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A Phase 2 Trial to Test Safety and Efficacy of ST-100, a Unique Collagen Mimetic Peptide Ophthalmic Solution for Dry Eye Disease

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Objective: Dry eye disease (DED) is a worldwide source of ocular discomfort. This first-in-human phase 2 clinical study determined the efficacy of treating signs and symptoms of DED using an ophthalmic solution of synthesized mimetic of human collagen (ST-100).

Design: This double-masked, randomized, study compared high (60 μg/mL) and low (22 μg/mL) dose ST-100 to vehicle utilizing the Ora, Inc. Controlled Adverse Environment (CAE) during a 28-day period.

Participants: Participants included males and females ≥ 18 years of age with signs and symptoms of DED for ≥ 6 months that worsened during CAE exposure who were not taking any topical prescription therapeutic.

Intervention: Participants applied ST-100 or vehicle placebo topically to both corneas (1 drop) twice daily via a blow-fill-sealed preservative-free container.

Main Outcome Measures: The prespecified primary efficacy sign end point was mean change from baseline (CFB) in total corneal fluorescein staining, and the primary symptom end point was mean CFB in ocular discomfort. A secondary prespecified efficacy end point was CFB in unanesthetized Schirmer's test for tear film production.

Results: Of 160 subjects in the intent-to-treat population (112 female, 48 male, median age 64), 146 completed the study. Total corneal fluorescein staining CFB improved for high-dose ST-100, with superiority over vehicle when both eyes were considered together (2-sample *t* test: $P = 0.0394$). High-dose ST-100 was superior to vehicle in Schirmer's CFB for the study eye (least squares mean difference [confidence interval] = 2.3 [0.6, 4.0], $P = 0.0094$). For study eyes, the proportion of Schirmer's test responders (CFB ≥ 10 mm, Schirmer's responder rate) was 12.2% for high-dose ST-100 versus 0.0% for vehicle ($P = 0.0266$). The CFB for ocular discomfort score improved in study eyes for high- and low-dose ST-100 (paired *t* test, $P = 0.0133$, $P = 0.0151$, respectively) but without superiority over vehicle (ANCOVA: $P = 0.5696$, $P = 0.8968$, respectively). ST-100 Schirmer's responders also demonstrated total elimination of worsening of corneal fluorescein stain during the stress of CAE sessions.

Conclusions: ST-100 significantly improved tear production and related outcomes in DED and was well-tolerated in reducing symptoms.

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Supplemental material available at www.ophthalmologyscience.org.

Dry eye disease (DED) is a multifactorial, progressive disorder involving insufficient tear coverage of the ocular surface that results in inflammation and damage to the corneal epithelial, basement membrane, and stromal layers.[1] The disease is characterized by complex and highly variable etiologies, clinical manifestations, severity, and clinical course, creating a challenge for therapeutic treatment.[2] Even so, commonality in signs and symptoms is indicative of a shared underlying pathophysiology. Damage to the ocular surface and the corneal nerves subserving its function, both superficial and deep, directly reflects clinical signs and symptoms.[3] Even in the early stages of DED, damage penetrates well beyond the surface epithelial layer, involving disruptions to the collagen found in Bowman's layer and the underlying corneal stroma and in the epithelial basement membrane.[4–6] Similarly, disruptions in extracellular matrix (ECM) lead to degradation of the integrity of corneal layers, structures, and membranes (e.g., Bowman's) and allow inflammatory cell infiltration.[4] Furthermore, structural compromise of collagen throughout corneal layers could allow exposure of inflammatory ligand binding sites, further exacerbating progression.[7]

Despite the link between corneal nerve damage in DED and ensuing inflammation,[8] therapeutics thus far have targeted other pathways. These include glandular dysfunction (both meibomian and lacrimal), inadequate lid

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closure, eyelid margin disease, exposure to topical irritants, and fundamental changes in tear film composition and production, including hyperosmolarity with secondary epithelial cell apoptosis.[9,10] These approaches, although expedient, ignore disruptions to corneal collagen and nerve integrity as potential common pathologies.[7] Available therapeutic options that stimulate tear production or reduce inflammation do not directly address underlying tissue or nerve damage.[10] Thus, effective management of DED also requires recognition of the importance of corneal nerve recovery in accelerating healing[11] and in the protection of the ECM from environmental stressors that compromise nerve bed homeostasis.[12] Our work suggests that ST-100, a collagen mimetic peptide, could fill this void as it rapidly repairs damaged collagen triple helices, thus quickly reversing damage to the ocular surface and ECM itself and repairing corneal nerve function as it heals the epithelium.[4,13] By healing the underlying collagen matrix that envelopes corneal innervation, ST-100 could also reduce the necessity for long-term use of traditional anti-inflammatory regimens. In support of this hypothesis, proof-of-concept studies in mice demonstrated that ST-100 induced significant recovery of the corneal epithelium, the subbasal and epithelial corneal nerve plexus, the stromal matrix, and the Bowman's layer after acute wounds and desiccation-induced damage.[6,13] This phase 2 trial assessed the efficacy of topical delivery of ST-100 in human DED.

Methods

Informed Consent and Ethics Review

Informed consent was obtained before any study-related procedures (visit 1). The protocol, informed consent form, Health Insurance Portability and Accountability Act form, and other forms as well as subject diary instructions were reviewed by the institutional review board (Alpha institutional review board). The study was performed in accordance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization, Good Clinical Practice, and in accordance with all applicable local, state, and federal requirements relevant to the use of investigational medicinal products.

Trial Registration

The study is registered via ClinicalTrials.gov with registration number NCT05241470 (https://clinicaltrials.gov/ct2/show/NCT05241470?term=Stuart+Therapeutics&draw=2&rank=1). A full trial protocol is available upon request from the authors.

Study Design

We designed a phase 2 randomized clinical study to compare the safety and efficacy of ST-100 to vehicle for the treatment of DED. One hundred sixty subjects were randomly assigned to 1 of the 3 groups (1:1:1): high-dose ST-100 (60 μg/mL), low-dose ST-100 (22 μg/mL), or vehicle as topical ophthalmic drops administered bilaterally, twice daily via a blow-fill-sealed preservative-free container for 4 weeks. The vehicle formulation was a standard ophthalmic water solution containing 3% D-mannitol and 0.29% sodium chloride as stabilizing and tonicity agents, 0.1% polysorbate 80 emulsifier, and 0.28% L-histidine buffer with pH adjusted to 6.5. All subjects, investigators, and study personnel involved with the conduct of the study were masked to treatment assignments.

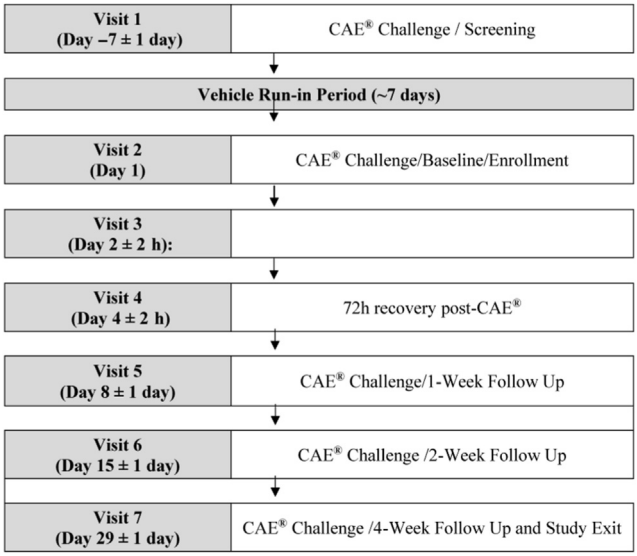

Figure 1. Flow chart summarizing visits and exposures to the Ora, Inc. Controlled Adverse Environment (CAE).

This was a multicenter study at 3 investigative sites in the United States (site 1, Andover, MA, 29 subjects; site 2, Raynham, MA, 43 subjects; site 3, Memphis, TN, 88 subjects), each with an independent investigator. The study consisted of 2 periods: a 7-day run-in period and a 4-week treatment period (Fig 1). During the screening, two 90-minute exposures utilizing the Ora, Inc. Controlled Adverse Environment (CAE)[14,15] were conducted to ascertain eligibility to enter the study, conducted at visit 1 (day 7 ± 1) and visit 2 (day 1, which we defined as baseline). Subjects who qualified after this initial screening at visit 1 entered the run-in phase, where they self-administered vehicle twice daily for approximately 7 days. Those who remained qualified at visit 2 (day 1) were randomized to receive either high- or low-dose ST-100 or vehicle for 28 days. Subjects self-administered drops twice daily and completed daily diary assessments as instructed. Further exposure to the CAE occurred subsequently at visit 5 (day 8 ± 1), visit 6 (day 15 ± 1), and visit 7 (day 29 ± 2), with pre-CAE, during CAE, and post-CAE assessments of ocular signs and symptoms (Fig 1). At visit 3 (day 2 ± 2 h) and visit 4 (day 4 ± 2 h), no CAE exposure occurred, but signs and symptoms were assessed for 24-hour recovery post-CAE and 72-hour recovery post-CAE. At visit 7, subjects exited from the study, and test treatment was discontinued.

