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CLINICAL TRIAL REPORT

# The Phase 3 INVIGORATE Trial of Reproxalap in Patients with Seasonal Allergic Conjunctivitis

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**Purpose:** There is an unmet need for new treatments for allergic conjunctivitis.

**Objective:** To assess the activity of reproxalap, a novel reactive aldehyde species modulator, in a real-world model of seasonal allergen exposure.

**Methods:** The INVIGORATE Trial, a prospective, quadruple-masked, vehicle-controlled, crossover, sequence-randomized Phase 3 trial, tested the efficacy of reproxalap in adults with a history of moderate to severe allergic conjunctivitis, ragweed pollen allergy, and allergen chamber-induced ocular itching and redness. Patients were randomly assigned (1:1) to receive 0.25% reproxalap ophthalmic solution or vehicle, followed by a 2-week washout period before crossing over to the other test article. The primary endpoint was ocular itching from 110 to 210 minutes after chamber entry; the key secondary endpoint was ocular redness over the chamber duration (0–4 scales for both endpoints).

**Results:** Of the 95 randomly assigned patients, 89 completed all visits (reproxalap to vehicle: n = 46; vehicle to reproxalap: n = 43). Primary and key secondary endpoints were met: reproxalap significantly reduced ocular itching (mean [SE]: −0.50 [0.03], $p < 0.001$) and redness (−0.14 [0.01], $p < 0.001$) relative to vehicle. Responder analyses confirmed the clinical relevance of both end points. Reproxalap was safe and well tolerated. No clinically significant changes in safety assessments were observed. No serious or severe treatment-emergent adverse events (TEAEs) were reported. The most commonly reported TEAE was mild and transient installation site irritation after reproxalap versus vehicle administration.

**Conclusion:** In this well-controlled allergen chamber trial, reproxalap was statistically superior to vehicle across typical symptoms and signs of allergic conjunctivitis.

**Trial Registration:** NCT04207736.

**Keywords:** allergic conjunctivitis, reactive aldehyde species, reproxalap, ocular itching, ocular redness

## Introduction

Reproxalap, a novel chemical entity in late-stage clinical development for the treatment of ocular inflammation, chemically sequesters a class of small molecules known as reactive aldehyde species (RASP). Via covalent binding to amine and thiol residues, RASP modulate the structure and function of proteins in an analog manner[1] depending on the amount of binding, and are upregulated during inflammation before cytokine release, leading to the potentiation of nuclear factor kappa B, inflammasomes, and other proteins.[2,3] RASP are associated with the systemic and ocular allergic response,[4,5] and RASP-mediated induction of inflammation facilitates the post-histaminic pathology of allergy, including cellular infiltrate and other sub-acute physiological characteristics of allergic conjunctivitis.[6] By targeting RASP, reproxalap represents a first-in-class therapeutic approach that does not directly activate or inhibit a specific protein, but rather indirectly modulates systems of proteins involved in the inflammatory cascade. The systems-based mechanism of action of reproxalap affords a theoretical advantage of pluripotent utility in inflammatory ocular disease broadly, reflected not only in allergic conjunctivitis,[7–9] but also noninfectious anterior uveitis[10] and dry eye disease.[11,12]

Despite an increasing prevalence of allergic conjunctivitis that includes more than 20% of the US population,[13] drugs to treat allergic conjunctivitis with novel mechanisms of action have not been introduced in decades. Furthermore,

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although most patients with allergic conjunctivitis are treated at least initially with topical antihistamines, the need for adjunctive therapy is common. For example, in one study, up to 60% of patients required non-antihistaminic medication,[14] which often includes topical corticosteroids, a class of drugs associated with potentially serious ocular toxicity including elevation of intraocular pressure and development of glaucoma.[15]

Topical conjunctival allergen challenge has typically been used in development of allergic conjunctivitis drugs.[7] However, the clinical relevance of applying concentrated allergen to the ocular surface can be questioned, and the assessment of duration of drug activity after challenge is often limited to less than 10 minutes,[16] possibly due to waning drug effect resulting from rapidly declining histamine levels, which peak within 5 to 10 minutes of allergen exposure.[17] Investigational allergic conjunctivitis drugs may also be tested in field trials, but exposure to pollen is variable, unpredictable, and depends heavily on weather and patient behaviors.[9] Allergen chambers, on the other hand, represent a combination of direct conjunctival challenge and field trials, allowing for a controlled exposure to allergens in a simulated real-world setting. In a small, proof-of-concept clinical trial using an allergen chamber, reproxalap demonstrated preliminary activity in patient-reported symptoms and investigator-assessed ocular redness.[8] Accordingly, the Phase 3 INVIGORATE Trial was performed to assess the activity of reproxalap in a real-world model of seasonal allergen exposure. Based on published clinical relevance thresholds for patient-reported ocular itching and redness,[9] two different within-patient responder analysis methods were used to confirm the clinical relevance of the results.

