AMERICAN ACADEMY OF OPHTHALMOLOGY®

Lotilaner Ophthalmic Solution 0.25% for *Demodex* Blepharitis

Randomized, Vehicle-Controlled, Multicenter, Phase 3 Trial (Saturn-2)

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Purpose: To evaluate the safety and efficacy of lotilaner ophthalmic solution 0.25% compared with vehicle for the treatment of *Demodex* blepharitis.

Design: Prospective, randomized, double-masked, vehicle-controlled, multicenter, phase 3 clinical trial.

Participants: Four hundred twelve patients with *Demodex* blepharitis were assigned randomly in a 1:1 ratio to receive either lotilaner ophthalmic solution 0.25% (study group) or vehicle without lotilaner (control group).

Methods: Patients with *Demodex* blepharitis treated at 21 United States clinical sites were assigned either to the study group (n = 203) to receive lotilaner ophthalmic solution 0.25% or to the control group (n = 209) to receive vehicle without lotilaner bilaterally twice daily for 6 weeks. Collarettes and erythema were graded for each eyelid at screening and at all visits after baseline. At screening and on days 15, 22, and 43, 4 or more eyelashes were epilated from each eye, and the number of *Demodex* mites present on the lashes was counted with a microscope. Mite density was calculated as the number of mites per lash.

Main Outcome Measures: Outcome measures included collarette cure (collarette grade 0), clinically meaningful collarette reduction to 10 collarettes or fewer (grade 0 or 1), mite eradication (0 mites/lash), erythema cure (grade 0), composite cure (grade 0 for collarettes as well as erythema), compliance with the drop regimen, drop comfort, and adverse events.

Results: At day 43, the study group achieved a statistically significant ($P < 0.0001$) higher proportion of patients with collarette cure (56.0% vs. 12.5%), clinically meaningful collarette reduction to 10 collarettes or fewer (89.1% vs. 33.0%), mite eradication (51.8% vs. 14.6%), erythema cure (31.1% vs. 9.0%), and composite cure (19.2% vs. 4.0%) than the control group. High compliance with the drop regimen (mean ± standard deviation, 98.7 ± 5.3%) in the study group was observed, and 90.7% of patients found the drops to be neutral to very comfortable.

Conclusions: Twice-daily treatment with lotilaner ophthalmic solution 0.25% for 6 weeks generally was safe and well tolerated and met the primary end point and all secondary end points for the treatment of *Demodex* blepharitis compared with vehicle control.

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Blepharitis, a chronic inflammation of the eyelid margin, can have varying causes, with the most common being an infestation of *Demodex* mites in the eyelid tissue found at the base of the eyelashes and in the meibomian glands.[1,2] Two recent studies found that 55% to 58% of all eye care patients and 62% to 69% of those with a blepharitis diagnosis, or about 25 million United States adults, have *Demodex* blepharitis.[3,4]

Demodex blepharitis is caused by mites that infest the eyelash follicles, where they cause damage via mechanical, chemical, and bacterial mechanisms.[5,6] Mite infestation contributes to the formation of cylindrical, sleeve-like

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coatings at the base of the eyelashes. In continuity with previous phase 2 and 2b/3 publications, the term *collarette* is used herein to describe these sleeve-like coatings.[7–11] These collarettes, composed of partially digested epithelial cells, mite waste, and eggs, are considered to be the pathognomonic sign of *Demodex* blepharitis.[6,12–15] Collarettes can be identified easily by an eye care provider by focusing the slit lamp on the upper lid margin and instructing patients to look down so that there is a clear view of the base of the upper lashes. Additional clinical manifestations of *Demodex* blepharitis include lid margin redness, lid edema, keratitis, eyelash misalignment or loss, meibomian gland dysfunction, and tear film disruptions.[14] Patients typically experience itching of the eyelid, feelings of dryness, blurred vision, and ocular irritation. Eighty percent of patients report a negative impact on daily life.[16] In the more advanced stages of *Demodex* blepharitis, patients may experience recurrent chalazia or pterygia, peripheral corneal vascularization, or corneal opacity.[17]

Current management includes lid scrubs, warm compresses, shampoos or lid hygiene products, off-label topical antibiotics or steroids, or in-office mechanical debridement of the lid margin.[18] Although varying degrees of temporary symptomatic improvement may be seen with the aforementioned approaches, they have not been demonstrated actually to eradicate the mites and thereby cure the disease. Currently, no therapies for *Demodex* blepharitis have been approved by the Food and Drug Administration.

Lotilaner is an antiparasitic agent that induces spastic paralysis and death in *Demodex* mites by selectively inhibiting parasite-specific γ-aminobutyric acid chloride channels, but not human γ-aminobutyric acid chloride channels.[19] After an extensive phase 2 and 2b/3 clinical trial program that tested the use of lotilaner in humans,[7–9,20,21] the current pivotal phase 3 clinical trial of TP-03 (lotilaner ophthalmic solution 0.25%; Tarsus Pharmaceuticals) was conducted for the treatment of patients with *Demodex* blepharitis.

Methods

This prospective, randomized, double-masked, vehicle-controlled phase 3 trial was conducted at 21 United States clinical sites (Appendix 1, available at www.aaojournal.org) from April 2021 through March 2022. (ClinicalTrials.gov identifier, NCT04784091). The study adhered to the tenets of the Declaration of Helsinki and was approved by Alpha IRB (San Clemente, CA). All enrolled patients provided written informed consent using the institutional review board–approved informed consent forms.

Eligibility criteria for participating in the study included age of 18 years or older, willingness to sign the informed consent form and comply with all study procedures, and availability for the duration of the study. Patients with blepharitis were eligible for recruitment into the study if they had all of the following signs present in the same eye: (1) more than 10 lashes with collarettes present on the upper lid (collarette scale grade 2 or worse), (2) at least mild erythema of the upper eyelid margin, and (3) mite density of 1.5 mites per lash or more (upper and lower eyelids combined). In addition, patients were required to have a corrected distance visual acuity of 0.7 logarithm of the minimum angle of resolution or better as assessed by the Early Treatment Diabetic Retinopathy Study chart in each eye at screening.