Participant Selection and Inclusion and Exclusion Criteria

Subjects were included if they had a reported history of DED and use or desire to use eye drops for ≥ 6 months before visit 1, except as noted in the subsequent exclusion criterion. To be included, each subject had to report a score of ≥ 2 on the Ora Calibra Ocular Discomfort and 4-symptom questionnaire[15] in ≥ 1 symptom before the CAE and Schirmer's test score ≤ 10 mm and ≥ 1 mm at visits 1 and 2. Other inclusion criteria at pre-CAE visits 1 and 2 included a conjunctival redness score ≥ 1 according to the Ora Calibra Conjunctival Redness for Dry Eye Scale[14] in ≥ 1 eye, a corneal fluorescein staining score of ≥ 2 in ≥ 1 region (e.g., inferior,

superior, or central), a sum corneal fluorescein staining score of $\geq$ 4, based on the sum of the inferior, superior, and central regions, and a total Lissamine green conjunctival score of $\geq$ 2, based on the sum of the temporal and nasal regions after the Ora Calibra Corneal and Conjunctival Scale.[15] In addition, each must have demonstrated a response to the CAE at visits 1 and 2 through a $\geq$ 1 point increase in fluorescein staining in the inferior region in $\geq$ 1 eye after CAE exposure and reporting an Ora Calibra Ocular Discomfort Score $\geq$ 3 at 2 or more consecutive times in $\geq$ 1 eye during CAE exposure (if a subject had an ocular discomfort rating of 3 at time = 0 for an eye, the subject was required to report an ocular discomfort rating of 4 for 2 consecutive measurements for that eye; note: a subject could not have an ocular discomfort score of 4 at time = 0).

Subjects were excluded at visit 1 for active blepharitis, meibomian gland dysfunction (due to confounding treatments), lid margin inflammation, active ocular allergies requiring therapeutic treatment, ocular infection, active ocular inflammation, laser-assisted in situ keratomileusis surgery within the previous 12 months, use of eye drops within 2 hours of the visit, contact lens use within 7 days of visit 1 or anticipated use during the study, or use of Restasis, Xiidra, or Cequa ophthalmic solutions within 45 days of visit 1. In addition, exclusion extended to subjects with planned ocular surgery over the study period or within the last 6 months, prior use of punctal plugs within 30 days of visit 1 or anticipated use during the study, and current topical ophthalmic prescriptions or over-the-counter solutions, artificial tears, gels or scrubs, or moisture chamber. Pregnant women were excluded, as were those taking omega-3 supplements within the last 3 months.

Study Assessments

Primary End Points. To assess the efficacy of ST-100, total corneal fluorescein staining score on the Ora Calibra scale, measured by mean change from baseline (visit 2) pre-CAE to day 29 (visit 7) pre-CAE, and Ocular Discomfort Score (Ora Calibra Ocular Discomfort Scale), measured by mean change from baseline (visit 2) pre-CAE to visit 7 pre-CAE, were selected as the primary sign end point and primary symptom end point, respectively.

Corneal fluorescein staining was conducted at all visits, both pre- and post-CAE, 3 to 5 minutes after the application of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. Grading of the inferior, superior, central, temporal, and nasal region was conducted using the Ora Calibra Corneal and Conjunctival Staining Scale from 0 to 4 with the use of half grade (0.5) increments, where grade 0 = none, 1 = trace, 2 = mild, 3 = moderate, and 4 = severe. The corneal sum score was the sum of scores from the inferior, superior, and central regions. The conjunctival sum score was the sum of scores from the nasal and temporal regions. The total eye stain score was the sum of scores from all 5 regions, representing the combination of corneal and conjunctival sums.

Overall ocular discomfort and dry eye symptoms were assessed at all scheduled visits and time points (pre-CAE, post-CAE, change from pre-CAE to post-CAE). Daily, subjects graded the severity of their dry eye disease symptoms in their diary in the morning and in the evening before instilling the study drug or vehicle. Subjects rated the severity of each of the following symptoms with reference to how both eyes felt (Ora Calibra Ocular Discomfort Scale and 4-Symptom Questionnaire): overall ocular discomfort, burning, dryness, grittiness and stinging according to the following 6-point (0−5) scale where 0 = none and 5 = worst.

Secondary Efficacy and Safety End Points. We measured tear production using Schirmer's test on both unanesthetized eyes pre-CAE at visit 1 (day 7), visit 2 (day 1), and visit 7 (day 29) with a sterile TearFlo strip placed in the lower temporal lid margin of each eye, with the length of the moistened area recorded in millimeters. Using this, we determined Schirmer's responder rate, defined as the proportion of patients achieving at least a 10-mm increase in tear production unanesthetized. This is considered demonstrable efficacy for the treatment of DED when exceeding the vehicle rate in 2 independent trials,[14] per draft United States Food and Drug Administration Guidance for Industry.[16] Other secondary outcomes not described herein include Ocular Surface Disease Index at visits 3, 4, 5, 6, and 7 (pre-CAE) and Lissamine green staining (Ora Calibra scale) at visits 3, 4, 5, 6, and 7 (pre-CAE, post-CAE, and pre- to post-CAE) for central, superior, inferior, temporal, and nasal regions that comprise the corneal sum, conjunctival sum, and total eye score. For this assessment, a Lissamine strip was placed into the inferior conjunctival cul-de-sac for approximately 30 seconds before evaluating the staining. Safety measures evaluated were best corrected visual acuity, slit-lamp evaluation, adverse event query, intraocular pressure, and undilated fundoscopy.

Statistical Analysis

The intent-to-treat (ITT) population included all randomized subjects. The primary analysis was performed on the ITT population with the multiple imputation methodology for missing values. The study eye was the eye with more severe disease, as determined before treatment, or the right eye. Certain results were demonstrated using both study eye plus fellow eye versus vehicle (placebo). Statistical programming and analyses were performed using Statistical Analysis System (SAS) version 9.4. Randomization and double masking were selected as state-of-the-art measures to reduce bias.

The primary efficacy analysis compared the CFB in total corneal fluorescein staining (Ora Calibra Scale) in the study eye and ocular discomfort (Ora Calibra Ocular Discomfort Scale), which were analyzed separately using an analysis of covariance (ANCOVA) model with terms for baseline value and treatment group. For each end point, CFB was calculated for each subject as visit minus baseline such that a positive difference indicated a worsening of dry eye signs or symptoms. In addition, treatment comparisons between ST-100 (high dose or low dose) and vehicle were calculated as active minus placebo, such that a negative result indicated a better score for the ST-100 (high dose or low dose) (i.e., the ST-100 [high dose or low dose] had a smaller increase in dry eye signs or symptoms than the vehicle group). The least squares (LS) mean of the intrasubject differences for each treatment group and the LS mean difference between treatment groups were presented from the model together with standard errors, 2-sided *P* values, and 2-sided 95% confidence intervals.

As further robustness analyses, 2-sample *t* tests and Wilcoxon rank sum tests were conducted to compare differences in CFB of total corneal fluorescein staining and staining scores at visit 7 (day 29) pre-CAE between each dose level of ST-100 with vehicle and as described for various ad hoc analyses. Wilcoxon rank sum tests were only conducted using analysis populations with observed data only and in place of Welch's *t* tests when normality failed. For the secondary efficacy end points and other ad hoc analyses, the LS means, LS mean differences, standard errors, 2-sided 95% confidence intervals for LS means, 2-sided 95% confidence intervals for the difference in means, and 2-sided *P* values were reported from the ANCOVA models. Pairwise 2-sample *t* tests and Wilcoxon rank sum tests were used as sensitivity for CFB at each visit and time point. Paired *t* tests were used to analyze CFB within each treatment group. Other statistics are as described for the relevant result.

