

**Bilastine 0.6% preservative-free eye drops, an effective once-daily treatment to reduce signs and symptoms of allergic conjunctivitis: A pooled analysis of two randomized clinical trials**

**Short title:** Bilastine randomized clinical trials

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## Abstract

**Background and objective:** Allergic conjunctivitis is the most common type of ocular allergy.

The objective of this study was to evaluate the efficacy of a new once-daily, preservative-free, bilastine 0.6% eye drop formulation for the treatment of allergic conjunctivitis.

**Methods:** Two double-masked, vehicle controlled, clinical studies (a Phase 2 Dose Ranging Study and a Phase 3 Efficacy Study) were conducted to assess the efficacy of bilastine ophthalmic solution for the treatment of signs and symptoms of allergic conjunctivitis. Both studies used the Ora-CAC<sup>®</sup> Conjunctival Allergen Challenge (CAC) Model to allow observations of allergic responses under controlled conditions. Each study was analyzed separately and then combined to create an integrated dataset.

**Results:** Efficacy was achieved for the primary efficacy endpoint of ocular itching for three bilastine concentrations (0.2%, 0.4%, and 0.6%) at 15 minutes and 8 hours post-instillation and bilastine 0.6% ophthalmic solution was also efficacious at 16 hours post-instillation. Bilastine 0.6% ophthalmic solution demonstrated non-inferiority to ketotifen 0.025% at the onset of action. From the integrated data set, differences between vehicle and bilastine 0.6% were significant at all time points both at onset (15 minutes) and at a prolonged duration (16 hours) after instillation.

**Conclusion:** This multi-trial assessment suggests that bilastine 0.6% ophthalmic solution is efficacious for the treatment of the signs and symptoms of allergic conjunctivitis, with a rapid and prolonged duration of action, and has a favorable safety profile. The added convenience of a once-a-day dosing regimen may contribute to patient adherence and improve their quality of life.

**Key words:** Allergic conjunctivitis. Antihistamine. Bilastine. Preservative-free. Once-daily.

## Resumen

**Justificación y objetivo:** La conjuntivitis alérgica es el tipo más común de alergia ocular. El objetivo de este estudio fue evaluar la eficacia de una nueva formulación oftálmica de bilastina 0,6 %, de administración única diaria y sin conservantes, para el tratamiento de la conjuntivitis alérgica.

**Métodos:** Se realizaron dos estudios clínicos doble ciego, controlados por vehículo (un estudio de búsqueda de dosis de fase 2 y un estudio de eficacia de fase 3) para evaluar la eficacia de la solución oftálmica de bilastina para el tratamiento de los signos y síntomas de la conjuntivitis alérgica. Ambos estudios utilizaron el modelo de provocación conjuntival Ora-CAC<sup>®</sup> *Conjunctival Allergen Challenge*, para evaluar las respuestas alérgicas bajo condiciones controladas. Cada estudio se analizó por separado y luego se combinaron para crear un conjunto de datos integrado.

**Resultados:** Se logró el objetivo de eficacia para el criterio principal de valoración del prurito ocular para tres concentraciones de bilastina (0,2%, 0,4% y 0,6%) a los 15 minutos y 8 horas después del tratamiento. La solución oftálmica de bilastina al 0,6% también fue eficaz a las 16 horas después de su aplicación inicial. La solución oftálmica de bilastina al 0,6% demostró no ser inferior al ketotifeno 0,025% al inicio de la acción. A partir del conjunto de datos integrados, las diferencias entre el vehículo y bilastina al 0,6% fueron significativas en todos los tiempos analizados, tanto al inicio (15 minutos) como durante un tiempo prolongado (16 horas) después de su aplicación.

**Conclusión:** Esta evaluación de múltiples ensayos sugiere que la solución oftálmica de bilastina al 0,6% es eficaz para el tratamiento de los signos y síntomas de la conjuntivitis alérgica, con una acción rápida y de duración prolongada, y tiene un buen perfil de seguridad. La ventaja adicional de un régimen de dosificación de una vez al día puede contribuir a la adherencia del paciente al tratamiento y a mejorar su calidad de vida.

**Palabras clave:** Conjuntivitis alérgica. Antihistamínico. Bilastina. Sin preservantes. Una vez al día.

## Summary box

### What do we know about this topic?

Bilastine, a second-generation antihistamine, is approved for the treatment of allergic rhinoconjunctivitis and urticaria in its oral formulation. An ophthalmic formulation was developed for the treatment of signs and symptoms of allergic conjunctivitis, and its efficacy was evaluated here.

### How does this study impact our current understanding and/or clinical management of this topic?

This multi-trial assessment shows that the newly developed once-daily and preservative-free ophthalmic formulation of bilastine 0,6% is efficacious for rapid reduction of ocular itching and safe in patients with allergic conjunctivitis. The once-a-day dosing regimen may contribute to patient adherence.

## Introduction

Allergic conjunctivitis is the most common type of ocular allergy (~80 to 90%), with a global prevalence ranging from 15 to 40% of the population [1]. Approximately 50% of patients who seek treatment for allergies present with ocular symptoms [2]. Allergic conjunctivitis results from a predominantly IgE-mediated inflammatory reaction in the conjunctiva or immediate hypersensitivity mechanism [3], and often coexists with other allergic diseases, such as asthma, allergic dermatitis, or food allergy, and particularly with allergic rhinitis [4]. Therefore, the term rhinoconjunctivitis is often used interchangeably to refer to both entities [5,6].

Topical therapies are preferred when symptoms are mainly ocular, because of their faster onset of action. Some oral antihistamines for allergic conjunctivitis treatment result in side effects such as fatigue and somnolence [7,8], while other, less sedating oral treatments can result in ocular dryness [9]. Administration via the topical ocular route reduces the risk of fatigue and somnolence associated with oral administration due to the lower systemic bioavailability of drugs via eye drops. Topical ophthalmic treatments often show superiority to oral and nasal treatments when local symptoms are predominant [10], and the European Academy of Allergy and Clinical Immunology (EAACI) recommends topical treatment for allergic conjunctivitis [11]. In addition, oral and topical administration of corticosteroids is associated with systemic side effects such as cataracts or elevated intraocular pressure (IOP) [12]. Moreover, certain topical vasoconstrictor eye drops can also result in a rebound effect of ocular redness [13]. Therefore, novel therapeutics alternatives are needed.