Patients were excluded if they had used any prescription antibacterial, antiparasitic, or anti-inflammatory steroid treatment within 14 days of screening or had used topical tea tree oil, hypochlorous acid, or any other lid hygiene products within the previous 14 days or were unwilling to forego the use of these products during the study. Patients also were excluded if they had used a topical prostaglandin analog (PGA) to promote eyelash growth within the last 30 days, had initiated PGA treatment for medical reasons within the past 30 days, or planned to change or discontinue PGA treatment during the study. Patients also were excluded if they had used artificial eyelashes or eyelash extensions or had other cosmetic eyelash or eyelid procedures (e.g., eyeliner tattooing, eyelash tinting, eyelash curling perm) within the previous 7 days or were unwilling to forego the use of these products during the study. Patients with lid structural abnormalities, acute ocular infection, active ocular inflammation other than blepharitis, severe dry eye in the opinion of the investigator, or hypersensitivity to lotilaner or any of the formulation components were excluded, as were those who had undergone ocular surgery within 3 months of screening; those who had unstable or uncontrolled cardiac, pulmonary, renal, oncologic, neurologic, metabolic, or other systemic conditions; or those who were pregnant or lactating.

Of note, patients who participated in the Saturn-1 clinical trial (ClinicalTrials.gov identifier, NCT04475432) were not allowed to participate in the Saturn-2 clinical trial. Using a computer-generated blocked randomization schedule, eligible patients were assigned randomly in a 1:1 ratio to receive bilaterally either the TP-03 study medication (lotilaner ophthalmic solution 0.25%; study group) or the vehicle formulation without lotilaner (control group). An interactive response technology system was used for the study and assigned the treatment based on the randomization.

The study drugs (TP-03 and vehicle) were indistinguishable based on appearance, consistency, and packaging (bottle size, shape, color, labeling, etc.). On day 1 (baseline visit), the first dose of study medication or vehicle was administered in the clinic. Subsequent doses were applied by the patients at home, 1 drop in each eye twice daily (morning and evening) for 6 weeks. Because the *Demodex* life cycle, from egg through adult stage, has been estimated to be approximately 3 weeks, the 6-week treatment duration was intended to provide acaricidal dosing across 2 full life cycles of *Demodex*.[5,22,23]

The patients were evaluated on days 8, 15, 22, and 43. All patients, investigators, and site personnel performing study assessments were masked to treatment allocation throughout the study. Patients were instructed to refrain from using any cosmetics (e.g., eye shadow, eyeliner, mascara, foundation, etc.) in the vicinity of the lid margin on the day of a study visit; any prescription antibacterial, antiparasitic, or anti-inflammatory steroid treatment, unless medically indicated; topical tea tree oil or hypochlorous acid treatment; lid hygiene products such as lid scrubs; artificial eyelashes, eyelash extensions, or other cosmetic eyelash or eyelid procedures (e.g., eyeliner tattooing, eyelash tinting, eyelash curling perm, etc.); or products used to promote eyelash growth (e.g., PGAs) during the study.

The patients were instructed how to administer the drops properly and to record the instillation of drops each day in a diary. Administration compliance was verified through an in-office review of the daily diary at each study visit. Noncompliance or overcompliance with the drug administration schedule was defined as having less than 80% or more than 125% of the expected number of drug administrations.

Collarettes and erythema were graded for each eyelid at screening and at all visits after baseline. The scale used to assign the collarette score ranged from 0 to 4 and that of erythema ranged from 0 to 3 in whole-unit increments (Tables S1 and S2, available at www.aaojournal.org). *Demodex* mites were counted at screening and on days 15, 22, and 43. Counting of *Demodex* mites was performed using a slit-lamp biomicroscope to select 2 or more lashes from each of the upper and lower eyelids of each eye (i.e., 1 lash from each half of each eyelid). Lashes with visible collarettes, if present, were targeted for epilation. Using fine forceps, the targeted lashes were twirled based on the Mastrota technique,[24] in which the eyelash is rotated like a propeller around its own axis with gentle tensioning, and then extracted. The lashes from each lid were placed in a drop of an artificial tear with an emulsifier (Refresh Optive Advanced or Refresh Optive Mega 3; Allergan) on separate glass slides. The epilated lashes were examined under a microscope. The number of *Demodex* mites observed and the number of lashes epilated were recorded, and mite density was calculated as the number of mites per lash. Mite eradication was defined as a mite density of 0 mites/lash.

One eye of each patient was chosen as the analysis eye. If both eyes met the inclusion criteria, the eye with the higher mite density at the screening visit was considered the analysis eye; if both eyes had equal mite density, the right eye was the analysis eye.

The primary efficacy end point was the proportion of patients with collarette cure, based on a collarette grade of 0 ($\leq$ 2 lashes with collarettes) of the upper eyelid of the analysis eye on day 43. The secondary efficacy end points were the percentage of patients with mite eradication (0 mites/lash for the analysis eye) on day 43 and composite cure, a combination of collarette and erythema grades (grade 0 for both collarettes and erythema on day 43 for the upper eyelid of the analysis eye). Additionally, the proportion of patients with erythema cure (erythema score of 0 for the upper eyelid of the analysis eye on day 43) was included as a secondary end point after the trial commencement but before the trial was completed and data were unmasked.