Results

Subject Disposition

Overall, 335 subjects were screened for the study, of whom 160 were enrolled and randomized; 146 subjects (91.3%) completed the study, and 14 subjects (8.8%) discontinued (Fig 2). From the 160 randomized subjects, 159 subjects (comprising 53, 52, and 54 subjects in the high-dose ST-100, low-dose ST-100, and vehicle groups, respectively) were included in the safety population. A total of 27 subjects (17.0%) reported 33 ocular adverse events (AEs). This included 9 subjects (17.0%) in the high-dose ST-100 group who reported 11 ocular AEs, 6 subjects (11.5%) in the low-dose ST-100 group who reported 6 ocular AEs, and 12 subjects (22.2%) in the vehicle group reported 16 ocular AEs. Overall, 8 subjects in the high-dose ST-100 group (15%) reported 9 ocular treatment-emergent adverse events (TEAEs), with only 1 TEAE causing discontinuation, compared with 11 subjects in the vehicle group (20%) reporting 15 TEAEs, of which 2 caused discontinuation. Thus, ST-100 was comparable with vehicle in terms of AEs (details in Table S1, available at www.ophthalmologyscience.org).

Demographics of ITT Population

A detailed summary of demographic data in the ITT population is described in Table 2. The age and gender distribution in the overall study population reflected the indication under study, with a bias toward females (70%) and a median age of 63.2 years. The baseline disease characteristics of randomized subjects were similar among all treatment groups. The population was characterized by subjects who had a mean total corneal fluorescein staining score of 4.82, a mean ocular discomfort score on the Ora Calibra Ocular Discomfort Scale of 2.5, and a mean unanesthetized Schirmer's test value of 4.9 mm at baseline pre-CAE with no statistically significant differences between groups.

Corneal and Conjunctival Staining and Ocular Discomfort

The primary efficacy sign end point was mean CFB (pre-CAE) at day 29 in the study eye corneal fluorescein staining, defined as the sum of stain scores in central, inferior, and superior regions. Both high- and low-dose ST-100 improved, with decreases of −0.18 and −0.16, respectively (paired *t* test, $P = 0.2889$ and 0.3542). However, neither dose showed superiority over vehicle and therefore failed to meet the primary end point (ANCOVA: $P = 0.9741$, $P = 0.9575$, respectively). Similarly, for total fluorescein stain (corneal plus conjunctival sums) in study eyes alone, mean CFB did not differ between high-dose ST-100 and vehicle ($P = 0.60$, Fig 3A) nor between low-dose ST-100 and vehicle ($P = 0.921$; Table S3, available at www.ophthalmologyscience.org). However, for fellow eyes, the CFB difference was significant for high-dose ST-100 compared with vehicle ($P = 0.02$; Welch's *t* test), as it was for low-dose ($P = 0.015$, Mann−Whitney rank sum test). Consequently, for high-dose study and fellow eyes combined, mean CFB in total stain was significant compared with vehicle ($P = 0.039$); this was not the case for low dose ($P = 0.073$; Table 4). For conjunctival total stain (nasal and temporal conjunctiva combined), high-dose ST-100 exceeded vehicle for study and fellow eyes combined ($P = 0.012$, Fig 3B), largely due to differences in temporal conjunctival staining ($P = 0.022$), whereas low-dose ST-100 did not ($P = 0.063$). Again, the high-dose did not differ between sites (analysis of variance on ranks, $P = 0.86$). Finally, high-dose ST-100 eyes combined had significant improvement in the sum of corneal staining compared with baseline ($P = 0.0197$), as did low dose ($P = 0.008$), whereas vehicle eyes did not ($P = 0.94$, Fig 3C and Table 4).

As a secondary efficacy end point, Lissamine green staining generally showed improvement from baseline to various visits and locations. For example, the CFB at days 15 and 29 (pre-CAE) for conjunctival sum all improved for high-dose ST-100 ($P \leq 0.037$), whereas vehicle did not ($P \geq 0.06$; Table S5, available at www.ophthalmologyscience.org). In some instances, low-dose ST-100 demonstrated an early influence compared with vehicle. For example, the CFB in pre- to post-CAE at day 8 for low dose was significant and exceeded vehicle for superior cornea (−0.35 vs. 0.15, respectively, $P = 0.001$); a similar result for the same measure was observed for low-dose ST-100 compared with vehicle for corneal sum ($P = 0.0392$) and total eye sum ($P = 0.0333$).

The coprimary efficacy symptom end point of our study was ocular discomfort, measured by the mean CFB (pre-CAE) at day 29 using the Ora Calibra Ocular Discomfort Scale (Table 6). High- and low-dose ST-100 treatment groups each demonstrated significant improvement with CFB of −0.4 ($P = 0.0133$, $P = 0.0151$, respectively) but did not show superiority over vehicle ($P = 0.5696$, $P = 0.8968$, respectively). ST-100 gave statistically significant improvement from baseline in overall discomfort assessed by the 4-symptom questionnaire (Ora Calibra Scale) at various visits (Table 6). Furthermore, high-dose ST-100 demonstrated superiority over vehicle in CFB to day 15 in overall ocular discomfort among subjects ($P = 0.0332$).

Schirmer's Test and Schirmer's Responder Rate

A prespecified secondary efficacy sign end point was unanesthetized Schirmer's score (pre-CAE) at day 29 (visit 7). At baseline (day 1), Schirmer's score in the ITT population for cohorts intended for high- and low-dose ST-100 did not differ from vehicle (Table 7, $P \geq 0.27$). Of the 49 subjects in the high-dose arm completing the trial, 24 (47%) had improved Schirmer's score at day 29 for the study eye compared with 46% in the low-dose arm and 31% in the vehicle group. For the high-dose group, the improved score was significant compared with vehicle ($P = 0.0127$); low dose did not differ from vehicle ($P = 0.33$; Table 7). The high-dose ST-100 group demonstrated superiority to the vehicle group in mean Schirmer's test CFB at day 29 for the study eye ($P = 0.0094$), whereas the low dose did not ($P = 0.24$).

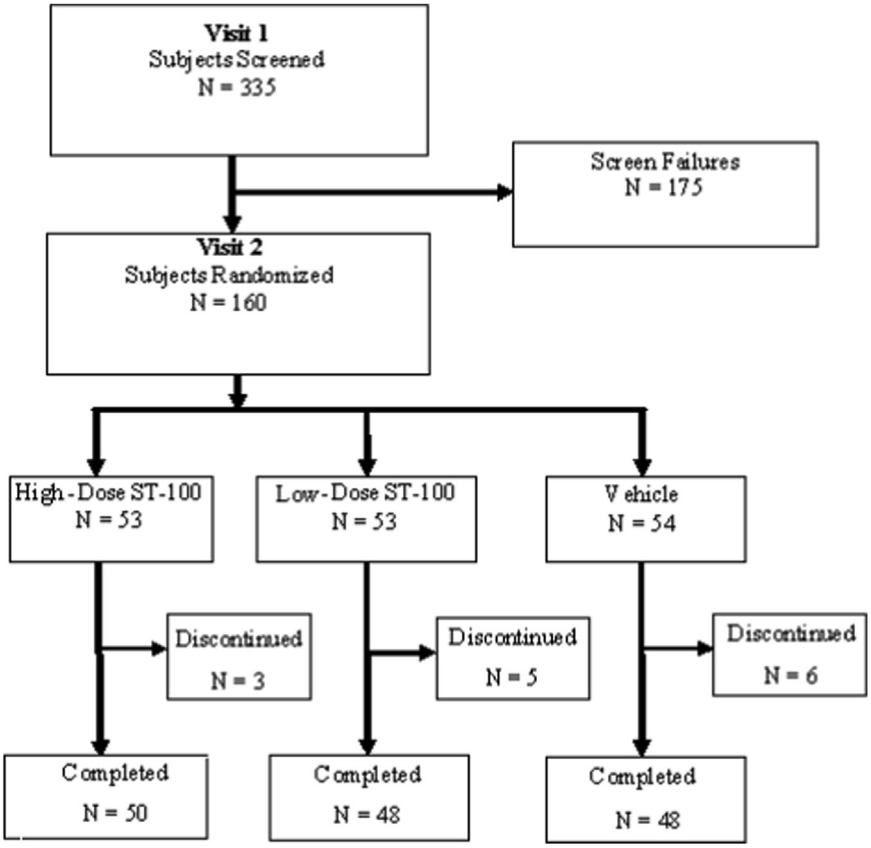

Figure 2. Subject disposition during the study as described in the text.