Bilastine is a second-generation, non-sedating, selective antihistamine that was initially developed as an oral formulation for the treatment of allergic rhinoconjunctivitis and urticaria. It

has a chemical structure similar to piperidinyl-benzimidazole but it is not structurally derived from it, nor is it a metabolite or enantiomer of any of the existing antihistamines, but rather an original molecule. Bilastine is authorized worldwide (28 European countries and over 94 non-European countries) for the symptomatic treatment of allergic rhinoconjunctivitis (seasonal and perennial) and urticaria in adults and adolescents. Bilastine has been shown to be effective in controlling the ocular signs and symptoms of allergic conjunctivitis [14].

Based on the demonstrated efficacy and safety of the oral formulation and the superiority of topical to oral antihistamines for the treatment of ocular allergy, an ocular formulation of bilastine was developed. This is a preservative and phosphates-free formulation, containing sodium hyaluronate in a multi-dose bottle presentation for the treatment of allergic conjunctivitis. The most frequently used preservative, benzalkonium chloride (BAK), has demonstrated toxic effects in laboratory, experimental, and clinical studies, and can cause irritation, discomfort, and chronic inflammation [15,16]. Therefore, preservative free eye drops are preferred to use whenever possible [11].

Here, we report the results of two clinical trials conducted to evaluate the efficacy of bilastine ophthalmic solution for treatment of the signs and symptoms of allergic conjunctivitis.

## **Methods**

### ***Study Design***

Two clinical trials, a single site (Phase 2 Dose Ranging Study, ClinicalTrials.gov number NCT03231969) and a multi-center (Phase 3 Efficacy Study, ClinicalTrials.gov number NCT03479307), were conducted to assess the efficacy of bilastine ophthalmic solution for

treatment of the signs and symptoms of allergic conjunctivitis. Both studies used the Ora-Conjunctival Allergen Challenge (Ora-CAC<sup>®</sup>) Model, which involves instillation of allergens directly into the eye to allow observations of acute allergic responses under controlled conditions [17].

Similar clinical protocols were employed for both trials, with the Phase 2 Dose Ranging Study encompassing a 1:1:1:1 (0.2% bilastine:0.4% bilastine:0.6% bilastine:vehicle) enrollment ratio and a 6 to 10 week assessment period and the Phase 3 Efficacy Study encompassing a 2:2:1 (0.6% bilastine:0.025% ketotifen:vehicle) enrollment ratio and a 5 to 9 week assessment period. The Phase 2 Dose Ranging Study included 8 office visits and the Phase 3 Efficacy Study included 6 office visits, outlined in **Table 1**.

Both studies included one follow-up phone conversation one-week after the last office visit. Institutional review of the protocol, protocol amendments, and informed consent were conducted in compliance with Good Clinical Practices, including the International Conference on Harmonisation Guidelines.

### ***Subjects***

To be enrolled in either study, subjects must have had a history of ocular allergies and a positive skin test reaction to a seasonal (grass, ragweed, and/or tree pollen) or perennial allergen (cat dander, dog dander, dust mites, or cockroach). Criteria for inclusion included providing written informed consent; being 18 years of age or older, been willing to discontinue wearing contact lenses for at least 72 hours prior to the start of the study and for the duration of the study, if female and of childbearing potential, having submitted a negative urine pregnancy test and

agreed to use an adequate methods of birth control for the duration of the study, having a calculated visual acuity of 0.7 logarithm of the minimum angle of resolution or better in each eye; having a positive bilateral post-CAC reaction within 10 minutes of instillation of allergen at Visit 2, and having a positive bilateral post-CAC reaction for at least two out of the first three time points following the challenge at Visit 3.

Subjects were excluded if they had any sensitivities to the investigational product, any ocular condition that could have affected the subject's safety or trial parameters, had ocular surgical intervention within the past 3 months or refractive surgery within the past 6 months, or been taking any disallowed medication including ophthalmological topical treatments (artificial tears, antihistamines, antihistamine/mast cell stabilizers, antihistamine-vasoconstrictor drug combinations, nonsteroidal anti-inflammatory drugs, or corticosteroids).

### ***Study Objectives***

The primary objectives of the Phase 2 Dose Ranging Study were to assess the relative efficacy, safety, and duration of action of three concentrations of bilastine ophthalmic solution (0.2%, 0.4%, and 0.6%) over vehicle for the treatment of the signs and symptoms of allergic conjunctivitis. The results of this study justified the optimal concentration and dosing regimen for phase 3 development. The primary objectives of the Phase 3 efficacy study were to demonstrate the superiority of bilastine 0.6% ophthalmic over vehicle for the treatment of patient-assessed ocular itching (assessed 3, 5, and 7 minutes post-CAC) at the onset of action and 16-hour duration of action, and to demonstrate non-inferiority of multidose bilastine 0.6% preservative-free to multidose ketotifen 0.025% with preservatives at the onset of action visit.



Ketotifen 0.025% is indicated for twice daily dosing and therefore comparisons to bilastine at 16 hours were not made.

A secondary objective of the Phase 3 Efficacy study was to demonstrate superiority of bilastine to vehicle for the treatment of conjunctival redness.

### ***Efficacy Variables***

The primary efficacy measure for the Phase 2 Dose Ranging Study was ocular itching evaluated by the subject post-CAC at the onset of action (15 minutes post-instillation) and duration of action (8 hours and 16 hours post-instillation) using the Ora Calibra® ocular itching scale from 0 to 4, where 0=none and 4=very severe. The primary efficacy measure for the Phase 3 Efficacy Study was ocular itching evaluated by the subject post-CAC at the onset of action (15 minutes post-instillation) and duration of action (16 hours post-instillation) using the same 0-4 scale.

Ocular itching at onset of action was then compared to ketotifen to evaluate non-inferiority of bilastine 0.6% to ketotifen 0.025%. Secondary efficacy measures included conjunctival redness evaluated by the investigator using the Ora Calibra® ocular redness scale (0-4 scale; 0=none, 4=extremely severe), measured in both the Phase 2 Dose Ranging Study and the Phase 3 Efficacy Study.