Safety parameters included assessment of adverse events (AEs); changes in corrected distance visual acuity; intraocular pressure; endothelial cell assessments; fluorescein corneal staining; slit-lamp biomicroscopy findings; and hematologic, blood chemistry, and urinalysis findings. The collection of AEs began after the patient's first instillation of study drug and continued until 7 days (for nonserious AEs) or 30 days (for serious AEs) after the patient's last study visit. In a subset of patients, AEs were recorded up to day 57. Adverse events were classified by the study investigator as not related, potentially related, or definitely related to the study treatment. Potentially related or definitely related AEs were considered treatment-related AEs. Corrected distance visual acuity was assessed at all visits using either the patient's own spectacles or a pinhole occluder, with an Early Treatment Diabetic Retinopathy Study visual acuity chart at a distance of 4 m. The number of letters read correctly was used to compute the patient's logarithm of the minimum angle of resolution corrected distance visual acuity. A change of more than 2 lines on the Early Treatment Diabetic Retinopathy Study chart ($\geq$ 0.22 logarithm of the minimum angle of resolution) was considered clinically meaningful. Bilateral intraocular pressure was assessed at baseline and on days 1 and 43 using Goldmann or Perkins applanation tonometry. At selected study centers, noncontact specular microscopy was conducted on days 1 and 43 to determine the central corneal endothelial cell density. Bilateral slit-lamp biomicroscopy was performed at every study visit, with the results of each assessed parameter being reported as normal, abnormal not clinically significant, or abnormal clinically significant. Bilateral corneal fluorescein staining was performed at all visits after screening using 1.0-mg sodium fluorescein strips. Staining in each of the 5 areas of the cornea was graded based on the National Eye Institute scale.[25] At selected study centers, blood and urine were collected at baseline, before study drug administration, for standard hematologic, blood chemistry, and urine analyses by a central laboratory and were collected again at day 43. Drop comfort was assessed at all visits through day 43. Patients rated the comfort of the study medications as very comfortable, slightly comfortable, neither comfortable nor uncomfortable, slightly uncomfortable, or very uncomfortable.

Sample size calculations were based on the response rates achieved in previous clinical studies of TP-03 for the treatment of *Demodex* blepharitis.[10] A sample size of 300 patients (150 in each arm) provided 99% power to establish the superiority of TP-03 to vehicle in patients meeting the primary and the secondary efficacy end points using a Pearson chi-square test with a 1-sided significance level of 0.025. Given that the coronavirus disease 2019 pandemic was active at the time of planning the study, a 30% discontinuation rate was used to calculate the sample size. Accordingly, approximately 209 participants per study drug group (approximately 418 participants total) were planned to be randomized.

All statistical analyses were performed using SAS software (SAS Institute, Inc.) version 9.4 or higher. Descriptive statistics were used to provide an overview of the efficacy and safety results. Categorical variables were summarized by frequency counts and percentages for each category, whereas continuous and ordinal variables were summarized using the mean, standard deviation, median, minimum, and maximum values for the data collected at each applicable visit. A 2-sample t test or its nonparametric counterpart, the Wilcoxon rank-sum test, was used as appropriate to assess the statistical significance of the difference between treatment groups in the mean comparisons. The comparisons between the proportions were made using a difference in proportions test. Where inferential testing was conducted, the statistical tests were 1-sided with an α level of 0.025. When comparing the means between the two groups and the change from baseline, the statistical tests were 2-sided at 95% confidence.

Results

A total of 412 patients were enrolled in the study, including 203 in the study group and 209 in the control group. Eighteen patients (9 in the study group and 9 in the control group) discontinued the study before the day 43 visit, and 1 patient missed the day 43 visit; thus, 393 patients (193 in the study group and 200 in the control group) completed the 6-week treatment interval with the study drug or vehicle control (Fig S1, available at www.aaojournal.org). The demographic and baseline characteristics of all patients are shown in Table 3. At baseline, the mean collarette score, mite density, and erythema score were similar in both groups.

Collarette Cure and Collarette Grade

The proportion of patients in each group who achieved collarette cure (collarette grade of 0 in the upper eyelid of the analysis eye) on day 43 was 56.0% in the study group versus 12.5% in the control group ($P < 0.0001$; Fig 2A). Figure 2B shows collarette grade 4 in an eye at baseline, which improved to grade 0 on day 43 after treatment.

On day 43, 89.1% of patients in the study group achieved clinically meaningful collarette reduction to 10 collarettes or fewer (defined as a collarette grade of 0 or 1 in the upper eyelid of the analysis eye) compared with 33.0% in the control group

Table 3. Demographic and Baseline Characteristics

	TP-03 (n = 203)	Vehicle (n = 209)
Age (yrs)		
Mean ± SD	63.9 ± 15.15	65.1 ± 13.35
< 65	84 (41.4)	80 (38.3)
≥ 65	119 (58.6)	129 (61.7)
Sex		
Male	106 (52.2)	106 (50.7)
Female	97 (47.8)	103 (49.3)
Childbearing potential*		
Yes	10 (10.3)	8 (7.8)
No	87 (89.7)	95 (92.2)
Ethnicity		
Hispanic or Latino	17 (8.4)	17 (8.1)
Not Hispanic or Latino	186 (91.6)	192 (91.9)
Race		
American Indian or Alaska Native	1 (0.5)	1 (0.5)
Asian	3 (1.5)	3 (1.4)
Black or African American	20 (9.9)	15 (7.2)
Native Hawaiian or other Pacific Islander	2 (1.0)	0
White	176 (86.7)	187 (89.5)
Other	0	3 (1.4)
Multiple race	1 (0.5)	0
Collarette score	2.9 ± 0.77	3.0 ± 0.80
Collarette grade		
0	0	0
1	0	0
2	72 (35.5)	64 (30.6)
3	80 (39.4)	77 (36.8)
4	51 (25.1)	68 (32.5)
Mite density	3.16 ± 1.42	3.33 ± 1.71
Erythema score	1.6 ± 0.64	1.6 ± 0.63

SD = standard deviation.
Data are presented as no. (%) or mean ± SD.
*Percentages based on the total number of female participants.

($P < 0.0001$; Fig 3A). Figure 3B shows collarette grade 4 in an eye at baseline, which improved to grade 1 on day 43 after treatment.

Table S4 (available at www.aaojournal.org) summarizes the mean change in collarette grade from baseline for the upper eyelid of the analysis eyes at each follow-up visit. The difference in the collarette grade between the study and control groups was statistically significant ($P < 0.05$) as early as day 8 and highly significant ($P < 0.0001$) from day 15 onward. On day 43, 96.4% of patients in the study group versus 66.5% of patients in the control group demonstrated at least a 1-grade collarette improvement in the upper eyelid of the analysis eye ($P < 0.0001$; Fig S4, available at www.aaojournal.org).