# Materials and Methods

## Trial Design

Patients with seasonal allergic conjunctivitis were enrolled in the single-center, crossover, placebo-controlled, sequence-randomized, quadruple-masked (patient, care provider, outcomes assessor, investigator) Phase 3 INVIGORATE Trial. The trial was performed in Canada (Cliantha Research, Mississauga, Ontario) in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Patients, the International Council for Harmonization Guideline on Good Clinical Practice, and all applicable local regulatory requirements and laws. Institutional review board (IRB)/ethics committee approval was obtained from Advarra, Inc. (Columbia, MD, USA). Informed consent was obtained from the patients, and the research followed Health Insurance Portability and Accountability Act and Personal Information Protection and Electronic Documents Act regulations. The clinical trial is registered in the National Library of Medicine database (http://www.clinicaltrials.gov; NCT04207736).

The trial was designed to evaluate the activity and clinical utility of 0.25% topical ocular reproxalap relative to vehicle. Patients were exposed to ambient aerosolized ragweed pollen in an allergen chamber for 3.5 hours, which approximates the average maximum duration of allergen exposure that patients are generally willing to withstand. As described previously,[18] commercially obtained ragweed pollen (*Ambrosia artemesiifolia*; Greer Laboratories, Lenoir, NC, USA) was aerosolized in HEPA-filtered fresh air and maintained at 3500 ± 500 grains/$m^3$ with rigorous temperature and humidity controls.

The trial consisted of four visits: a medical screening visit that included ragweed pollen skin testing; an allergen chamber exposure qualification visit to elicit conjunctival redness and itching; and 2 treatment visits during which patients were treated topically with one drop of test article in each eye within 5 minutes before chamber entry and again 90 minutes after chamber entry, when peak symptoms are typically apparent. The second dose was designed to recapitulate a typical patient-driven scenario during prolonged symptomatic antigen exposure. All allergen chamber visits were separated by 2 weeks ± 3 days. Patients were randomly assigned (1:1) via a predetermined randomization sequence to one of two sequences for the test article visits: reproxalap then placebo or placebo then reproxalap. Reproxalap and vehicle were matched in volume and appearance, and patients and investigators were masked to treatment assignment throughout the trial.

## Patient Population

As previously described,[8] adult patients aged ≥18 years with a history of ≥2 years of moderate to severe allergic conjunctivitis, a positive skin test result (≥3 mm) for ragweed pollen, and chamber-induced ocular itching and redness

scores of ≥2.5 and ≥2 (both scales range from 0 to 4), respectively, were eligible. Patients were excluded for any clinically significant slit lamp findings or a history of inflammatory or infectious disease that could interfere with the conduct of the trial. Other exclusions were use of antihistamines within 7 days before screening and use of corticosteroid or immunotherapeutic agents within 14 days before screening. Patients were excluded or deferred to a later visit if ocular itching and redness scores were >0.5 or >1, respectively, in either eye before any allergen chamber visit.

## Study Assessments and Endpoints

Ocular itching and tearing scores were recorded by patients using an electronic tablet at approximately 2, 7, 12, 17, 22, 32, 42, 52, 72, 92, 97, 102, 107, 112, 122, 132, 142, 152, 172, 192, and 212 minutes after chamber entry on a scale ranging from 0 (no itching) to 4 (severe), allowing half-unit increments, for itching and from 0 (no tearing) to 3 (severe) for tearing. On a similar schedule offset by at least 3 minutes, for the nasal and temporal aspect of each eye, trained technicians visually assessed conjunctival redness without a slit lamp based on a validated descriptive and photographic redness scale from 0 (none) to 4 (severe) with 0.5 increments.[19] Similar assessments of ocular itching, tearing, and redness were performed at approximately 2, 7, 12, 17, 22, 32, 42, and 62 minutes after chamber exit. Safety assessments were visual acuity, slit lamp biomicroscopy, undilated fundoscopy, intraocular pressure (non-contact intraocular pressure tonometry), and adverse events. Patients were monitored for symptoms and signs of asthma or other conditions that could affect study conduct or interpretation.