### ***Statistical Methods***

All randomized subjects who received study medication were included in the intent-to-treat population as used for all analyses. The primary efficacy analyses were conducted using analysis of covariance (ANCOVA) models with lost observation carried forward (LOCF) for missing

data. These models included treatment and the average of the subjects' post-CAC scores at Visit 3 as covariates. Least square (LS) means were estimated for each treatment and for the difference between each active treatment and vehicle at each visit and time point. In addition, ANCOVA models were run at 15 minutes, 8 hours (for the Phase 2 Dose Ranging Study), and 16 hours post-instillation, with treatment, time point, and time-appropriate baseline as covariates for adjustment (accounting for repeated measurements). LS means for each treatment and for the difference between each active treatment and vehicle (along with the corresponding 95% CI) were calculated at each visit and time point from these repeated measures ANCOVA models. Two-sample t-tests were used as unadjusted sensitivity analyses at each post-CAC time point. The number of subjects with ocular itching scores reduced by 50% were summarized with counts and percentages by treatment group for each post-CAC time point at 15 minutes, 8 hours (for the Phase 2 Dose Ranging Study only), and 16 hours post-instillation. The proportion of responders at each visit was compared between treatment groups using a Fisher's exact test. To demonstrate the non-inferiority of bilastine 0.6% compared to ketotifen 0.025% for the treatment of ocular itching, bilastine 0.6% had to demonstrate statistical non-inferiority to ketotifen 0.025% within 0.4 units for all 3 post-CAC time points: 3, 5, and 7 minutes at 15 minutes post-instillation (for the Phase 3 Efficacy Study). Analyses for conjunctival redness were performed using the same populations and missing data methods as for the primary endpoint.

## Results

### *Study Population*

A total of 349 subjects were randomized (bilastine 0.2%, n=30; bilastine 0.4%, n=30; bilastine 0.6%, n=122; ketotifen 0.025%, n=90; or vehicle, n=77), and 343 subjects completed the two studies and were included in the data analysis. A CONSORT flow diagram showing the progress of patients throughout the trial is included in **Figure 1**.

The demographic profile and baseline characteristics were similar across both studies and treatment groups (**Table 2**).

### *Efficacy Analysis*

#### *Phase 2 Dose Ranging Study*

The primary efficacy measures of ocular itching induced by the CAC was assessed by subjects in each eye using the Ora Calibra<sup>®</sup> ocular itching scale (0-4, where 0=none, 4= extremely severe) at 15 minutes, 8 hours, and 16 hours post-instillation. All three bilastine concentration significantly reduced ocular itching compared to vehicle for all three post-CAC time points, for both onset of action and duration of action (**Figure 2**). Bilastine 0.6% demonstrated the greatest statistically significant treatment differences from vehicle, with  $p < 0.0001$  at all three post-CAC time points for all three time points post-instillation.

In addition to the statistical assessments of itch responses, clinical significance was also examined using the 50% responder rate as a metric of efficacy. This measure of clinical effectiveness establishes a cutoff of 50% reduction in individual subject itch scores as an indication of a clinically significant reduction. As shown in **Figure 3A**, significant clinical relief

of ocular itching was observed at 15 minutes and 8 hours post-instillation as compared to placebo for all doses of bilastine (15 minutes: 71.4% of subjects with >50% reduction in ocular itching for bilastine 0.2%, 75.0% for bilastine 0.4%, and 83.3% for bilastine 0.6%; 8 hours: 44.8% for bilastine 0.2%, 35.7% for bilastine 0.4%, and 76.7 for bilastine 0.6%; 16-hours: 10.0% for bilastine 0.2%, 13.3% for bilastine 0.4%, and 58.1% for bilastine 0.6%). Bilastine 0.6% also provided clinically significant relief at 16 hours post-instillation as compared to placebo (58.1% of subjects with >50%). Based on these results, bilastine 0.6% was found to be the optimal dose and was selected for use in the Phase 3 efficacy study.

For the key secondary efficacy endpoint of conjunctival redness, treatment differences resulted in p-values of less than 0.05 for bilastine 0.6% compared to vehicle at 7 minutes post-CAC at 15 minutes, 8 hours and 16 hours post-instillation, and for bilastine 0.4% at 7 minutes post-CAC at 15 minutes post-instillation (data not shown). Bilastine ophthalmic solution appears to be safe and well tolerated. Similar numbers of treatment emergent adverse events (TEAEs) were reported in the bilastine 0.2% group (8 TEAEs) and the vehicle group (7 TEAEs), with fewer TEAEs reported in the bilastine 0.4% group (3 TEAEs) and the bilastine 0.6% group (1 TEAE).

#### *Phase 3 Efficacy Study*

The primary efficacy measure of ocular itching was assessed after CAC by subjects in each eye 15 minutes and 16 hours post-instillation. All itch measures were significantly lower for bilastine 0.6% than for vehicle ( $p < 0.0001$  for 3, 5, and 7 minutes post-CAC) for 15 minutes and 16 hours post-instillation (**Figure 4**). These improvements were  $\geq 1$  unit compared to vehicle at all three time points 15 minutes post-instillation, with the greatest mean treatment difference occurring at

5 minutes post-CAC (unadjusted mean treatment difference = -1.183, LS mean treatment difference = -1.208).

To demonstrate the non-inferiority of bilastine 0.6% compared to ketotifen 0.025% for the treatment of ocular itching, bilastine 0.6% had to demonstrate statistical non-inferiority to ketotifen 0.025% within 0.4 units for all 3 post-CAC time points: 3, 5, and 7 minutes at 15 minutes post-instillation (Visit 5). Comparison of the bilastine 0.6% and ketotifen 0.025% groups demonstrated that bilastine 0.6% was non-inferior at all three post-CAC time points for onset of action (15 minutes post-instillation), based on an inferiority margin of 0.4 (mean treatment difference= 0.009, -0.077, and -0.159 for 3, 5, and 7 minutes, respectively, post-CAC for 15 minutes post-instillation).