Mite Eradication and Density

The proportion of patients in each group who achieved mite eradication (mite density of 0 mites/lash in the analysis eye) is shown in Figure 5A. The proportion of study group patients achieving mite eradication was statistically significantly higher than in the control group at all visits at which mite density was measured (day 15 onward; $P < 0.0001$). On day 43, 51.8% of the patients in the study group achieved mite eradication, compared with 14.6% of patients in the control group ($P < 0.0001$).

Table S5 (available at www.aaojournal.org) presents the change in mean mite density from baseline for the analysis eye at each follow-up visit. The study group demonstrated a statistically significantly lower ($P < 0.0001$) mite density compared with the control group from day 15 onward. On day 43, 86.5% of the eyes in the study group showed a mean mite density of 0.5 mites/lash or less, compared with 34.7% in the control group ($P < 0.0001$; Fig 5B).

Erythema Cure

The proportion of patients who achieved an erythema cure (grade 0 erythema) on day 43 was significantly higher in the study group compared with the control group (31.1% vs. 9.0%; $P < 0.0001$; Fig 6A). Figure 6B shows erythema of the eyelid margin in a patient (grade 2) at baseline and no erythema (grade 0) after treatment on day 43. Table S6 (available at www.aaojournal.org) presents the change in mean erythema score from baseline for the analysis eye at each follow-up visit.

Composite Cure

The proportion of patients who achieved a composite cure (grade 0 collarette and grade 0 erythema) on day 43 was significantly higher in the study group compared with the control group (19.2% vs. 4.0%; $P < 0.0001$; Fig 7). The primary (collarette cure) and secondary (mite eradication, erythema cure, and composite cure) efficacy end points with imputed missing data are described in Table S7 (available at www.aaojournal.org). Even after imputing missing data, the two groups showed a statistically significant difference in the percentage of patients achieving a cure on day 43.

Adverse Events

The proportion of patients with at least 1 ocular treatment-emergent AE that was considered to be study drug related was 19.2% (39/203) in the study group and 12.4% (26/209) in the control group. The most common study drug-related ocular treatment-emergent AE in the study group was instillation site pain (7.9% in the study group and 6.7% in the control group). Other study drug-related ocular treatment-emergent AEs, with 1.0% or more incidence in either the study or control group, included dry eye (1.5% vs. 0.5%), visual acuity reduced (0.5% vs. 1.4%), chalazion (1.0% vs. 0%), conjunctival hyperemia (1.0% vs. 0%), eyelid pruritus (1.0% vs. 0%), photophobia (1.0% vs. 0%), visual impairment (0.0% vs. 1.0%), instillation site irritation (1.0% vs. 0%), and vital dye staining of the cornea present (1.0% vs. 0%; Table S8, available at www.aaojournal.org). None of the study drug-related ocular events were considered serious.

Additional Safety Outcomes

No clinically significant adverse effects occurred in the study drug or the vehicle control groups for multiple safety measures, including intraocular pressure, endothelial cell density, and corneal staining (Table S9, available at www.aaojournal.org). No clinically relevant changes occurred from baseline in median values for systemic clinical laboratory values (hematologic analysis, clinical chemistry analysis, and urinalysis) in the study or control groups on day 43.

Drop Comfort

Figure 8 demonstrates the proportion of patients in the study and control groups who rated the drop as neutral to very comfortable on days 1 (baseline visit), 8, 15, 22, and 43. On day 43, 90.7% of patients in the study group compared with 88.5% of patients in the control group found the drops to be neutral to very comfortable.

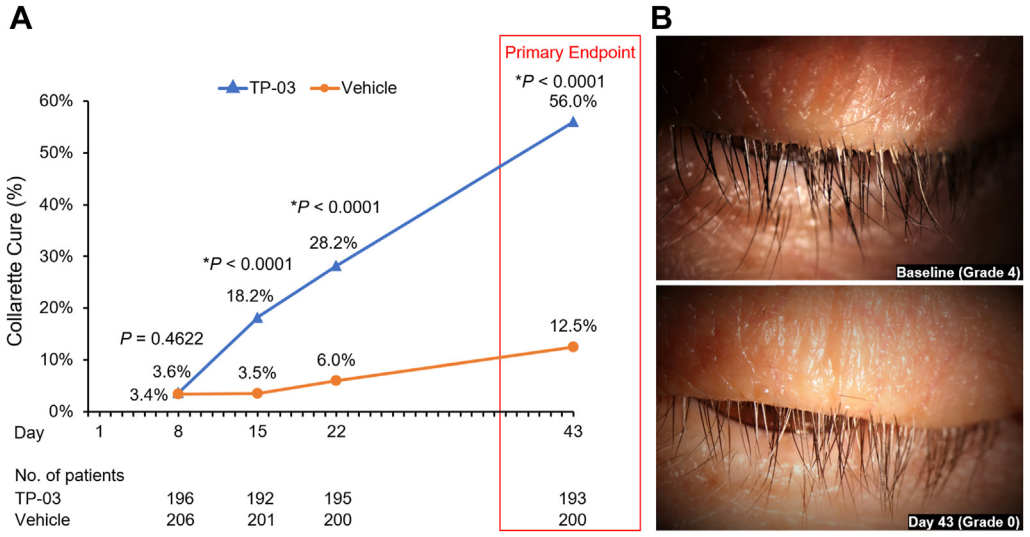

Figure 2. **A**, Graph showing the proportion of patients with collarette cure (grade 0 [≤ 2 lashes with collarettes]) in the upper eyelid of the analysis eye in the study and control groups (observed data). **B**, Photographs of an eye before and after treatment showing collarette improvement from grade 4 at baseline to grade 0 on day 43.

Compliance

Overall, the mean dosing compliance was 98.6% ± 5.4% (98.7% ± 5.3% in the study group) (Table S10, available at www.aaojournal.org). All but 3 patients across both groups met the definition of compliance (i.e., used at least 80% and not more than 125% of the expected number of assigned study drug administrations).