Table 2. Patient Demographics

Variable	High-Dose ST-100	Low-Dose ST-100	Vehicle	All Subjects
Age, yrs: n	53	53	54	160
Min, max	33, 84	40, 91	30, 85	30, 91
Mean (SD)	61.9 ± 11.60	64.4 ± 10.29	63.4 ± 13.28	63.2 ± 11.77
Median	61.0	65.0	66.0	64.0
< 65: n (%)	30 (56.6%)	26 (49.1%)	26 (48.1%)	82 (51.3%)
≥ 65: n (%)	23 (43.4%)	27 (50.9%)	28 (51.9%)	78 (48.8%)
Sex: n (%)				
Male	24 (45.3%)	13 (24.5%)	11 (20.4%)	48 (30.0%)
Female	29 (54.7%)	40 (75.5%)	43 (79.6%)	112 (70.0%)
Ethnicity: n (%)				
Hispanic or Latino	1 (1.9%)	1 (1.9%)	2 (3.7%)	4 (2.5%)
Not Hispanic or Latino	52 (98.1%)	52 (98.1%)	52 (96.3%)	156 (97.5%)
Race: n (%)				
American Indian or Alaska Native	0	0	0	0
Asian	0	1 (1.9%)	0	1 (0.6%)
Black or African American	15 (28.3%)	15 (28.3%)	18 (33.3%)	48 (30.0%)
Native Hawaiian or Other Pacific Islander	0	1 (1.9%)	0	1 (0.6%)
White	38 (71.7%)	35 (66.0%)	36 (66.7%)	109 (68.1%)
Other	0	0	0	0
Multiple	0	1 (1.9%)	0	1 (0.6%)

n = number of subjects in the respective treatment group for the given measure, with percentages based on the total number of subjects in each respective treatment group. Subjects who had selected more than 1 race were summarized in the multiple race group. Age was calculated using the following equation: Age = (Informed Consent Date − Date of Birth)/365.25, truncated as an integer.
SD = standard deviation.

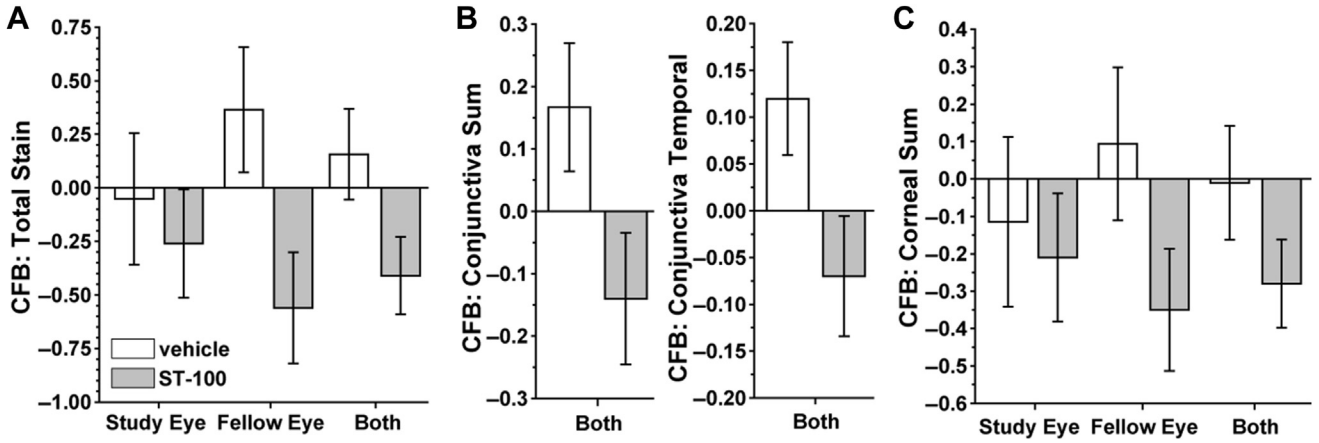

Figure 3. Ad hoc analysis of corneal and conjunctival staining. **A**, Mean change from baseline (CFB) at day 29 in total fluorescein eye stain defined as the total of the corneal (central, inferior, and superior regions) and conjunctival (nasal and temporal) sums, as measured on the Ora Calibra scale pre-Controlled Adverse Environment (CAE), for high-dose ST-100 and vehicle study, fellow, and both eyes. **B,** Mean CFB at day 29 for the sum of conjunctival fluorescein (left) and temporal conjunctival (right) stains for high-dose ST-100 and vehicle. **C,** Mean CFB at day 29 for the corneal sum of fluorescein stain for study, fellow, and both eyes. Significance as described in the text.

Table 4. Ad Hoc Analysis of Fluorescein Staining, Both Eyes

Measurement (Pre-CAE)	High-Dose ST-100	Low-Dose ST-100	Vehicle
Total stain CFB at day 29: n	100	96	96
Minimum, maximum	−6.0, 4.0	−5.0, 5.0	−4.5, 6.5
Mean ± SD	−0.42 ± 1.81	−3.3 ± 1.67	0.16 ± 2.08
Two-sided 95% CI	(−0.78, −0.06)	(−0.67, 0.00)	(−0.26, 0.58)
P value (paired *t* test, vs. baseline)	**0.0224**	0.0521	0.4625
ST-100 − vehicle, difference in means ± SE	−0.58 ± 0.278	−0.49 ± 0.271	
Two-sided 95% CI	(−1.12, −0.03)	(−1.02, 0.05)	
P value (vs. vehicle, 2-sample *t* test)	**0.0394**	0.0727	
Conjunctival sum CFB at day 29: n	100	96	96
Minimum, maximum	−4.0, 2.5	−2.0, 2.5	−3.0, 2.5
Mean ± SD	−0.14 ± 1.06	−0.01± 0.79	0.17± 1.01
Two-sided 95% CI	(−0.35, 0.07)	(−0.17, 0.16)	(−0.04, 0.37)
P value (paired *t* test, vs. baseline)	0.1883	0.9488	0.1082
ST-100 − vehicle, difference in means ± SE	−0. 31 ± 0.148	−0.17 ± 0.131	
Two-sided 95% CI	(−0.60, −0.02)	(−0.43, 0.09)	
P value (vs. vehicle, Wilcoxon rank sum test)	**0.0121**	0.0629	
Temporal conjunctival CFB at day 29: n	100	96	96
Minimum, maximum	−2.0, 2.0	−1.0, 1.0	−1.5, 1.5
Mean ± SD	−0.07 ± 0.644	−0.07 ± 0.479	0.12 ± 0.593
Two-sided 95% CI	(−0.20, 0.06)	(−0.16, 0.03)	(0.00, 0.24)
P value (paired *t* test, vs. baseline)	0.279	0.169	0.051
ST-100 − vehicle, Difference in means ± SE	−0.19 ± 0.088	−0.19 ± 0.078	
Two-sided 95% CI	(−0.36, −0.02)	(−0.34, −0.03)	
P value (vs. vehicle)	**0.0332**	**0.0169**	
P value (vs. vehicle, Wilcoxon rank sum test)	**0.0224**	**0.0187**	
Superior cornea: CFB at day 29: n	100	96	96
Minimum, maximum	−1.5, 2.5	−2.0, 1.0	−2.0, 2.0
Mean ± SD	−0.12 ± 0.686	−0.21 ± 0.597	−0.05 ± 0.769
Two-sided 95% CI	(−0.26, 0.02)	(−0.33, −0.09)	(−0.21, 0.10)
P value (paired *t* test, vs. baseline)	0.0832	**0.0009**	0.5088
Corneal um: CFB at day 29: n	100	96	96
Minimum, maximum	−3.0, 3.5	−3.5, 3.0	−4.5, 4.0
Mean ± SD	−0.28 ± 1.181	−0.33 ± 1.192	−0.01 ± 1.492
Two-sided 95% CI	(−0.51, −0.05)	(−0.57, −0.09)	(−0.31, 0.29)
P value (paired *t* test, vs. baseline)	**0.0197**	**0.0083**	0. 9456

Bold values indicate statistical significance.
CAE = controlled adverse environment; CFB = change from baseline; CI = confidence interval; n = number of eyes in the respective treatment group for the given measure; SD = standard deviation; SE = standard error.

