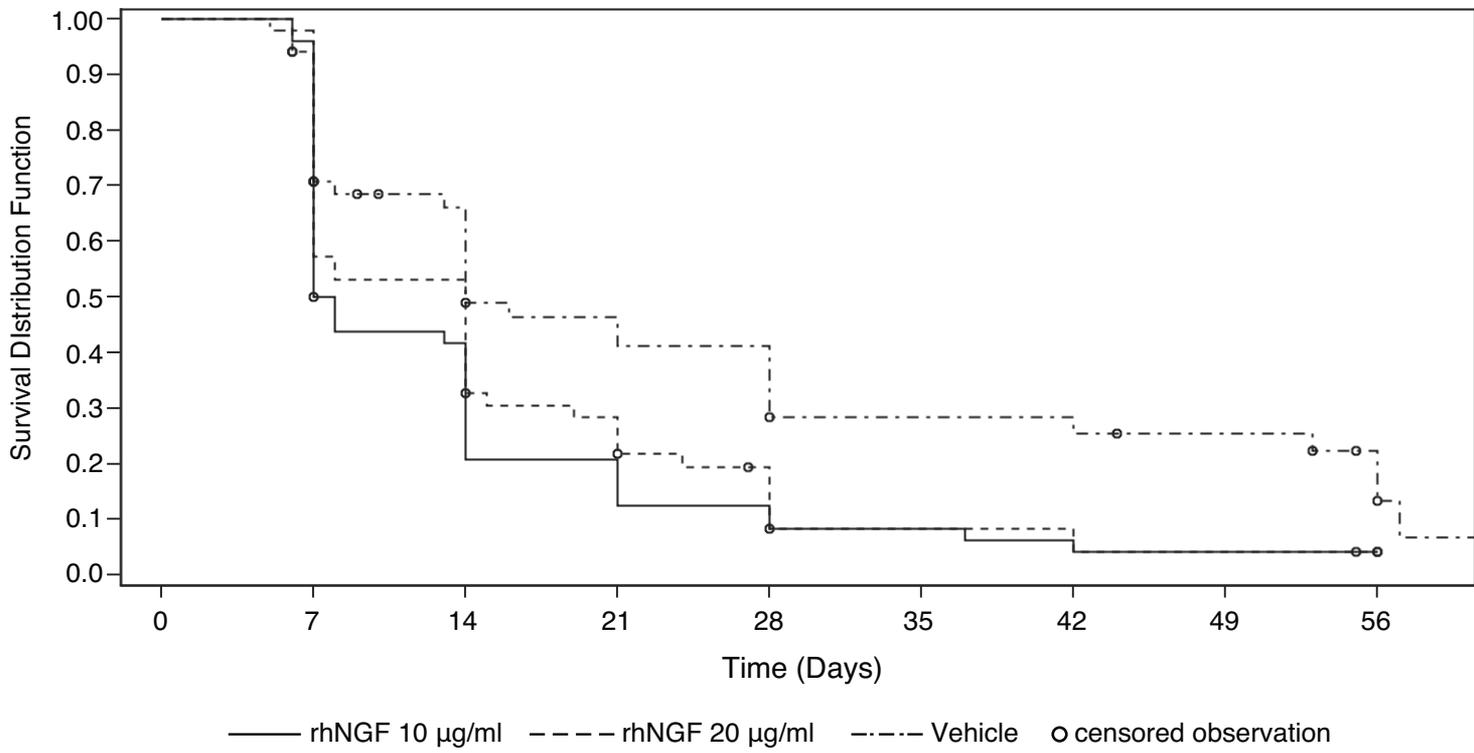
Vehicle

rhNGF 10 µl/ml

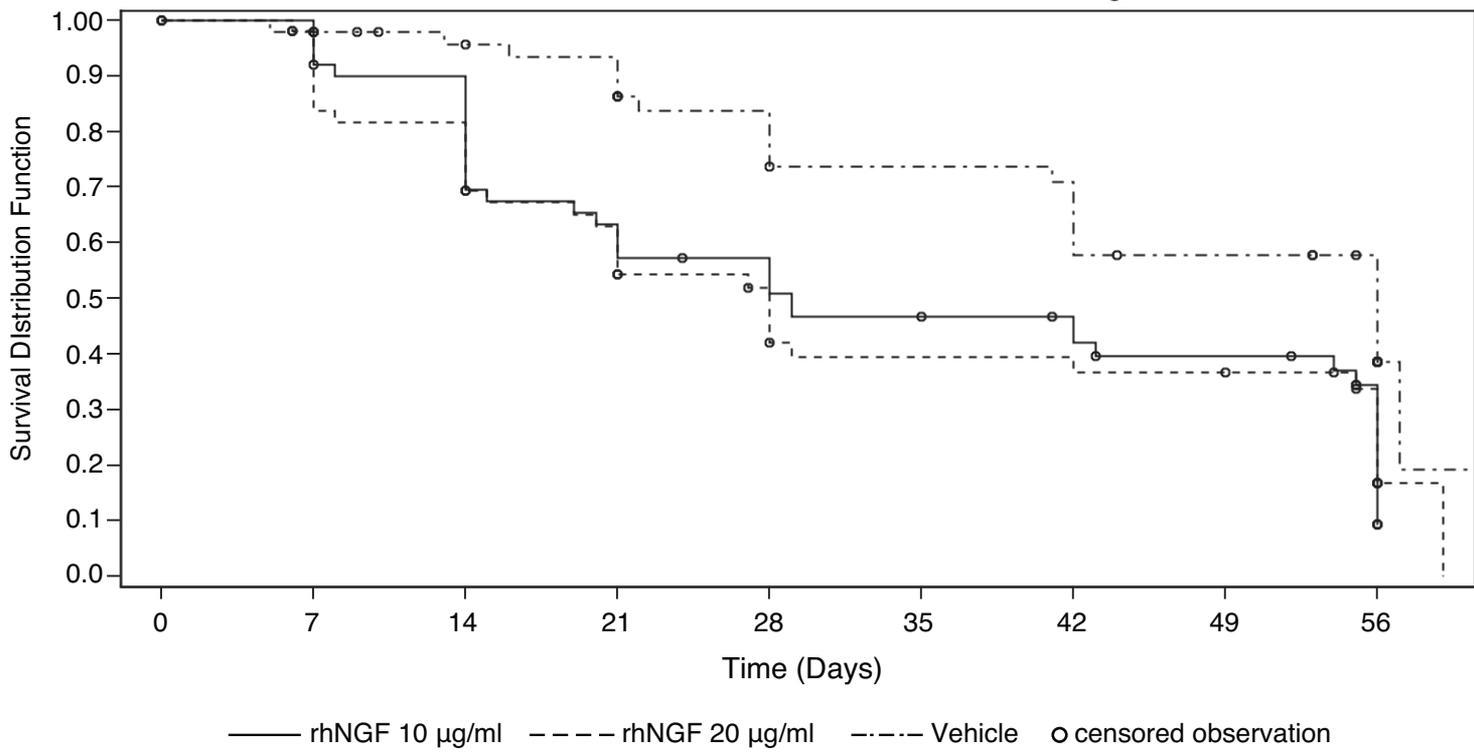
rhNGF 20 µl/ml



Controlled Treatment Period: Time to Onset of Corneal Healing



Controlled Treatment Period: Time to Corneal Healing



**Table 1. Patient demographics and baseline characteristics.**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; N = number of patients who received each treatment in the specified study period; N' = number of events reported, n = number of patients; rhNGF = recombinant human nerve growth factor.