Discussion

In this prospective, randomized, vehicle-controlled trial, lotilaner ophthalmic solution 0.25% (TP-03) was statistically superior to vehicle in the treatment of *Demodex* blepharitis. Six weeks of twice-daily treatment with TP-03 resulted in collarette cure (0–2 lashes with collarettes on the upper eyelid of the analysis eye) in 56% of patients and clinically meaningful collarette reduction to 10 collarettes or fewer on the upper eyelid of the analysis eye in 89% of patients. All prespecified primary and secondary end points in the study were met, with statistically significant differences ($P < 0.0001$) between TP-03 and vehicle at all end points.

The severity of collarettes is associated with more severe infestation by *Demodex* mites.[1,12,26,27] Patients in this study showed an average baseline collarette severity of grade 3,

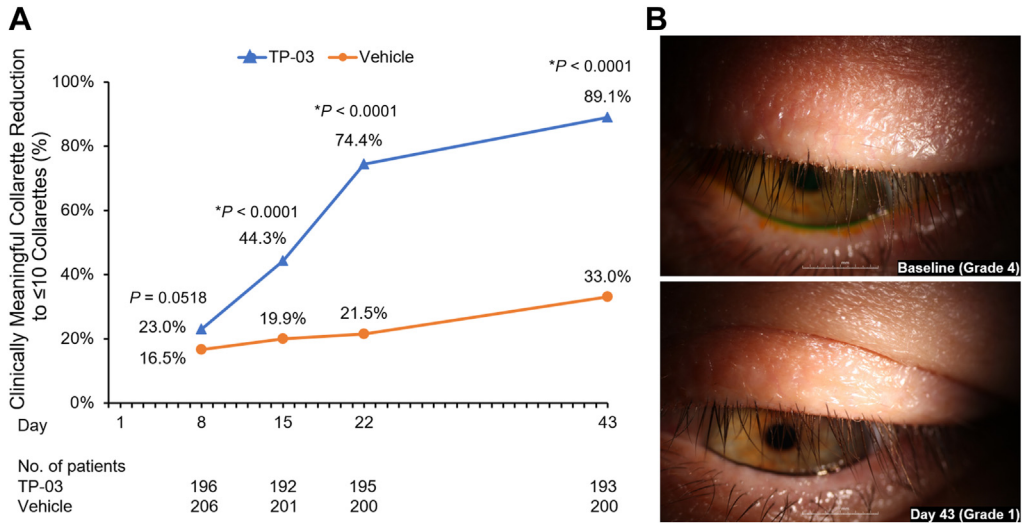

Figure 3. **A**, Graph showing the proportion of patients with clinically meaningful collarette reduction to 10 collarettes or fewer (grade 0–1) in the upper eyelid of the analysis eye in the study and control groups (observed data). **B**, Photographs of an eye before and after treatment showing collarette improvement from grade 4 at baseline to grade 1 on day 43.

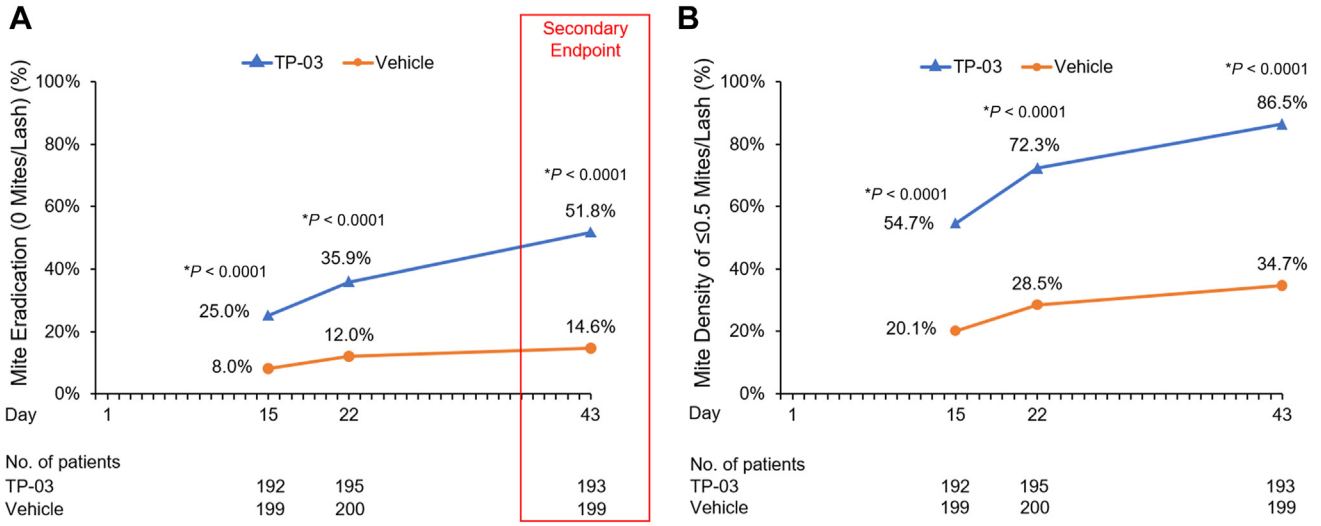

Figure 5. Graphs showing the proportion of patients (**A**) with mite eradication (mite density of 0 mites/lash) in the analysis eye in the study and control groups (observed data) and (**B**) achieving mite density of 0.5 mites/lash or less.

representing approximately 100 lashes with collarettes per lid or between one third and two thirds of all the lashes on the upper lid having collarettes. By study conclusion at week 6, the mean collarette score was 0.6, with nearly all (96.4%) of those treated with TP-03 achieving an improvement in collarettes of at least 1 grade. Significant and clinically meaningful improvements began as early as day 8 (Table S4, available at www.aaojournal.org).