## Statistical Analysis

The primary endpoint was change from baseline in ocular itching score from 110 to 210 minutes after chamber entry. Achievement of the endpoint necessitated that ocular itching for the majority of prespecified time points during the 110 to 210 minutes after chamber entry was statistically significantly lower in reproxalap-treated patients than in vehicle-treated patients. The key secondary end point was change from baseline in ocular redness, averaged across both regions and both eyes, over the duration of the chamber. Achievement of the key secondary end point necessitated that, across all chamber time points assessed in aggregate, ocular redness was statistically significantly lower in reproxalap-treated patients than in vehicle-treated patients. The additional secondary endpoint was ocular tearing score over the duration of the chamber.

Assuming a treatment difference from vehicle of 0.5 units in itching score change from baseline, and assuming an estimated standard deviation of 1.35 units, a sample size of 100 participants provided a power of 96% at 2-sided significance level of 0.05 for a crossover design. Mixed-effect models for repeated measures (MMRM) were used to assess each endpoint, with sequence, visit, treatment group, time after chamber entry, and the interaction of treatment group and time as factors. Baseline was defined as the average of scores just before chamber entry. Rank-based nonparametric testing for longitudinal data was used as a sensitivity analysis for the MMRM assessments.[20] To assess clinical relevance, based on previously reported clinical relevance threshold estimates of 0.5 units for ocular itching and redness,[9] within-patient (reproxalap minus vehicle) time to ocular itching and redness scores of ≥0.5 in the allergen chamber were calculated and compared via 1-way *t* test to 0 (no difference between reproxalap and vehicle). Binomial generalized estimating equation (GEE) modeling with the same factors as the MMRM analyses was used to assess differences in 0.5-unit responder proportions over the duration of the chamber for ocular itching and redness. Safety assessments were characterized with summary statistics.

# Results

## Patient Disposition and Characteristics

Patient disposition is diagrammed in Figure 1. A total of 568 patients with allergic conjunctivitis were screened, and 95 were randomly assigned and treated between October 15, 2019, and February 16, 2021. As is typical with allergen chamber trials, the high rate of screening failures was due to exclusions related to allergen chamber itching and redness requirements, potentially reflecting perennial allergy or clinically insignificant ocular allergy to ragweed. There were 6 discontinuations: 3 patients were discontinued because of protocol violations (vehicle to reproxalap sequence), 1 patient voluntarily withdrew (vehicle to reproxalap sequence), 1 patient was discontinued because of scheduling conflicts

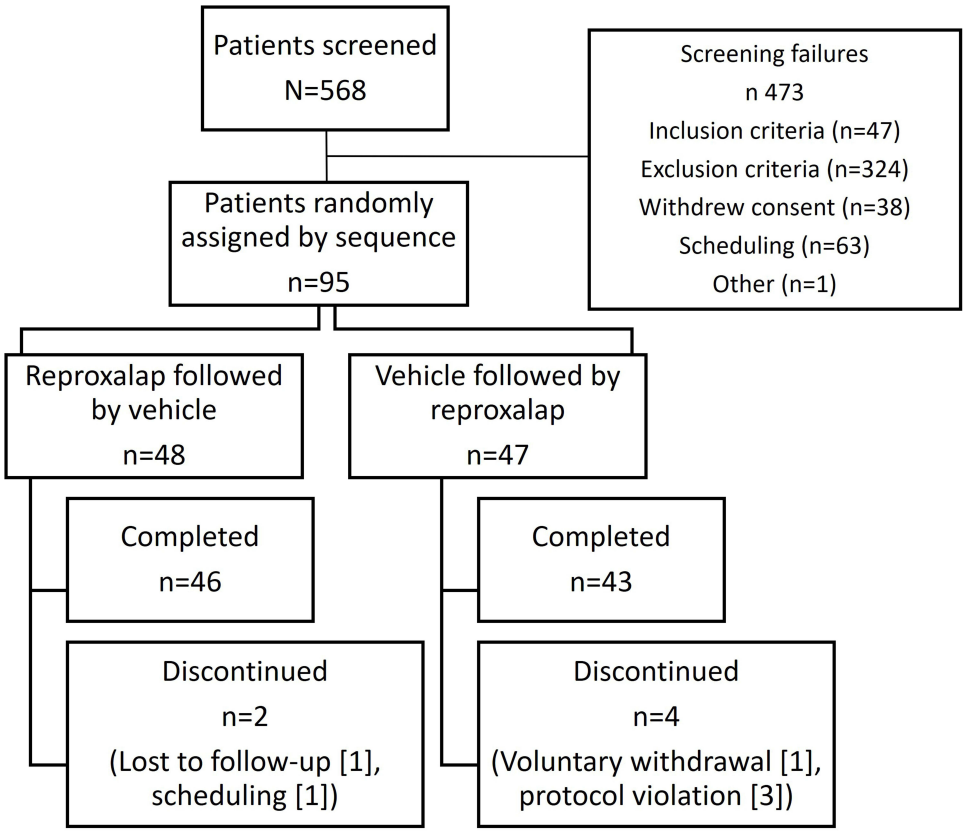


**Figure 1** Patient disposition. N = number of randomly assigned patients in the population; n = number of patients with data.