Table 6. Ocular Discomfort

Measurement (Study Eye, Pre-CAE)	High-Dose ST-100	Low-Dose ST-100	Vehicle
Baseline Ora Calibra scale (day 1): n	53	53	54
Minimum, maximum	0, 4	0, 4	0, 4
Mean ± SD	2.4 ± 1.04	2.6 ± 1.06	2.5 ± 1.11
Two-sided 95% CI	(2.1, 2.7)	(2.3, 2.9)	(2.2, 2.8)
ST-100 − vehicle, difference in means	−0.1	0.1	
Two-sided 95% CI	(−0.5, 0.3)	(−0.3, 0.5)	
P value (vs. vehicle, Two-Sample *t* test)	0.5589	0.5591	
CFB at day 29 Ora Calibra scale: n	53	53	54
Minimum, maximum	−3, 2	−3, 2	−3, 3
Mean ± SD	−0.4 ± 1.07	−0.4 ± 1.08	0.12 ± 0.593
Two-sided 95% CI	(−0.7, −0.1)	(−0.7, −0.1)	(−0.7, 0.0)
P value, paired *t* test (vs. baseline)	**0.0133**	**0.0151**	0.0624
ANCOVA LS mean ± SE (mm)	−0.4 ± 0.13	−0.3 ± 0.14	−0.3 ± 0.14
LS mean difference (CI), ANCOVA model (ST-100 −vehicle)	−0.1 (−0.5, 0.3)	0.0 (−0.4,0.4)	
P value (vs. vehicle)	0.5696	0.8968	
CFB at day 8 (overall, 4-symptom questionnaire): n	51	49	50
Minimum, maximum	−3, 1	−4, 2	−3, 1
Mean ± SD	−0.2 ± 0.91	−0.6 ± 1.17	0.2 ± 0.96
Two-sided 95% CI	(−0.5, 0.0)	(−0.9, −0.2)	(−0.4, 0.1)
P value, paired *t* test (vs. baseline)	0.0700	**0.0019**	0.2420
CFB at day 8 (burning, 4-symptom questionnaire): n	51	49	50
Minimum, maximum	−2, 1	−4, 2	−3, 1
Mean ± SD	−0.3 ± 0.82	−0.3 ± 0.93	0.0 ± 0.71
Two-sided 95% CI	(−0.4, 0.0)	(−1.0, −0.2)	(−0.4, 0.1)
P value, paired *t* test (vs. baseline)	**0.0270**	**0.0051**	0.2314
CFB at day 15 (overall, 4-symptom questionnaire): n	51	49	50
Minimum, maximum	−2, 2	−3, 2	−1, 1
Mean ± SD	−0.3 ± 0.82	−0.3 ± 0.93	0.0 ± 0.71
Two-sided 95% CI	(−0.6, −0.1)	(−0.5, 0.0)	(−0.2, 0.2)
P value, paired *t* test (vs. baseline)	**0.0053**	0.051	> 0.9999
ANCOVA LS mean ± SE	−0.4 ± 0.15	−0.2 ± 0.11	0.0 ± 0.11
LS mean difference (CI), ANCOVA model (ST-100 −vehicle)	−0.3 (−0.6, 0.0)	−0.1 (−0.5, 0.2)	
P value (vs. vehicle)	**0.0332**	0.3444	
CFB at day 15 (burning, 4-symptom questionnaire): n	51	49	49
Minimum, maximum	−2, 2	−4, 3	−2, 2
Mean ± SD	−0.3 ± 0.90	−0.2 ± 1.34	0.0 ± 0.87
Two-sided 95% CI	(−0.5, 0.0)	(−0.6, 0.2)	(−0.3, 0.2)
P value, paired *t* test (vs. baseline)	**0.0238**	0.247	0.742
CFB at day 15 (dryness, 4-symptom questionnaire): n	51	49	49
Minimum, maximum	−2, 1	−2, 3	−3, 3
Mean ± SD	−0.3 ± 0.81	−0.2 ± 0.87	−0.1 ± 0.95
Two-sided 95% CI	(−0.5, −0.1)	(−0.4, 0.1)	(−0.4, 0.2)
P value, paired t test (vs. baseline)	**0.0081**	0.1974	0.3711
CFB at day 29 (overall, 4-symptom questionnaire): n	50	48	48
Minimum, maximum	−3, 2	−4, 2	−3, 2
Mean ± SD	−0.4 ± 1.12	−0.5 ± 1.11	−0.2 ± 1.09
Two-sided 95% CI	(−0.7, −0.1)	(−0.8, −0.2)	(−0.5, 0.1)
P value, paired *t* test (vs. baseline)	**0.0153**	**0.0044**	0.1921
CFB at day 29 (grittiness, 4-symptom questionnaire): n	50	48	48
Minimum, maximum	−2, 2	−3, 3	−3, 3
Mean ± SD	−0.4 ± 0.88	−0.3 ± 1.06	−0.3 ± 1.17
Two-sided 95% CI	(−0.6, −0.1)	(−0.6, 0.0)	(−0.6, 0.1)
P value, paired *t* test (vs. baseline)	**0.0054**	**0.0342**	0.0964
CFB at day 29 (burning, 4-symptom questionnaire): n	50	48	48
Minimum, maximum	−2, 4	−4, 2	−3, 2
Mean ± SD	−0.4 ± 1.14	−0.3 ± 1.27	−0.3 ± 1.17
Two-sided 95% CI	(−0.7, 0.0)	(−0.7, 0.1)	(−0.6, 0.0)
P value, paired *t* test (vs. baseline)	**0.0300**	0.1185	0.0897

Bold values indicate statistical significance.
ANCOVA = analysis of covariance; CAE = controlled adverse environment; CFB = change from baseline; CI = confidence interval; LS = least squares; n = number of eyes in the respective treatment group for the given measure; SD = standard deviation; SE = standard error.

Table 7. Unanesthetized Schirmer's Test and Responder Rate: Intent-to-Treat Population

Measurement (Pre-CAE)	High-Dose ST-100	Low-Dose ST-100	Vehicle
Baseline Schirmer's test (day 1, study eye): n	53	53	54
Minimum, maximum (mm)	1, 10	1, 10	1, 10
Mean ± SD (mm)	5.2 ± 2.8	4.4 ± 2.8	5.0 ± 2.9
Two-sided 95% CI	(4.4, 6.0)	(3.6, 5.2)	(4.2, 5.8)
ST-100 − vehicle, difference in means (SE)	0.2 ± 0.55	−0.6 ± 0.55	
Two-sided 95% CI	(−0.9, 1.3)	(−1.7, 0.5)	
P value (vs. vehicle, 2-sample *t* test)	0.6828	0.2712	
Day 29 Schirmer's Test (study eye): n	49	48	48
Minimum, maximum (mm)	0, 25	1, 20	0, 17
Mean ± SD (mm)	6.9 ± 5.8	5.2 ± 4.1	4.5± 3.3
Two-sided 95% CI	(5.2, 8.6)	(4.0, 6.4)	(3.5, 5.4)
ST-100 − vehicle, difference in means ± SE	2.4 ± 0.95	0.7 ± 0.75	
Two-sided 95% CI	(0.5, 4.3)	(−0.8, 2.2)	
P value (vs. vehicle, 2-sample *t* test)	**0.0127**	0.3347	
Schirmer's test CFB at day 29 (study eye): n	49	48	48
Minimum, maximum (mm)	−7, 17	−5, 14	−7, 9
Mean ± SD (mm)	1.6 ± 5.6	0.8 ± 3.7	−0.5 ± 3.5
Two-sided 95% CI	(0.0, 3.2)	(−0.3, 1.8)	(−1.5, 0.5)
P value, paired *t* test (vs. baseline)	0.0507	0.1699	0.3063
ANCOVA LS mean ± SE (mm)	1.8 ± 0.6	0.5 ± 0.6	−0.5 ± 0.6
LS mean difference (CI), ANCOVA model (ST-100 − vehicle)	2.3 (0.6, 4.0)	1.0 (−0.7, 2.7)	
P value (vs. vehicle)	**0.0094**	0.2365	
Schirmer's responder rate (study eye): n (%)	6/49 (12.2%)	1/48 (2.1%)	0/48
Difference of proportions (ST100 − vehicle)	0.122	0.021	
Exact 2-sided 95% CI	(−0.075, 0.315)	(−0.187, 0.228)	
Fisher exact test *P* value	**0.0266**	> 0.9999	
Schirmer's responder rate (both eyes): n/total (%)	11/98 (11.2%)	4/96 (4.2%)	2/96 (2.1%)
ST100 − vehicle GEE model	5.94	2.04	
Covariance structure: unstructured odds ratio			
Two-sided 95% CI	(1.25, 28.20)	(0.32, 12.92)	
P value (odds ratio)	**0.0249**	0.4476	

Bold values indicate statistical significance.
ANCOVA = analysis of covariance; CAE = Controlled Adverse Environment; CFB = change from baseline; CI = confidence interval; GEE = generalized estimating equation; LS = least squares; mm = millimeters; n = number of subjects in the respective treatment group for the given measure, with percentages based on the total number of subjects in each respective treatment group; SD = standard deviation; SE= standard error.