Although counting mites from epilated lashes would be time-consuming and uncomfortable for patients in routine clinical practice, it long has been considered standard practice for confirming *Demodex* blepharitis in clinical trials.[12] In the present study, a set of trained mite counters performed mite counts across all sites to help ensure consistency. At baseline, mean mite density in the TP-03 group was 3.2 mites/lash, corresponding to potentially hundreds of mites infesting the upper lid. After treatment for 6 weeks, 86.5% of patients in the TP-03 group showed 0.5 mite per lash or fewer. For complete mite eradication (0 mites), a statistically significant difference ($P < 0.0001$) between the two groups was observed as early as week 2, and more than half of the patients treated with TP-03 achieved complete mite eradication by week 6. The eradication of mites closely followed the resolution of collarettes, thus confirming within this study the causative nature of *Demodex* infestation for this important sign. Owing to the close association between mite density and collarettes, slit-lamp examination of the upper eyelid margin to identify collarettes is considered an easy and viable alternative for identifying mite infestation and monitoring response to the

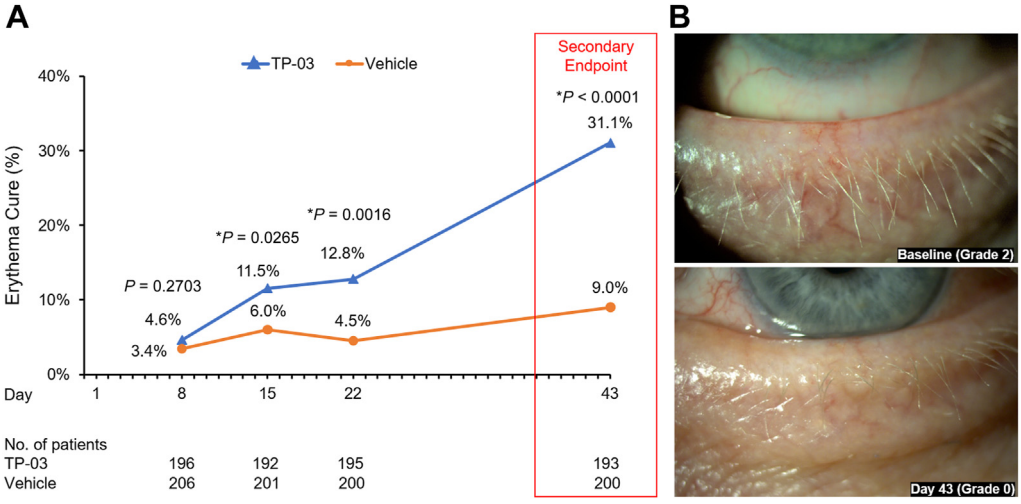

Figure 6. **A**, Graph showing the proportion of patients with erythema cure (grade 0) in the study and control groups (observed data). **B**, Photographs of an eye before and after treatment showing improvement in erythema of the eyelid margin from grade 2 at baseline to grade 0 on day 43.

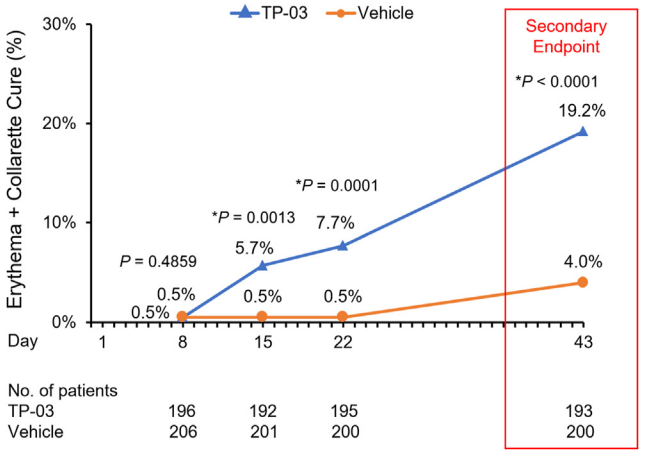

Figure 7. Graph showing the proportion of patients with composite cure (collarette cure plus erythema cure) in the study and control groups (observed data).

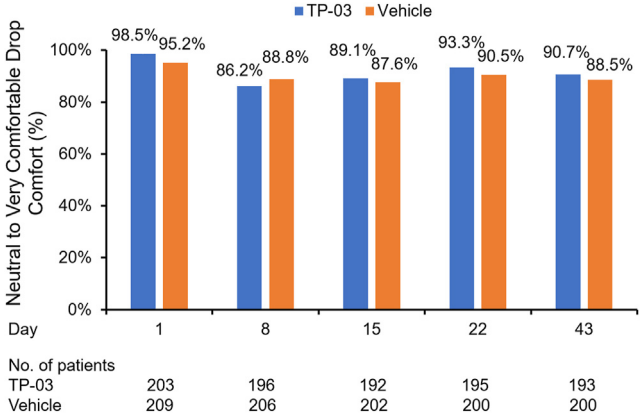

Figure 8. Bar graph showing the proportion of patients in the study and control groups who rated the drop as neutral to very comfortable.

treatment in routine clinical practice.[2,28] Patients were not allowed to perform any mechanical lid scrubbing or to use any other lid therapies during the study, suggesting that TP-03 itself, rather than any mechanical cleaning of the lid margin, was responsible for eradicating the mites.

Resolution of lid erythema lagged slightly behind the eradication of mites and elimination of collarettes in this study. Although statistically significant differences between the study and control groups were seen by week 2, the rate of erythema cure seemed to peak at later time points compared with collarette and mite eradication. We hypothesize that it may take time for inflammation to resolve after mites and collarettes have been eradicated or resolved. Nearly one third (31.1%) of patients treated with TP-03 achieved complete resolution of lid erythema by the conclusion of the study.

TP-03 generally was well tolerated, with a safety profile similar to that of its vehicle, among adults with *Demodex* blepharitis who used the study drug twice daily for up to 43 days. Drop comfort on instillation can affect patient compliance and therefore efficacy of treatment.[29] In the present study, compliance with the drop regimen was high (mean, 98.6 ± 5.4%), which in part may have been the result of good tolerability of the drops. More than 90% of patients reported the drop to be neutral to very comfortable.