As in the Phase 2 study, clinical significance of itch reduction was also examined using the greater than 50% responder rate as a metric of efficacy. As shown in **Figure 3B**, significant clinical relief of ocular itching was observed at 15 minutes (72.2% of subjects had a >50% reduction in itch) for bilastine 0.6% demonstrating the quick onset of action of the formulation. For the key secondary efficacy endpoint of conjunctival redness, treatment differences resulted in p-values of less than 0.05 for bilastine 0.6% compared to vehicle at 7, 15 and 20 minutes post-CAC at 15 minutes post-instillation. A total of 6 subjects (2.6%) experienced treatment-emergent adverse events in this study; all of these were mild in severity, and none were considered treatment-related.

#### *Integrated Dataset*

As a supplement to the individual results of the two studies, data from the Phase 2 Dose Ranging Study was combined with data from the Phase 3 Efficacy Study to create a larger, integrated

dataset. The integrated population mean itch scores exhibited the same efficacy as those from the individual studies: differences between vehicle and bilastine 0.6% were significant at all time points both at onset (15 minutes) and at a prolonged duration (16 hours) after instillation (**Figure 5**). The 2 subpopulations examined, perennial or seasonal qualifying allergen, also displayed a consistent high efficacy across all measures of ocular itching (**Figure 5**). This confirms that the overall results were not due to a strong preferential effect of the drug on itching due to a specific type of allergen.

As a final assessment of the integrated dataset, a responder analysis was conducted to determine the clinical significance of itch relief observed in the pooled datasets (**Figure 6**). While the two subpopulations derived from the pooled analysis differed in size (there are about twice as many subjects qualified with seasonal allergens as compared to those qualified with perennial allergens), they are similar in all other characteristics. This was also observed in the responder analysis, where clinical response was seen in the same proportion of subjects in all groups at the onset of action and at 16 hours duration of action measure.

## Discussion

Bilastine has previously been shown to be effective in the treatment of rhinitis and allergic conjunctivitis as an oral formulation [14,18], and the present work demonstrates the efficacy of bilastine as an ophthalmic solution for allergic conjunctivitis treatment. In a Phase 2 clinical trial, efficacy was achieved for the primary efficacy endpoint of ocular itching for three bilastine concentrations (0.2%, 0.4%, and 0.6%) at 15 minutes and 8 hours post-instillation [19]. In a subsequent Phase 3 study, bilastine 0.6% ophthalmic solution was efficacious at 15 minutes and

16 hours post-instillation, demonstrating both the onset and duration of action of bilastine ophthalmic solution. Moreover, bilastine 0.6% solution demonstrated non-inferiority to ketotifen 0.025% ophthalmic solution at 15 minutes post installation [20].

The integrated data set strongly supported the findings of the individual studies, showing subjects who received bilastine 0.6% ophthalmic solution demonstrated a significantly lower mean itch score at all post-CAC time points for both 15-minutes post instillation (onset of action) and 16 hours post-instillation (duration of action). Furthermore, a significantly larger percentage of subjects in the active group showed a greater than 50% reduction in individual itch scores than in the placebo group, demonstrating a significant improvement of itching associated with allergic conjunctivitis.

Bilastine 0.6% solution is the first direct formulated multidose non-preserved ophthalmic solution with sodium hyaluronate formulated for once-daily use for allergic conjunctivitis treatment. Moreover, to increase patient compliance the goal is to maximize patient convenience by reducing the number of instillations per day and minimizing the potential irritant and toxic effects of preservative compounds on the ocular surface. Further, ophthalmic solutions containing preservatives such as BAK cannot be used concurrently with contact lens use [11,21]. Since there is no preservative in bilastine ophthalmic solution, previous preclinical studies have demonstrated not interaction of the formulation with soft contact lens (data not shown).

A possible limitation that must be considered when interpreting the results of this study is that in the Phase 3 study, the administered ketotifen contained a preservative. The reason was that there were no multidose ophthalmic formulations of preservative-free ketotifen available in the market at the time this study was planned and conducted. In order to homogenize the administration

device in multidose containers to maintain a double-blind study, the ketotifen multidose formulation with preservatives was selected.

Across the two studies, bilastine ophthalmic solution demonstrated a favorable safety profile and efficacy for the treatment of ocular itching, and better comfort scores as reported by subjects, in experimental conjunctival challenge tests. Further studies are necessary to demonstrate similar outcomes in real clinical practice conditions. Overall, the results of these two studies are strongly supportive of the use of bilastine 0.6% ophthalmic solution for the treatment of ocular itching associated with allergic conjunctivitis while protecting the ocular surface homeostasis.

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### **Conflicts of interest**

PJG is an employee of Ora, Inc. JBC is a consultant to Ora, Inc. PA, AG, GH and NF are employees of FAES Farma.



## References

1. Azari AA, Barney NP. Conjunctivitis: a systematic review of diagnosis and treatment. *JAMA*. 2013;310:1721–9.
2. Owen CG, Shah A, Henshaw K, Smeeth L, Sheikh A. Topical treatments for seasonal allergic conjunctivitis: systematic review and meta-analysis of efficacy and effectiveness. *Br J Gen Pract*. 2004;54:451–6.
3. Ono SJ, Abelson MB. Allergic conjunctivitis: update on pathophysiology and prospects for future treatment. *J Allergy Clin Immunol*. 2005;115:118–22.
4. Naclerio RM, Pinto J, deTineo M, Baroody FM. Elucidating the mechanism underlying the ocular symptoms associated with allergic rhinitis. *Allergy Asthma Proc*. 2008;29:24–8.
5. Abelson MB, Welch DL. An evaluation of onset and duration of action of patanol (olopatadine hydrochloride ophthalmic solution 0.1%) compared to Claritin (loratadine 10 mg) tablets in acute allergic conjunctivitis in the conjunctival allergen challenge model. *Acta Ophthalmol Scand Suppl*. 2000;60–3.
6. Sánchez-Hernández MC, Montero J, Rondon C, Benitez del Castillo JM, Velázquez E, Herreras JM, et al. Consensus document on allergic conjunctivitis (DECA). *J Investig Allergol Clin Immunol*. 2015;25:94–106.
7. Singh-Franco D, Ghin HL, Robles GI, Borja-Hart N, Perez A. Levocetirizine for the treatment of allergic rhinitis and chronic idiopathic urticaria in adults and children. *Clinical Therapeutics*. 2009;31:1664–87.
8. Marmouz F, Giralt, Izquierdo I. Morning and evening efficacy evaluation of rupatadine (10 and 20 mg), compared with cetirizine 10 mg in perennial allergic rhinitis: a randomized, double-blind, placebo-controlled trial. *JAA*. 2011;27.
9. Ousler GW, Workman DA, Torkildsen GL. An open-label, investigator-masked, crossover study of the ocular drying effects of two antihistamines, topical epinastine and systemic loratadine, in adult volunteers with seasonal allergic conjunctivitis. *Clin Ther*. 2007;29:611–6.
10. Spangler DL, Abelson MB, Ober A, Gotnes PJ. Randomized, double-masked comparison of olopatadine ophthalmic solution, mometasone furoate monohydrate nasal spray, and fexofenadine hydrochloride tablets using the conjunctival and nasal allergen challenge models. *Clin Ther*. United States; 2003;25:2245–67.
11. Leonardi A, Silva D, Perez Formigo D, Bozkurt B, Sharma V, Allegri P, et al. Management of ocular allergy. *Allergy*. 2019;74:1611–30.
12. Abelson MB, Butrus S. Corticosteroids in ophthalmic practice. *Albert & Jakobiec's Principles and Practice of Ophthalmology*. 3 ed. Philadelphia: Saunders Elsevier; 2008.