Schirmer's responder rate, defined as the proportion of patients achieving at least a 10-mm increase in tear production unanesthetized, for study eyes was 12.2%, for high-dose ST-100, 2.1% for low dose, and 0% for vehicle (Table 7). Thus, high-dose ST-100 achieved the Schirmer's responder rate end point, compared with vehicle (P = 0.0266). The proportion of responders for study and fellow eyes considered together was 11.2% for high-dose ST-100 and 4.2% for low dose, with high dose demonstrating superiority over vehicle (2.1%; P = 0.0249), whereas low dose did not (P = 0.45). The mean Schirmer's CFB at day 29 for both eyes combined for high dose increased significantly, compared with a decrease for vehicle (1.32 mm vs. −0.59 mm: Mann−Whitney U Statistic, P = 0.028; Fig 4); the increase for low dose (0.80 ± 0.42 mm) was not significant compared with vehicle (Mann−Whitney U Statistic, P = 0.060). Furthermore, improvement in the fellow eye tracked significantly with improvement in the study eye for both high-dose ST-100 (P = 0.001) and low dose (P < 0 .001, Table S3), which was not the case for vehicle (P = 0.14).

Staining in Schirmer's Test Responders

For patients achieving Schirmer's responder rate at day 29, ST-100 had a protective effect on study eyes, by reducing incremental corneal fluorescein staining during the CAE sessions, measured as the difference from pre- to post-CAE for low and high doses considered together (Table 8). The mean increase in corneal sum at baseline was 3.43 compared with 1.64 at day 29, yielding a CFB of −1.79. This improvement significantly exceeded that for nonresponders (−0.50; P = 0.0274, difference in means 2-sample t test). Both central and superior cornea contributed to this improvement. The mean increase in central corneal stain from pre- to post-CAE was 0.71 at baseline and dropped to 0.0 at day 29, for a CFB of −0.71, compared with −0.07 for nonresponders (P = 0.0436, difference in means 2-sample t test). Similarly, for superior cornea, the CFB was −0.64, compared with −0.10 for nonresponders (P = 0.033, Wilcoxon rank sum test).

We defined total eye stain as the sum of corneal (central, inferior, and superior) and conjunctival (nasal and temporal)

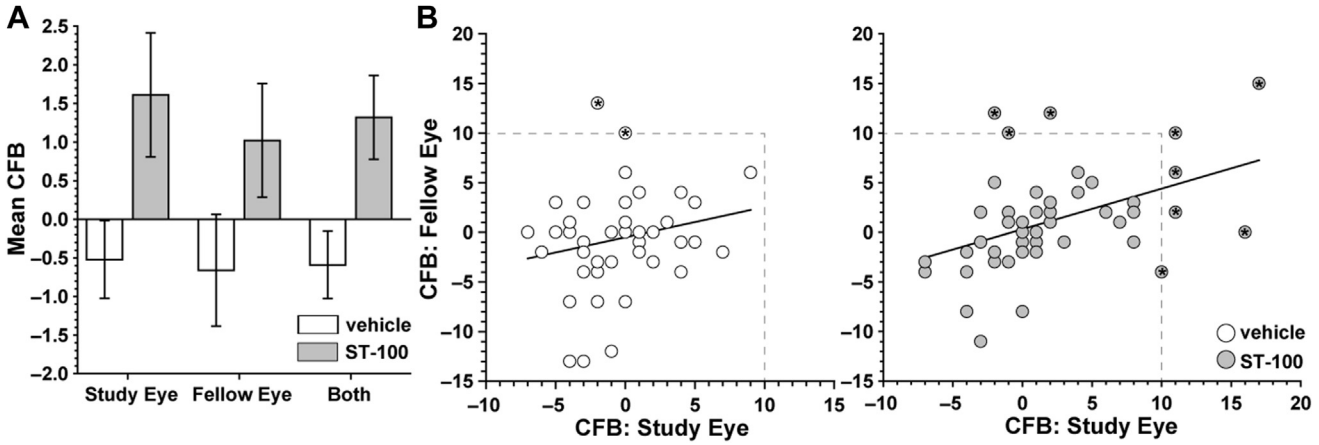

Figure 4. Schirmer's test change from baseline (CFB) and responder rate. **A,** Mean change from baseline in Schirmer's score for the study eye, fellow eye, and both eyes combined in vehicle vs. high-dose ST-100 subjects in the intent-to-treat population (mean ± standard error). **B,** CFB in fellow eye tracks with CFB in the study eye in the high-dose ST-100 cohort (right, Pearson product-moment correlation = 0.45, $P = 0.001$) but not in the vehicle cohort (Pearson product-moment correlation = 0.21, $P = 0.15$). Best-fitting regression line is shown for both. The dashed box indicates minimal CFB in Schirmer's test score (10 mm) for each eye; Schirmer's responders are indicated (*). Other significance as described in the text.

fluorescein stains. As mean Schirmer's CFB at day 29 increased for ST-100 high-dose responders (both eyes), the CFB in total stain decreased significantly (Pearson product-moment correlation, −0.73, $P = 0.011$); this was not so for nonresponders; Fig 5A). Similarly, the CFB at day 29 in corneal sum for responders also decreased significantly as Schirmer's CFB increased (Pearson product-moment correlation, −0.76, $P = 0.007$), whereas for nonresponders, this was not the case.

Given the significant improvement in Schirmer's test and total eye fluorescein stain for high-dose ST-100 (Tables 4, 7), we calculated the ratio of Schirmer's to total stain at day 29 and compared it with the same ratio at baseline (Fig 6). This ratio serves as an index of overall improvement because a higher value can result from an improvement in Schirmer's, diminished total stain, or both. When study eyes only are considered, the ratio at day 29 was significantly greater for ST-100 compared with vehicle: 0.67 ± 0.05 versus 0.56 ± 0.06, respectively ($P = 0.023$, Mann−Whitney rank sum test). The ratio at baseline did not differ ($P = 0.89$). Considering both eyes together widened the difference between ST-100 and vehicle: 1.01 ± 0.09 versus 0.65 ± 0.06, respectively ($P = 0.029$; Mann−Whitney rank sum test). Once again, the ratio at baseline did not differ ($P = 0.47$). In all cases, a higher ratio at baseline was predictive of a better outcome at day 29, as indicated by positive correlations for both vehicle and ST-100 ($P < 0.001$; Pearson product-moment correlation).

Finally, Schirmer's test responders showed a significant difference in CFB versus nonresponders at day 15 pre-CAE for temporal Lissamine green staining ($P = 0.0030$) and also at day 15 pre-CAE for total conjunctival Lissamine staining ($P = 0.0204$).

Tear Production and Ocular Discomfort

Given the improvements in tear production and ocular discomfort for high-dose ST-100 (Tables 6, 7), we related the 2 outcomes by subtracting ocular discomfort from Schirmer's score at day 29 and comparing the same difference at baseline (Fig 7). A higher value can reflect not only an improvement in Schirmer's score but also increased comfort. When study eyes only are considered, the difference at day 29 for ST-100 was significantly greater than vehicle: 4.86 ± 0.84 versus 2.11 ± 0.51, respectively ($P = 0.018$, Mann−Whitney rank sum test). The ratio at baseline did not differ ($P = 0.53$). Similarly, pooling both eyes yielded a similar result: 4.61 ± 0.59 for ST-100 versus 2.67 ± 0.44 for vehicle ($P = 0.019$; Mann−Whitney rank sum test). Once again, the difference at baseline did not differ ($P = 0.74$). In both cases, a greater difference at baseline was predictive of a better outcome at day 29, as indicated by positive correlations for both vehicle and ST-100 ($P < 0.001$; Pearson product-moment correlation).

ST-100 Reduces Ocular Discomfort During CAE Sessions

We found that ST-100 reduced ocular discomfort during the 90-minute CAE sessions on days 8, 15, and 29. During these sessions, patients reported ocular discomfort scores at regular intervals for a portion of the session. We subtracted this score from that taken the same day pre-CAE, so that a negative number indicates improvement. This difference was compared with that at the same interval at baseline, yielding a CFB. Next, we summarize this analysis by comparing the minimum interval during CAE at which improvement in ocular discomfort differed significantly from the same measurement at baseline (Table 9).