This study confirmed the results seen in earlier phase 2 and 2b/3 studies of TP-03 demonstrating the validity and repeatability of the treatment results.[7–10,21] TP-03 is a first-in-class antiparasitic treatment for *Demodex* blepharitis, a lid margin disease that, despite being highly prevalent with a significant psychosocial impact on patients, currently has no approved treatments.[4,16]

One limitation of this study and the previous phase 2b/3 study relates to the long-term follow-up of recurrence,[21] given that *Demodex* mites may reinfest the eyelids from their residence in other areas of the face. To address this, a long-term rollover study is in progress. The collarette and erythema grading scales used in the present study also have been used previously in phase 2 studies and a phase 2b/3 study; however, these scales have not been evaluated for interrater and intrarater reliability. This also can be considered a potential limitation of the present study.

The results of this pivotal Phase 3 study demonstrate that lotilaner ophthalmic solution 0.25% was superior to vehicle when dosed twice daily for 6 weeks for the treatment of *Demodex* blepharitis.

Footnotes and Disclosures

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*A list of members of the Saturn 2 study group are available in the Appendix (www.aaojournal.org).

HUMAN SUBJECTS: Human subjects were included in this study. The study was approved by Alpha IRB, San Clemente, California. All research adhered to the tenets of the Declaration of Helsinki. All enrolled patients provided written informed consent using the IRB-approved informed consent forms.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Gaddie, Donnenfeld, Karpecki, Baba, Holdbrook, Yeu

Analysis and interpretation: Gaddie, Donnenfeld, Karpecki, Vollmer, Berdy, Peterson, Simmons, Edell, Whitson, Ciolino, Baba, Holdbrook, Trevejo, Meyer, Yeu

Data collection: Vollmer, Berdy, Peterson, Simmons, Edell, Whitson, Meyer

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Overall responsibility: Gaddie, Donnenfeld, Karpecki, Vollmer, Berdy, Peterson, Simmons, Edell, Whitson, Ciolino, Baba, Holdbrook, Trevejo, Meyer, Yeu

Abbreviations and Acronyms:

AE = adverse event; **PGA** = prostaglandin analog.

Keywords:

Blepharitis, *Demodex* blepharitis, Lotilaner ophthalmic solution 0.25%, Lotilaner phase 3 clinical trial, Treatment of *Demodex* blepharitis.

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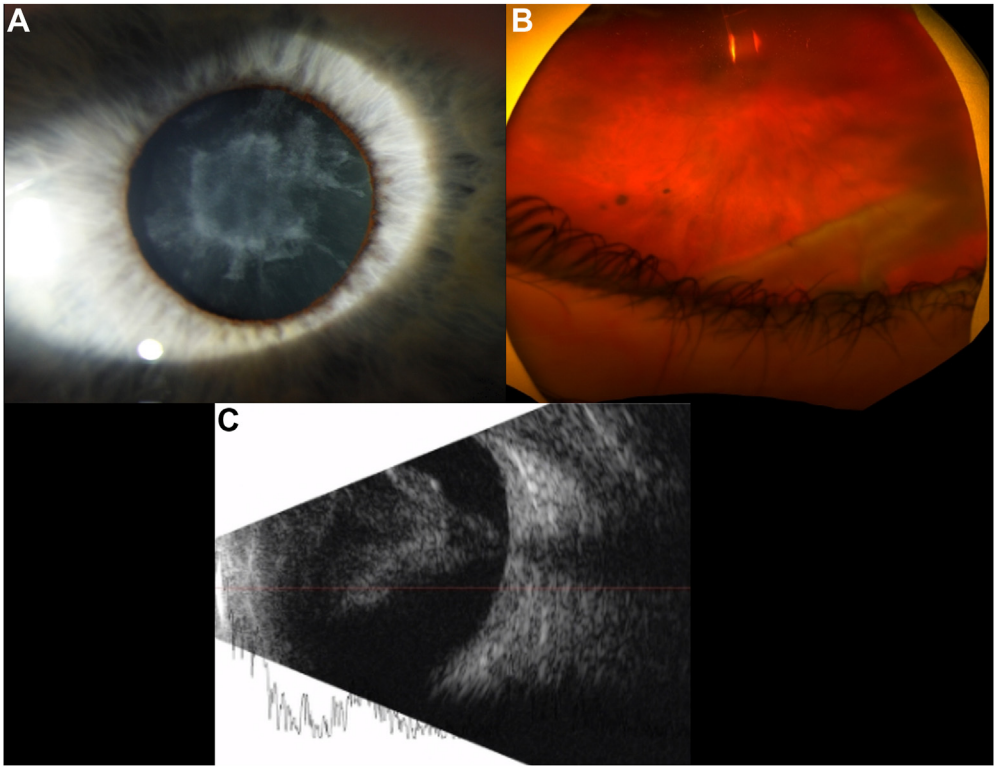
References