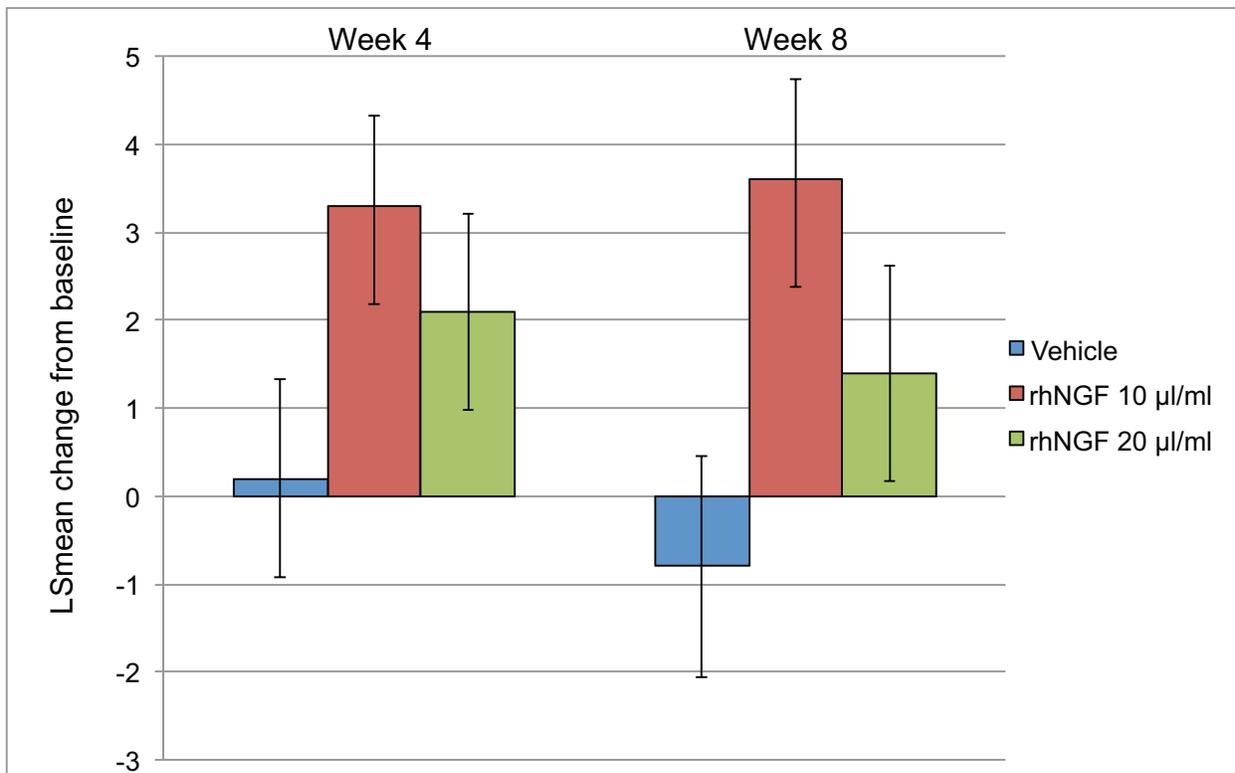
Percentages (%) are calculated using the population number in each treatment group (N) as the denominator.

\*Treatment-related AEs are those events recorded by the investigator as having a possible, probable, or highly probable relationship to study treatment.

† Disease progression was defined as increase in lesion size  $\geq$  1mm; decrease in best