For patients in the high-dose ST-100 cohort, ocular discomfort improved from pre-CAE immediately on day 8, during the first 5 minutes, exceeding the same score at baseline (paired *t* test, $P = 0.0216$). Significance in CFB for this cohort persisted through 50 minutes ($P \leq 0.036$). In contrast, low-dose ST-100 did not reach a significant CFB until minute 40 ($P = 0.0262$), whereas vehicle never did so

Table 8. Pre- to Post-CAE: ST-100 for Schirmer's Test Responders in Study Eyes

Fluorescein Stain	Group	Min, Max	Mean ± SD	Difference in Means ± SE	Two-Sided 95% CI	*P* Value
Corneal sum CFB at day 29	Responders	−4.0, 1.5	−1.79 ± 2.14	−1.29 ± 0.58	(−2.43, −0.15)	**0.0274**
	Nonresponders	−5.0, 4.0	−0.50 ± 1.45			
Central cornea CFB at day 29	Responders	−1.5, 0.5	−0.71 ± 0.91	−0.65 ± 0.32	(−1.28, −0.02)	**0.0436**
	Nonresponders	−2.0, 2.0	−0.07 ± 0.82			
Superior cornea CFB at day 29	Responders	−1.5, 1.0	−0.64 ± 0.80	−0.55 ± 0.27	(−1.08, −0.01)	**0.0451**
	Nonresponders	−2.0, 2.0	−0.10 ± 0.70			

CFB at day 29: change in pre- to post-CAE from baseline (day 2) to day 29.
P value: lesser of 2-sample test vs. Wilcoxon rank sum test for difference in means. Bold values indicate statistical significance.
CAE = Controlled Adverse Environment; CFB = change from baseline; CI = confidence interval; SE= standard error.

for that day ($P \geq 0.071$). On day 15, high-dose ST-100 achieved a significant CFB at the beginning of the session (time = 0, $P = 0.0420$), maintaining significant improvement for the entire 45-minute interval tested ($P \leq 0.047$). On the same day, low-dose ST-100 reached a significant CFB through 25 to 45 minutes ($P \leq 0.006$), comparable with vehicle ($P \leq 0.0471$). On day 29, high-dose ST-100 achieved a significant CFB from 10 to 70 minutes ($P \leq 0.0167$), although vehicle patients did not reach a significant CFB until minute 35 and then at sporadic intervals ($P \leq 0.0399$).

Finally, subjects in both ST-100 treatment groups saw improved visual function scores from baseline assessed by the Ocular Surface Disease Index at various visits. High-dose ST-100 achieved superiority over vehicle Ocular Surface Disease Index scores for blurred vision at day 4, non-CAE ($P = 0.0097$), gritty eyes at day 8, pre-CAE ($P = 0.0393$) and painful or sore eyes at day 15, pre-CAE ($P = 0.0137$), all compared with baseline (Table S3).

Discussion

Our preclinical studies demonstrate that ST-100 has broad reparative properties for both corneal acute injury and desiccation.[6,13] The capacity for collagen mimetic peptides to repair damaged collagen in the ECM promotes neuronal survival, both in the cornea and in the posterior segment.[13,17,18] Although DED involves diverse etiologies and clinical manifestations,[2] consistency in signs and symptoms point to a common pathogenic pathway that includes not only damage to the corneal surface but also to the underlying nerve bed.[3,8] These factors in conjunction with our earlier studies led us to test whether

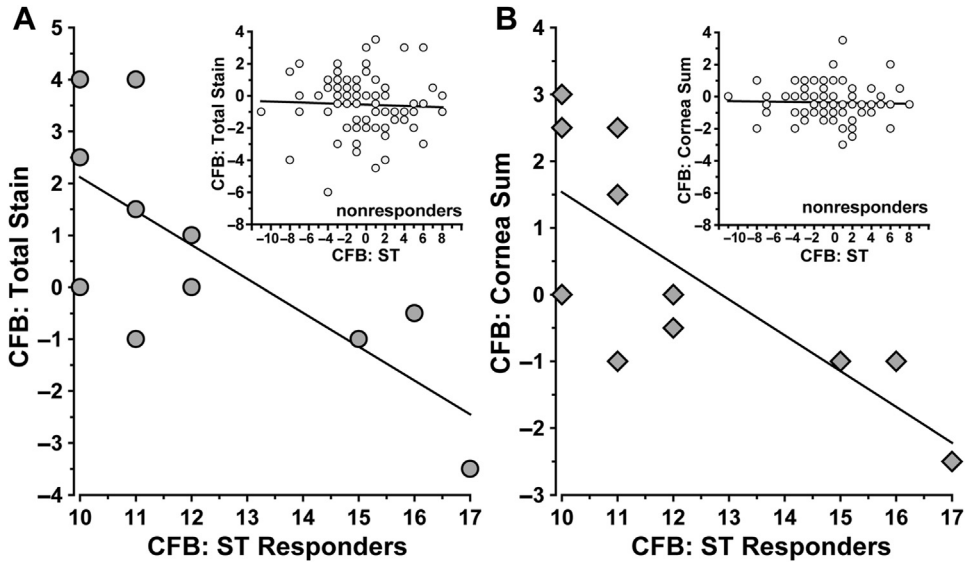

Figure 5. Schirmer's test change from baseline and responder rate. **A,** Change from baseline (CFB) at day 29 in total fluorescein eye stain diminishes significantly with increasing CFB in Schirmer's score for study and fellow eyes achieving the Schirmer's responder rate for ST-100 high-dose treatment (Pearson product-moment correlation, −0.73, $P = 0.011$); for nonresponder eyes (inset), the 2 measurements did not correlate (Pearson product-moment correlation, −0.043, $P = 0.696$). **B,** CFB at day 29 in the sum of corneal fluorescein stain similarly diminishes as CFB in Schirmer's score increases for responder study and fellow eyes together with ST-100 high dose (Pearson product-moment correlation, −0.76, $P = 0.007$); once again, for nonresponder eyes (inset), this was not the case (Pearson product-moment correlation, −0.031, $P = 0.78$).

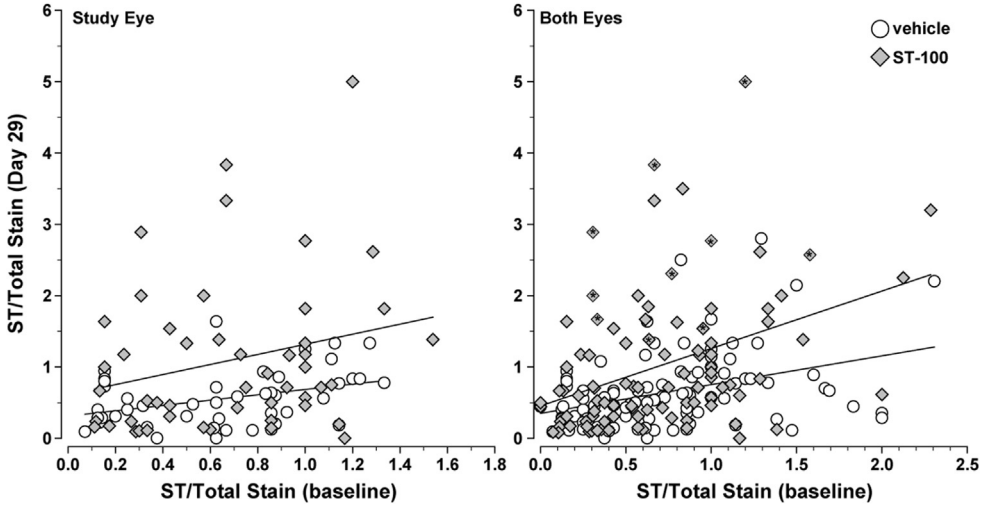

Figure 6. Ratio of Schirmer's test to total eye stain. Left: for study eyes, the ratio of Schirmer's test to total eye stain at day 29 compared with the same ratio at baseline. Right: for both eyes together, the ratio at day 29 compared with baseline; Schirmer's test responders for both eyes indicated by *; a higher ratio at baseline was predictive of a better outcome at day 29 for both vehicle (Pearson product-moment correlation, 0.38, $P < 0.001$) and ST-100 (Pearson product-moment correlation, 0.39, $P < 0.001$).

ST-100 could present an effective treatment for DED, presumably through its demonstratable ability to heal the corneal epithelium, restore the nerve bed, and increase corneal sensitivity and tear film integrity.[13] These factors in concert could explain reduced corneal staining and improved Schirmer's scores with ST-100 topical treatment. That Schirmer's score improved significantly in study eyes compared with vehicle, whereas fluorescein staining did not could be attributed to ST-100's direct effect on the corneal nerve bed, leading to improved tear production.[3]

We investigated ST-100 in 2 concentrations, high dose (60 μg/ml) and low dose (22 μg/ml) for safety and efficacy in the treatment of the signs and symptoms of DED when administered twice daily for 28 days. The baseline disease characteristics and demographic characteristics of the ITT population such as age, sex, race, and ethnicity were balanced between treatment groups and representative of a broad DED population in this first-in-human phase 2 trial. Patients had to demonstrate both signs and symptoms and generally exhibited "moderate" to "severe" disease characteristics during their screening.[14]

ST-100 showed early, consistent, significant, and clinically meaningful improvements in several signs and symptoms of DED. Although both low- and high-dose ST-100 demonstrated statistically significant findings, the high dose was generally more efficacious. The primary efficacy sign end point was the total corneal fluorescein staining score measured as CFB at day 29. Although high-dose and