1. Cheng AM, Hwang J, Dermer H, Galor A. Prevalence of ocular demodicosis in an older population and its association with symptoms and signs of dry eye. *Cornea*. 2021;40:995−1001.
2. Zhang AC, Muntz A, Wang MTM, et al. Ocular Demodex: a systematic review of the clinical literature. *Ophthalmic Physiol Opt*. 2020;40:389−432.
3. Teo A, Rosenberg E, Jacobson A. Prevalence of Demodex colonization in patients presenting to an outpatient clinic. *Invest Ophthalmol Vis Sci*. 2021;62:1236−1236.
4. Trattler W, Karpecki P, Rapoport Y, et al. The prevalence of Demodex blepharitis in US eye care clinic patients as determined by collarettes: a pathognomonic sign. *Clin Ophthalmol*. 2022;16:1153−1164.
5. Liu J, Sheha H, Tseng SC. Pathogenic role of Demodex mites in blepharitis. *Curr Opin Allergy Clin Immunol*. 2010;10:505−510.
6. Luo X, Li J, Chen C, Tseng S, Liang L. Ocular demodicosis as a potential cause of ocular surface inflammation. *Cornea*. 2017;36(Suppl 1):S9−S14.
7. Gonzalez-Salinas R, Yeu E, Holdbrook M, et al. Safety and efficacy of topical lotilaner ophthalmic solution 0.25% for the treatment of Demodex blepharitis: a pilot study. *J Ophthalmol*. 2021 Sep 21;2021:3862684. doi: 10.1155/2021/3862684. eCollection.
8. Gonzalez-Salinas R, Karpecki P, Yeu E, et al. Safety and efficacy of lotilaner ophthalmic solution, 0.25% for the treatment of blepharitis due to Demodex infestation: a randomized, controlled, double-masked clinical trial. *Cont Lens Anterior Eye*. 2022;45:101492.
9. Gonzalez-Salinas R, Yeu E, Holdbrook M, et al. Collarette elimination and Demodex mite eradication with topical lotilaner ophthalmic solution, 0.25. *J Ocul Pharmacol Ther*. 2021;37:479−484.
10. Yeu E, Holdbrook M, Baba SN, et al. Treatment of Demodex blepharitis: a prospective, randomized, controlled, double-masked clinical trial comparing topical lotilaner ophthalmic solution, 0.25% eyedrops to vehicle. *Ocul Immunol Inflamm*. 2022 Aug 1;1-9. doi: 10.1080/09273948.2022.2093755. Online ahead of print.
11. Yeu E, Wirta DL, Karpecki P, et al. Lotilaner ophthalmic solution, 0.25%, for the treatment of Demodex blepharitis: results of a prospective, randomized, vehicle-controlled, double-masked, pivotal trial (Saturn-1). *Cornea*. 2023;42:435−443.
12. Gao YY, Di Pascuale MA, Li W, et al. High prevalence of Demodex in eyelashes with cylindrical dandruff. *Invest Ophthalmol Vis Sci*. 2005;46:3089−3094.
13. Messaoud R, El Fekih L, Mahmoud A, et al. Improvement in ocular symptoms and signs in patients with Demodex anterior blepharitis using a novel terpinen-4-ol (2.5%) and hyaluronic acid (0.2%) cleansing wipe. *Clin Ophthalmol*. 2019;13:1043−1054.
14. Rabensteiner DF, Aminfar H, Boldin I, et al. Demodex mite infestation and its associations with tear film and ocular surface parameters in patients with ocular discomfort. *Am J Ophthalmol*. 2019;204:7−12.
15. Savla K, Le JT, Pucker AD. Tea tree oil for Demodex blepharitis. *Cochrane Database Syst Rev*. 2020;6:CD013333.
16. O'Dell L, Dierker DS, Devries DK, et al. Psychosocial impact of Demodex blepharitis. *Clin Ophthalmol*. 2022;16:2979−2987.
17. Zhang XB, Ding YH, He W. The association between Demodex infestation and ocular surface manifestations in meibomian gland dysfunction. *Int J Ophthalmol*. 2018;11:589−592.
18. Fromstein SR, Harthan JS, Patel J, Opitz DL. Demodex blepharitis: clinical perspectives. *Clin Optom (Auckl)*. 2018;10:57−63.
19. Ozoe Y, Asahi M, Ozoe F, et al. The antiparasitic isoxazoline A1443 is a potent blocker of insect ligand-gated chloride channels. *Biochem Biophys Res Commun*. 2010;391:744−749.
20. Yeu E, Holdbrook MJ, Baba SN, Gomes PJ. Psychosocial impact of Demodex blepharitis. *Invest Ophthalmol Vis Sci*. 2021;62:1261−1261.
21. Yeu E, Wirta DL, Karpecki P, et al. Lotilaner ophthalmic solution, 0.25%, for the treatment of Demodex blepharitis: results of a prospective, randomized, vehicle-controlled, double-masked, pivotal trial (Saturn-1). *Cornea*. 2023;42(4):435−443.

22. Cheng AM, Sheha H, Tseng SC. Recent advances on ocular Demodex infestation. *Curr Opin Ophthalmol*. 2015;26:295−300.
23. Rufli T, Mumcuoglu Y. The hair follicle mites Demodex folliculorum and Demodex brevis: biology and medical importance. A review. *Dermatologica*. 1981;162:1−11.
24. Mastrota KM. Method to identify Demodex in the eyelash follicle without epilation. *Optom Vis Sci*. 2013;90:e172−e174.
25. Lemp MA. Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes. *CLAO J*. 1995;21:221−232.
26. Aumond S, Bitton E. Palpebral and facial skin infestation by Demodex folliculorum. *Cont Lens Anterior Eye*. 2020;43:115−122.
27. Lopez-Ponce D, Zuazo F, Cartes C, et al. High prevalence of Demodex spp. infestation among patients with posterior blepharitis: correlation with age and cylindrical dandruff. *Arch Soc Esp Oftalmol*. 2017;92:412−418.
28. Ayres BD, Donnenfeld E, Farid M, et al. Clinical diagnosis and management of Demodex blepharitis: the Demodex expert panel of treatment and eyelid health (DEPTH). *Eye (Lond)*. 2023 Mar 24. doi: 10.1038/s41433-023-02500-4. Online ahead of print.
29. Nichols KK, Holland E, Toyos MM, et al. Ocular comfort assessment of lifitegrast ophthalmic solution 5.0% in OPUS-3, a phase III randomized controlled trial. *Clin Ophthalmol*. 2018;12:263.

Pictures & Perspectives



Massage Gun Ophthalmopathy

A 28-year-old man without psychiatric history/recent drug use presented with 2 months of painless vision loss in his left eye. He applied a Blusmart Massage Gun (image not included for copyright reasons) directly on and around each closed eye for more than an hour daily for several months to relieve the "feeling of eye pressure" (the patient had no history/knowledge of glaucoma). Visual acuity was 20/80 in the right eye and no light perception in the left eye. Intraocular pressure was 9 mmHg in the right eye and 23 mmHg in the left eye. In the right eye, the patient had an anterior subcapsular cataract (**A**) and dialysis/giant retinal tear-associated macula-on rhegmatogenous retinal detachment (**B**) successfully repaired with scleral buckling. In the left eye, the patient had early phthisis, white cataract, severe neovascularization of the iris, and an irreparable stiff funnel retinal detachment (**C**). (Magnified version of Figure **A**-**C** is available online at www.aaojournal.org).

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