corrected distance visual acuity (BCDVA) by  $>5$  Early Treatment Diabetic Retinopathy Study (ETDRS) letters; progression in lesion depth to corneal melting or perforation; or onset of infection.

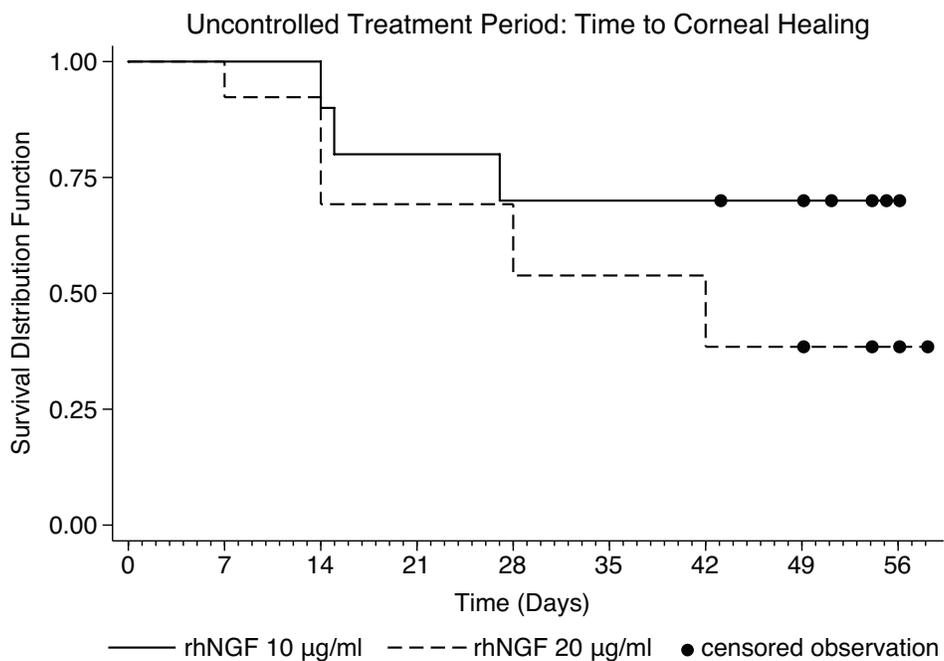
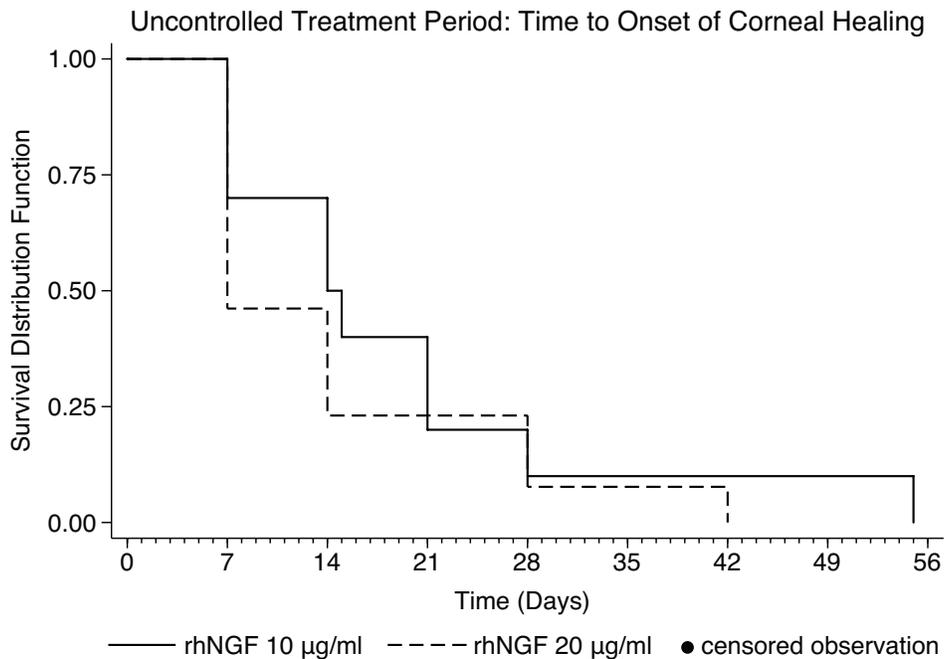
**Figure 4. Exploratory efficacy analysis of change in reflex tearing during the controlled treatment period.**