13. Spector SL, Raizman MB. Conjunctivitis medicamentosa. *J Allergy Clin Immunol*. 1994;94:134–6.
14. Bartra J, Mullol J, Montoro J, Jauregui I, del Cuvillos A, Davila I, et al. Effect of bilastine upon the ocular symptoms of allergic rhinoconjunctivitis. *J Investig Allergol Clin Immunol*. 2011;21 Suppl 3:24–33.
15. Baudouin C, Labbé A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. *Prog Retin Eye Res*. 2010;29:312–34.
16. Vaede D, Baudouin C, Warnet J-M, Brignole-Baudouin F. [Preservatives in eye drops: toward awareness of their toxicity]. *J Fr Ophthalmol*. 2010;33:505–24.
17. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. *Arch Ophthalmol*. 1990;108:84–8.
18. Wolthers OD. Bilastine: A New Nonsedating Oral H1 Antihistamine for Treatment of Allergic Rhinoconjunctivitis and Urticaria. *BioMed Research International*. 2013;626837.
19. Gomes PJ, Ciolino JB, Arranz P, Hernández G, Fernández N. Efficacy of once-daily ophthalmic bilastine for the treatment of allergic conjunctivitis: a dose-finding study. *J Investig Allergol Clin Immunol*. 2022;0.
20. Gomes PJ, Ciolino JB, Arranz P, Hernández G, Fernández N. Bilastine 0.6% preservative-free eye drops, a once-daily treatment for allergic conjunctivitis. *J Investig Allergol Clin Immunol*. 2023;0.
21. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo C-K, et al. TFOS DEWS II Definition and Classification Report. *The Ocular Surface* [Internet]. 2017 [cited 2021 Jul 5];15:276–83. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1542012417301192>

## Tables

**Table 1.** Study schedule outline.

Phase 2 Dose Ranging Study		Phase 3 Efficacy Study	
Visit	Schedule	Visit	Schedule
Visit 1 (Day -50 to -22)	Screening /Informed Consent	Visit 1 (Day -50 to -22)	Screening /Informed Consent
Visit 2 (Day -21±3)	Allergen Titration	Visit 2 (Day -21±3)	Allergen Titration
Visit 3 (Day -14±3)	Allergen Confirmation	Visit 3 (Day -14±3)	Allergen Confirmation
Visit 4a (Day 1)	Randomization/Enrollment and 1st Study Treatment Instillation	Visit 4a (Day 1)	Randomization/Enrollment and 1st Study Treatment Instillation
Visit 4b (Day 1, 16 hours after Visit 4a)	16-Hour Duration of Action	Visit 4b (Day 1, 16 hours after Visit 4a)	16-Hour Duration of Action
Visit 5a (Day 15±3)	2nd Study Treatment Instillation	Visit 5 (Day 8±3)	15-Minute Onset of Action
Visit 5b (Day 15 ±3, 8 hours after Visit 5a)	8-Hour Duration of Action	-	-
Visit 6 (Day 22 ±3)	15-Minute Onset of Action	-	-

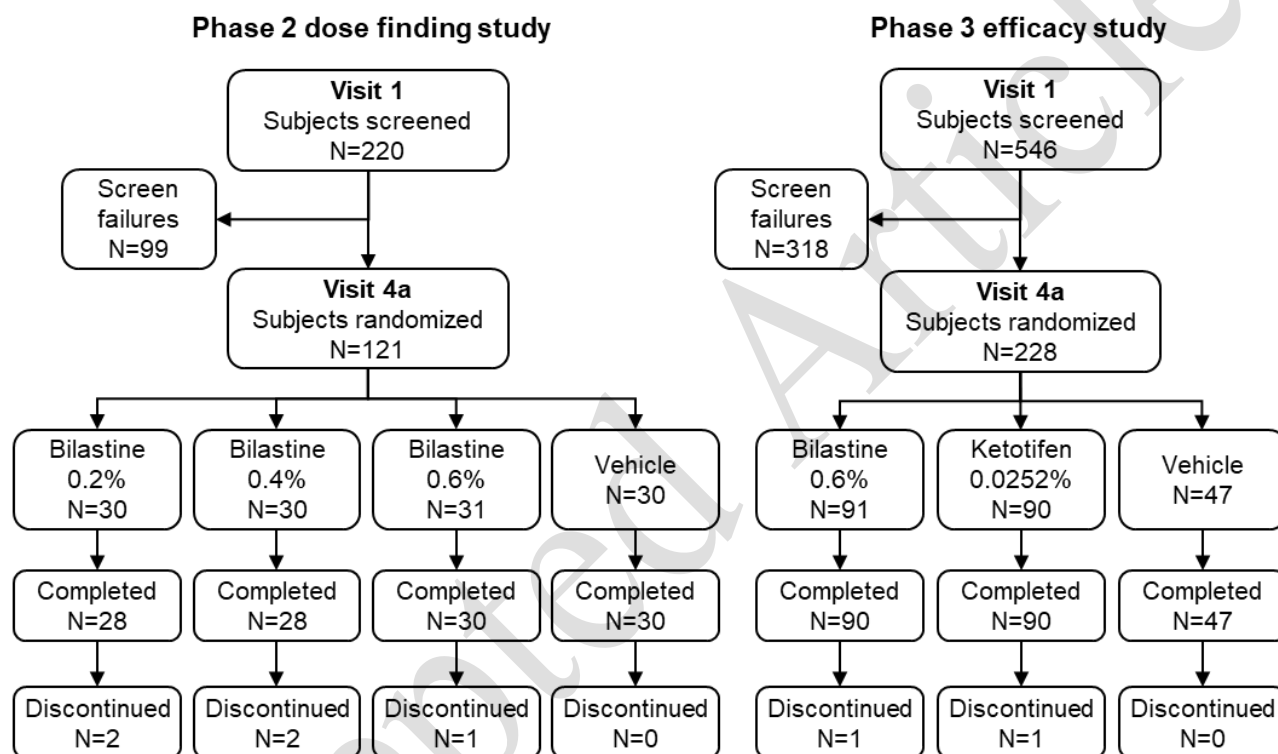
**Table 2.** Demographic profile and baseline characteristics of subjects.