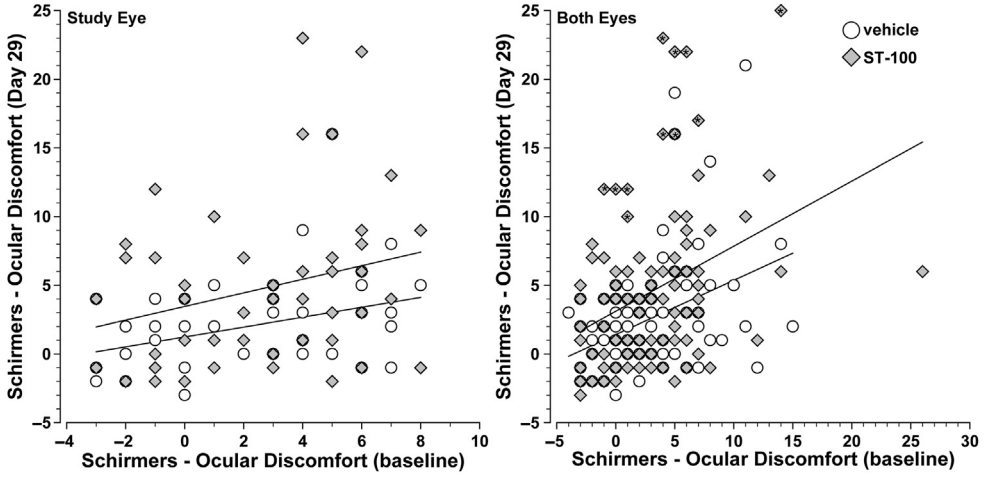

Figure 7. Schirmer's test score corrected by ocular discomfort. Left: for study eyes, Schirmer's test showed less ocular discomfort (Ora Calibra Ocular Discomfort Scale) at day 29 compared with the same difference at baseline. Right: for both eyes together, the same difference at day 29 compared with baseline; Schirmer's test responders for both eyes indicated by *; a difference at baseline was predictive of a better outcome at day 29 for both vehicles (Pearson product-moment correlation, 0.38, $P < 0.001$) and ST-100 (Pearson product-moment correlation, 0.37, $P < 0.001$).

Table 9. Ocular Discomfort (Ora Calibra Scale), During the CAE: Study Eyes

CFB to Visit and Time in CAE	High-Dose ST-100	Low-Dose ST-100	Vehicle
Day 8, 5–50 and 80 min tested: n	51	49	50
Minimum time to CFB significance (min)	**5**	40	–
Intervals of CFB significance (min)	5–50	80	–
Day 15, 0–45 min tested: n	48	48	49
Minimum time to CFB significance (min)	**0**	25	25
Intervals of CFB significance (min)	0–45	25–45	25–45
Day 29, 10–80 min tested: n	49	48	48
Minimum time to CFB significance (min)	**10**	35	35
Intervals of CFB significance (min)	0–70	35–45, 55, 65, 80	35–50,65–80

Bold values indicate statistical significance.
CFB = change from baseline in ocular discomfort measured during CAE compared with pre-CAE for the day indicated. Times indicate the minimal interval during the session in which this difference differed significantly from the same interval at baseline, with significance defined as $P \leq 0.05$ as calculated via paired t test.
CAE = Controlled Adverse Environment.

low-dose ST-100 study eyes demonstrated a trend of improvement (Fig 3), neither showed statistically significant superiority over vehicle study eyes. Even so, an ad hoc analysis of corneal and conjunctival fluorescein staining demonstrated that when study and fellow eyes are combined, the difference in mean CFB at day 29 for high-dose ST-100 total eye stain vs. vehicle was significant (Table 4, Fig 3). This was not due to some bias in ST-100 treatment between investigators, as the high-dose CFB did not differ between sites (Kruskal–Wallis 1-way analysis of variance on ranks, $P = 0.94$). Likewise, for total conjunctival staining (nasal and temporal combined), high-dose ST-100 exceeded vehicle in efficacy for study and fellow eyes combined, again with no difference between sites for high dose (Kruskal–Wallis 1-way analysis of variance on ranks, $P = 0.86$). Thus, ST-100 induced significant improvements in conjunctival fluorescein staining results, coupled with a noticeable improvement in corneal staining. These results are consistent with the known rapid healing and recovery of conjunctival epithelium after corneal injury, which often precedes that of corneal epithelium.[19]

As a prespecified efficacy sign end point, our study employed CFB at day 29 for unanesthetized Schirmer's test score. The high-dose ST-100 group demonstrated superiority in the study eye to the vehicle group both in improvement of mean Schirmer's score at day 29 and CFB (Table 7). Because Schirmer's was only measured at baseline and at the end of the study, there is some uncertainty as to when this beneficial and important effect of ST-100 treatment began. High-dose ST-100 also met the Schirmer's responder rate end point, which is defined as a significant difference in patients achieving a 10-mm increase or more in their unanesthetized Schirmer's test score (Table 7 , Fig 4). This is considered a clinically meaningful end point to demonstrate the efficacy of a candidate therapeutic in increasing tear production in DED when achieving superiority over vehicle.[16,20]

Typically, dry eye patients are less able to cope with environmental stresses and, when subjected to CAE, show a worsening of staining scores during exposure.[8,14,21] However, ST-100 subjects with improved Schirmer's test scores demonstrated other improvements as well (Table 8, Fig 5). High-dose ST-100 Schirmer's responders showed improvement in study eyes for corneal and conjunctival fluorescein staining during the CAE sessions. Responders exhibited strong correlation between improvement in Schirmer's CFB and a corresponding decrease in both total eye fluorescein stain and total corneal fluorescein stain. Furthermore, the ratio of Schirmer's score to total fluorescein stain, an index of overall improvement in ocular surface health, increased from baseline and at day 29 was significantly greater than vehicle (Fig 6). The therapeutic efficacy of ST-100 in this responder group shows that increased tear production leads to corneal tissue repair and recovery, characteristics consistent with our preclinical studies in mice.[6,7,13]

The coprimary efficacy symptom end point was CFB at day 29 in pre-CAE ocular discomfort. The study eye for high- and low-dose ST-100 treatment groups each demonstrated significant improvement from baseline (Table 6), with no difference between study sites (analysis of variance, $P = 0.64$), but did not show superiority over vehicle. Even so, ocular discomfort scores assessed by the 4-symptom questionnaire supported the superiority of high-dose ST-100 over vehicle in the improvement of overall ocular discomfort at day 15 and in other metrics. Schirmer's score scaled by subtracting ocular discomfort, showed improvements in tear production and comfort (Fig 7). This metric also showed significantly greater improvement for high-dose ST-100 at day 29 compared with vehicle, although baseline values did not differ. ST-100 also reduced ocular discomfort rapidly during CAE sessions measured in 5-minute intervals, measured as the difference from the same interval at baseline (Table 9). These results suggest that ST-100 provided resilience against ocular stress with increased comfort as the trial progressed.

Finally, ST-100 also demonstrated excellent tolerability. Comfort scores were low ($\leq$ 2.0 is considered "very comfortable") and equivalent or superior to both vehicle and well-known artificial tear products.[20] Overall, ST-100 demonstrated efficacy in both signs and symptoms of DED, with a fast onset of action within only 28 days of treatment and with a favorable comfort and safety profile

when used according to the study protocol. Taken together, these results confirm a therapeutic effect in subjects with DED, which represents an increasingly burdensome ocular disease.[21,22] ST-100 was safe compared with vehicle, as evidenced by a small number of TEAEs (Table S1), which were well balanced between treatment and vehicle groups and of only mild severity. These multiple improvement trends in efficacy sign and symptom end points suggest that an extended trial duration may yield additional significant results.

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Footnotes and Disclosures

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All authors have completed and submitted the ICMJE disclosures form.

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HUMAN SUBJECTS: Human subjects were included in this study. Informed consent was obtained before any study-related procedures (visit 1). The protocol, informed consent form, Health Insurance Portability and Accountability Act form, and other forms as well as subject diary instructions were reviewed by the institutional review board (Alpha institutional review board, San Clemente, CA). The study was performed in accordance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization, Good Clinical Practice, and in accordance with all applicable local, state, and federal requirements relevant to the use of investigational medicinal products.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Baratta, Schlumpf, Del Buono, DeLorey, Ousler, Calkins

Data collection: Ousler

Analysis and interpretation: Baratta, Schlumpf, Del Buono, Ousler, Calkins

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Abbreviations and Acronyms:
AEs = adverse events; **ANCOVA** = analysis of covariance; **CAE** = Controlled Adverse Environment; **CFB** = change from baseline; **DED** = dry eye disease; **ECM** = extracellular matrix; **ITT** = intent-to-treat; **LS** = least squares; **TEAEs** = treatment-emergent adverse events.

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