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



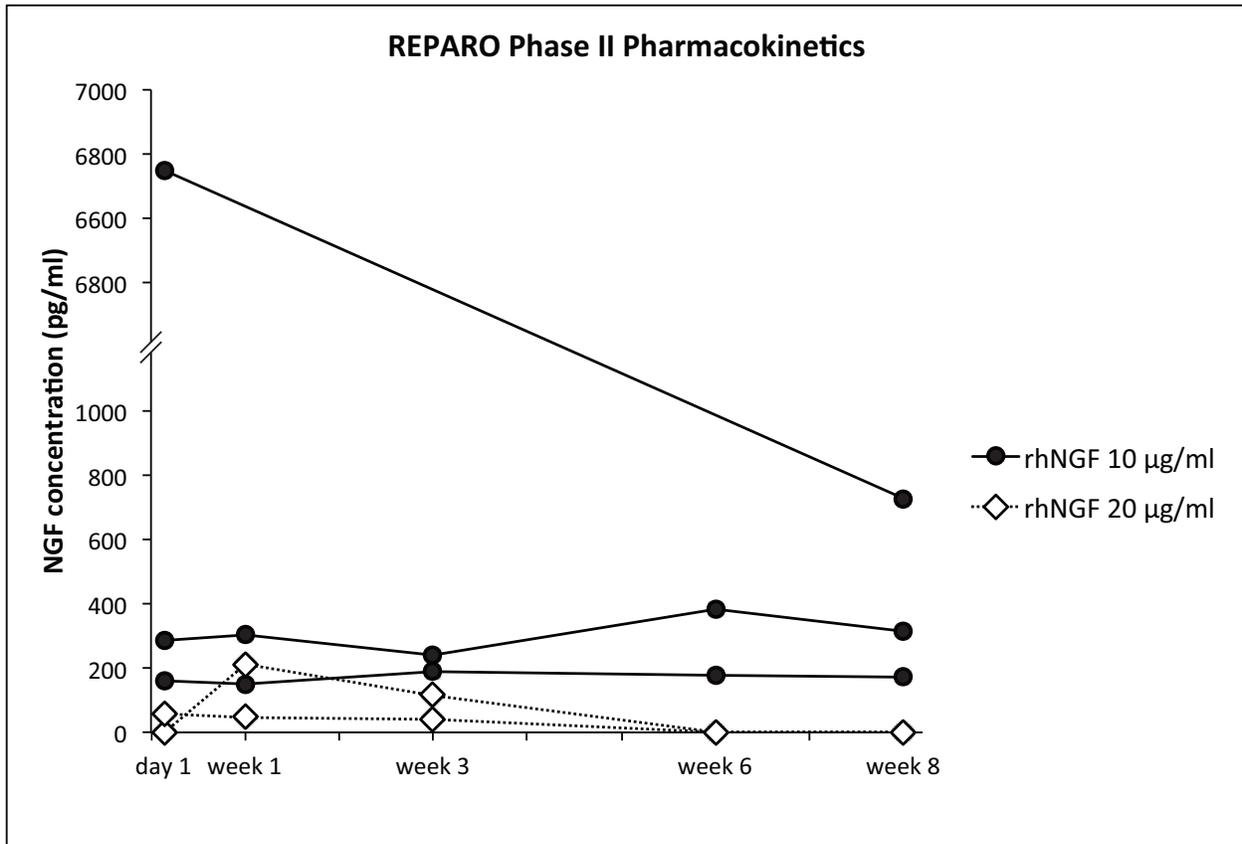
**Figure 6. Exploratory analyses of Kaplan-Meier time-to-event variables during uncontrolled treatment.**