<b>Phase 2 Dose Ranging Study</b>					
	<b>Bilastine 0.2% (N=30)</b>	<b>Bilastine 0.4% (N=30)</b>	<b>Bilastine 0.6% (N=31)</b>	<b>Vehicle (N=30)</b>	<b>All Subjects (N=121)</b>
Age (years), mean±SD	50.4±10.7	47.0±14.0	51.8±13.1	48.3±13.2	49.4±12.8
Sex (female), N (%)	16 (53.3)	18 (60.0)	15 (48.4)	17 (56.7)	66 (54.5)
Race, N (%)					
American Indian or Alaskan Native	0	1 (3.3%)	0	0	1 (0.8)
Asian	1 (3.3)	0	1 (3.2)	0	2 (1.7)
Black or African American	1 (3.3)	2 (6.7)	4 (12.9)	3 (10.0)	10 (8.3)
White	27 (90.0)	27 (90.0)	26 (83.9)	27 (90.0)	107 (88.4)
Multiple	1 (3.3)	0	0	0	1 (0.8)
Baseline Ocular Itching (Visit 3, Day -14), mean±SD					
Pre-CAC	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	–
3 Minutes Post-CAC	2.75±0.57	2.98±0.57	2.69±0.75	2.92±0.61	–
5 Minutes Post-CAC	3.14±0.53	3.32±0.46	2.99±0.60	3.31±0.56	–
7 Minutes Post-CAC	3.21±0.50	3.39±0.54	3.07±0.58	3.33±0.57	–
<b>Phase 3 Efficacy Study</b>					
	<b>Bilastine 0.6% (N=91)</b>	<b>Ketotifen 0.025% (N=90)</b>	<b>Vehicle (N=47)</b>	<b>All Subjects (N=228)</b>	
Age (years), mean±SD	45.9±12.88	41.7±12.10	45.1±16.03	44.1±13.38	
Sex (male), N (%)	58 (63.7)	53 (58.9)	25 (53.2)	136 (59.6)	
Race, N (%)					
American Indian or Alaskan Native	1 (1.1)	1 (1.1)	1 (2.1)	3 (1.3)	
Asian	14 (15.4)	13 (14.4)	6 (12.8)	33 (14.5)	
Black or African American	25 (27.5)	27 (30.0)	14 (29.8)	66 (28.9)	
White	49 (53.8)	48 (53.3)	26 (55.3)	123 (53.9)	
Multiple	2 (2.2)	1 (1.1)	0	3 (1.3)	
Baseline Ocular Itching (Visit 3, Day -14), mean ± SD					
Pre-CAC	0.00±0.00	0.00±0.00	0.00±0.00	–	
3 Minutes Post-CAC	2.71±0.61	2.71±0.70	2.61±0.6	–	
5 Minutes Post-CAC	2.98±0.58	2.99±0.56	2.91±0.47	–	
7 Minutes Post-CAC	3.09±0.59	3.04±0.54	2.97±0.51	–	

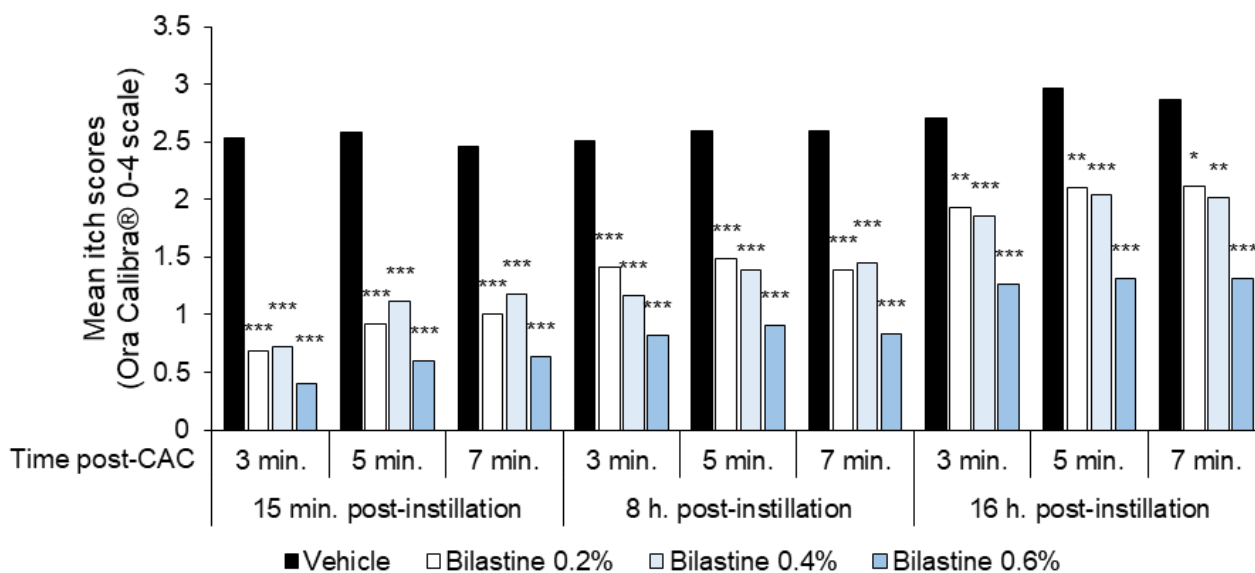
Abbreviations: SD, standard deviation.