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



**Figure 7. REPARO Phase II Pharmacokinetics.**

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



## **Appendix 1. REPARO Study Group and Administration**

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## **Appendix 2. REPARO Phase II Study: Complete Eligibility Criteria**

### **Inclusion Criteria**

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

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

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

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

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

### **Exclusion Criteria**

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

1. Patients with Stage 2 or 3 NK affecting both eyes.
2. Any active ocular infection (bacterial, viral, fungal or protozoal) or active ocular inflammation not related to NK in the affected eye.
3. Any other ocular disease requiring topical ocular treatment in the affected eye during the course of the study treatment period. No topical treatments other than the study medications provided by the study sponsor or allowed by the study protocol could be administered in the affected eye during the course of the study treatment periods.
4. Patients with severe vision loss in the affected eye with no potential for visual improvement in the opinion of the Investigator as a result of the study treatment.
5. Schirmer test without anesthesia  $\leq 3$  mm/5 minutes in the affected eye.
6. Patients with severe blepharitis and/or severe meibomian gland disease in the affected eye.
7. History of any ocular surgery (including laser or refractive surgical procedures) in the affected eye within the three months before study enrolment. (An exception to the preceding statement was allowed if the ocular surgery was considered to be the cause of the Stage 2 or 3 NK). Ocular surgery in the affected eye was not allowed during the study

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

8. Prior surgical procedure(s) for the treatment of NK (eg, complete tarsorrhaphy, conjunctival flap, etc) in the affected eye with the exception of amniotic membrane transplantation. Patients previously treated with amniotic membrane transplantation could only be enrolled 2 weeks after the membrane had disappeared within the area of the PED or corneal ulcer or at least 6 weeks after the date of the amniotic membrane transplantation procedure. Patients previously treated with Botox (botulinum toxin) injections used to induce pharmacologic blepharoptosis were eligible for enrolment only if the last injection was given at least 90 days prior to enrolment in the study.
9. Use of therapeutic contact lenses or contact lens wear for refractive correction during the study treatment periods in the eye with NK.
10. Anticipated need for punctal occlusion during the study treatment period. Patients with punctal occlusion or punctal plugs inserted prior to the study were eligible for enrolment provided that the punctal occlusion was maintained during the study.
11. Evidence of corneal ulceration involving the posterior third of the corneal stroma, corneal melting or perforation in the affected eye.
12. Presence or history of any ocular or systemic disorder or condition that might have hindered the efficacy of the study treatment or its evaluation, could possibly have interfered with the interpretation of study results, or could have been judged by the Investigator to be incompatible with the study visit schedule or conduct (eg, progressive or degenerative corneal or retinal conditions, uveitis, optic neuritis, poorly controlled diabetes, autoimmune disease, systemic infection, neoplastic diseases).

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

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

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

Abbreviations: rhNGF = recombinant human nerve growth factor

Percentages are calculated using the population number in each treatment group as the denominator.

Patients may be counted under more than one category.

## Appendix 4. REPARO Phase II Study: Additional Safety Results

### Treatment-related adverse events (TAEs)

#### *Controlled treatment period*

See text.

#### *Uncontrolled treatment period*

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

#### *Follow-up period*

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

### Serious adverse events (SAEs) leading to death

#### *Controlled treatment period*

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

#### *Uncontrolled treatment period*

No deaths occurred during the uncontrolled treatment period.

#### *Follow-up period*

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