## Figures

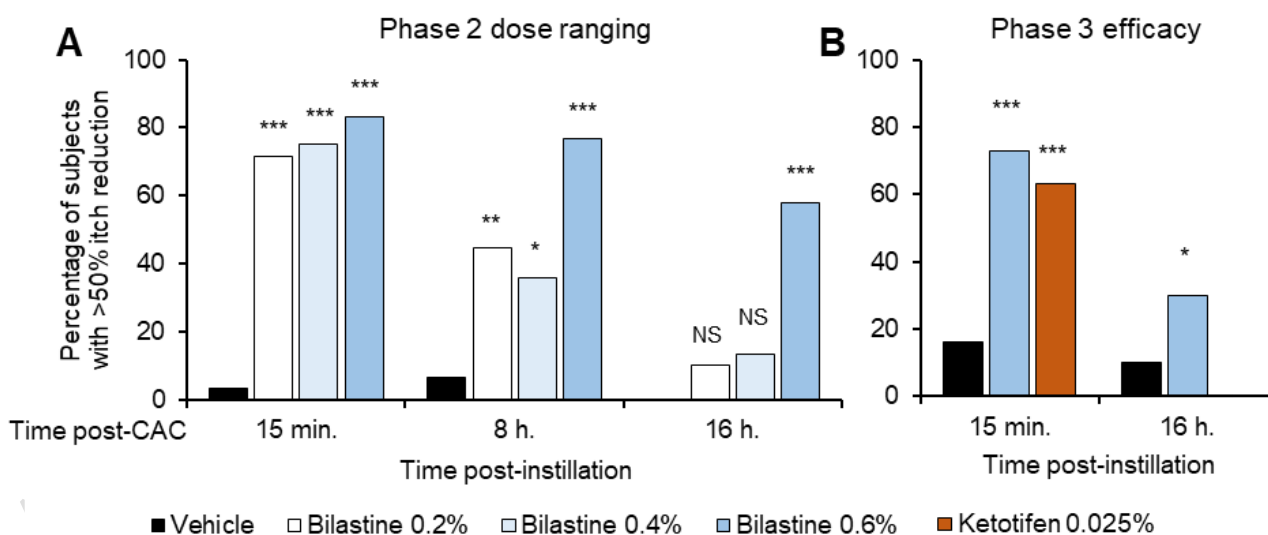
**Figure 1.** CONSORT flow chart.



**Figure 2.** Mean itch scores across concentrations of bilastine in the Phase 2 dose ranging study. Bars represent the ANCOVA LS mean itch score for vehicle and each concentration of bilastine (0.2%, 0.4%, and 0.6%) for each time point post-CAC (3, 5, and 7 minutes), for each study visit where 15 minutes post-installation is Visit 6, 8 hours post-installation is Visit 5b, and 16 hours post-installation is Visit 4b. Significance (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ , two-sample t-test) compared to the mean itch score of the vehicle. Abbreviations: CAC, conjunctival allergen challenge; h, hours; min, minutes.

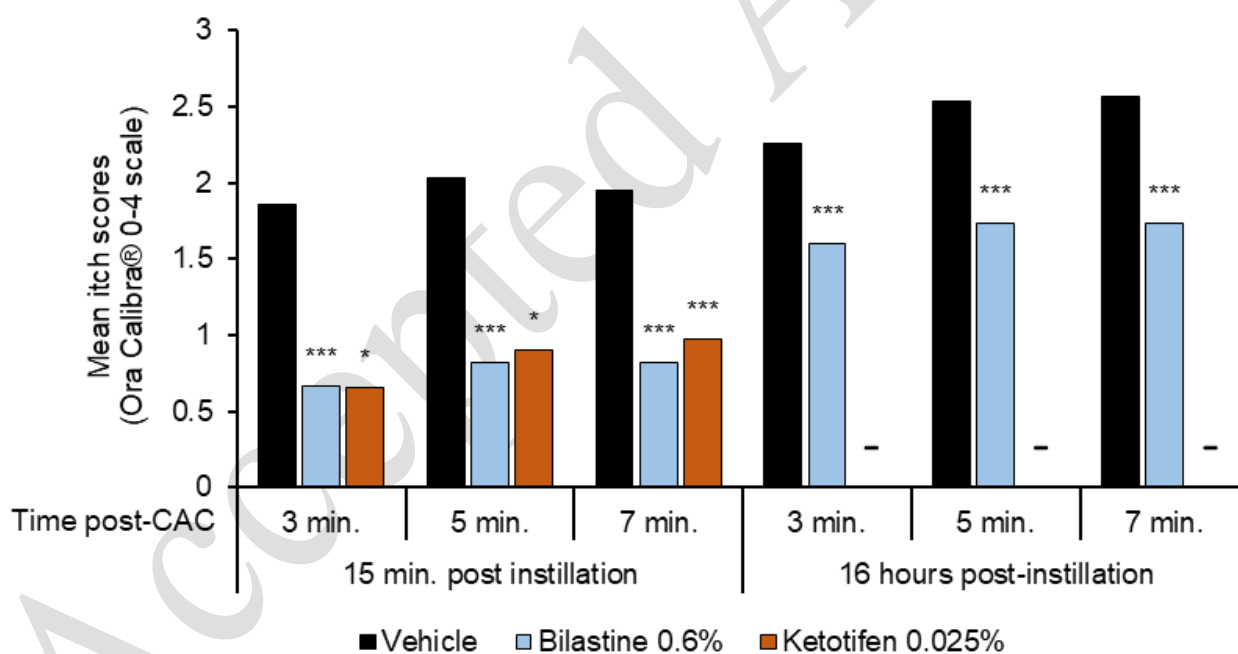


**Figure 3.** Responder analysis: percentage of subjects with > 50% itch reduction in the Phase 2 dose ranging study and the Phase 3 efficacy study. Bars represent the percentage of subjects with a greater than 50% reduction in mean itch score (as measured on the 0-4 scale), measured at A) 15 minutes post-instillation (Visit 6), 8 hours post-instillation (Visit 5b) 16 hours post-instillation (Visit 4b); and B) measured at 15 minutes post-instillation (Visit 5) and 16 hours post-instillation (Visit 4b). Significance (NS, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ , Fisher's exact test) compared to placebo in the percentage of subject with a greater than 50% reduction in itch score. Abbreviations: CAC, conjunctival allergen challenge; h, hours; min, minutes.



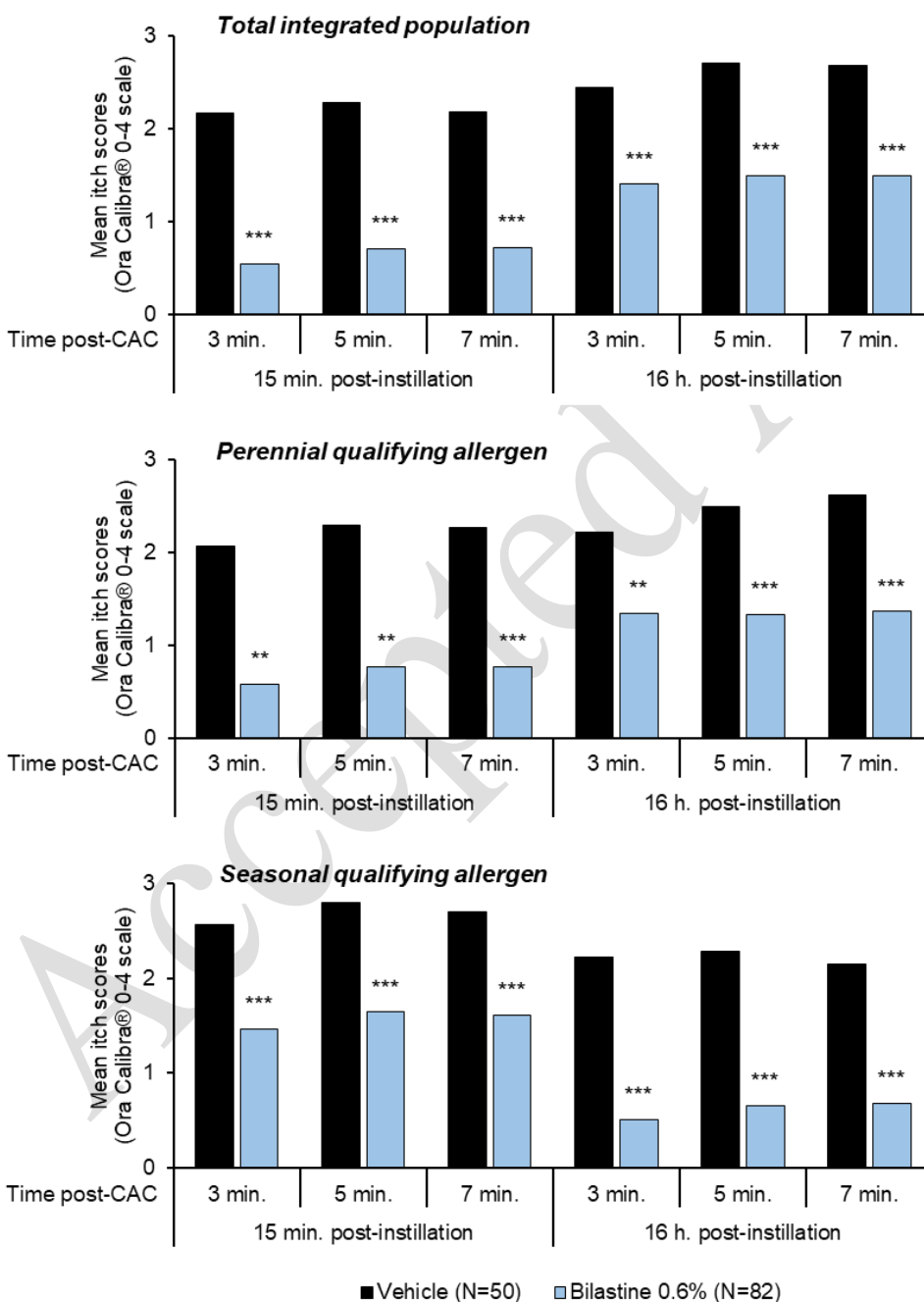
**Figure 4.** ANCOVA LS mean itch responses to bilastine or ketotifen, Phase 3 efficacy study.

Bars represent the ANCOVA LS mean itch score for vehicle, bilastine 0.6% and ketotifen 0.025% for each time point post-CAC (3, 5, and 7 minutes), for each study visit where 15 minutes post-installation is Visit 5 and 16 hours post-installation is Visit 4b. Significance (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ , two-sample t-test) compared to the mean itch score of the vehicle. Abbreviations: CAC, conjunctival allergen challenge; h, hours; min, minutes.





**Figure 5.** ANCOVA LS mean itch analysis for the integrated data set. Bars represent the ANCOVA LS mean itch score for vehicle and bilastine 0.6% for each time point post-CAC (3, 5, and 7 minutes), for each study visit where 15 minutes post-installation is Visit 5 and 16 hours post-installation is Visit 4b. Significance (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ , two-sample t-test) compared to the mean itch score of the vehicle. Abbreviations: CAC, conjunctival allergen challenge; h, hours; min, minutes.



**Figure 6.** Responder analysis. Percentage of subjects with >50% itch reduction at all time points, Integrated Dataset. Bars represent the percentage of subjects with a >50% reduction in mean itch score (as measured on the 0-4 scale), measured at 15 minutes post-instillation and 16 hours post-instillation. Significance (\*,  $p < 0.05$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ , Fisher's exact test) indicates difference compared to placebo in the percentage of subject with a greater than 50% reduction in itch score.