(reproxalap to vehicle sequence), and 1 patient was lost to follow up (reproxalap to vehicle sequence). Eighty-nine patients completed all treatment periods. Baseline demographic characteristics were generally comparable across sequence groups (Supplemental Table 1); patients identifying as white represented approximately half of the enrolled population, and patients identifying as Asian or Black each represented approximately 20% of the enrolled population.

## Efficacy

The primary endpoint of ocular itching was achieved: relative to vehicle, treatment with reproxalap led to lower levels of patient-reported ocular itching at all prespecified time points (Figure 2; $p < 0.001$ for each time point; Supplemental Table 2).

The key secondary endpoint of investigator-assessed conjunctival redness (Figure 3; $p < 0.001$; Supplemental Table 2) and the secondary endpoint of patient-reported ocular tearing (Figure 4; $p < 0.001$; Supplemental Table 2) were also achieved. Non-parametric assessments confirmed the MMRM results (Supplemental Table 2). Escalation of symptoms and redness after prechamber dosing was blunted with reproxalap. Although treatment with both reproxalap and vehicle during the chamber arrested the increase in symptoms and redness, scores after reproxalap treatment remained lower than those after vehicle treatment over the duration of the chamber, as well as over all time points after chamber exit.

## Clinical Relevance Assessments

Within-patient time to increase of 0.5 points[9] for ocular itching (reproxalap minus vehicle mean, 14.7; 95% CI, 4.6–24.8; $p = 0.005$) and redness (reproxalap minus vehicle mean, 22.1; 95% CI, 2.5–41.7; $p = 0.028$) in the allergen chamber was longer after reproxalap treatment than after vehicle treatment (Figure 5). The time to event analyses were confirmed with 0.5-unit GEE responder proportions over the duration of the chamber for ocular itching (Supplemental Figure 1; odds ratio [OR], 0.53; 95% CI, 0.35–0.81; $p = 0.003$) and redness (Supplemental Figure 2; OR, 0.58; 95% CI, 0.39–0.85; $p = 0.005$), indicating that patients were more likely to demonstrate an increase of 0.5 points after vehicle treatment than after reproxalap.

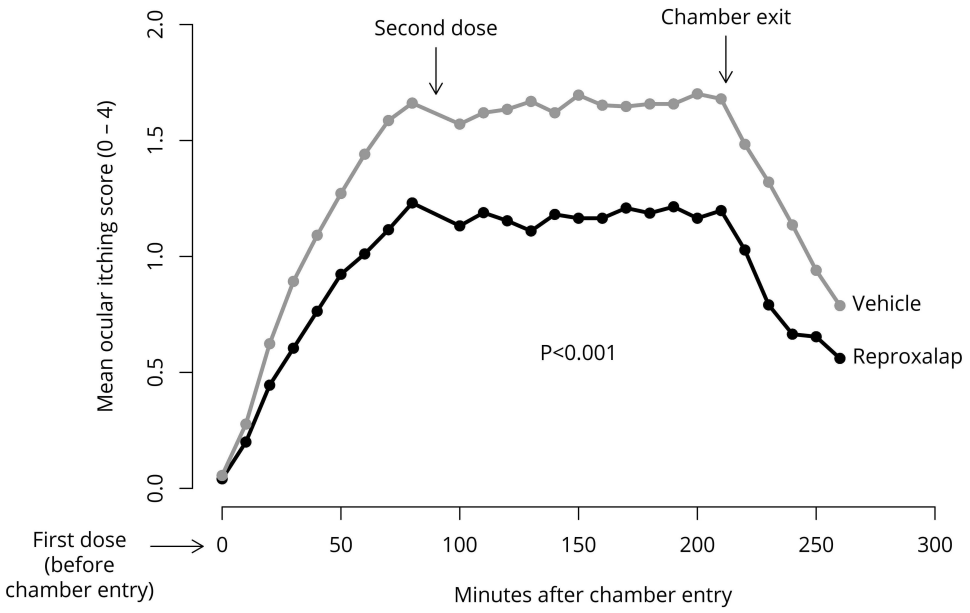


**Figure 2** Patient-reported ocular itching score. P value derived from MMRM analysis of change from baseline (just prior to chamber entry) over all time points in aggregate. MMRM = mixed-effect model for repeated measures.

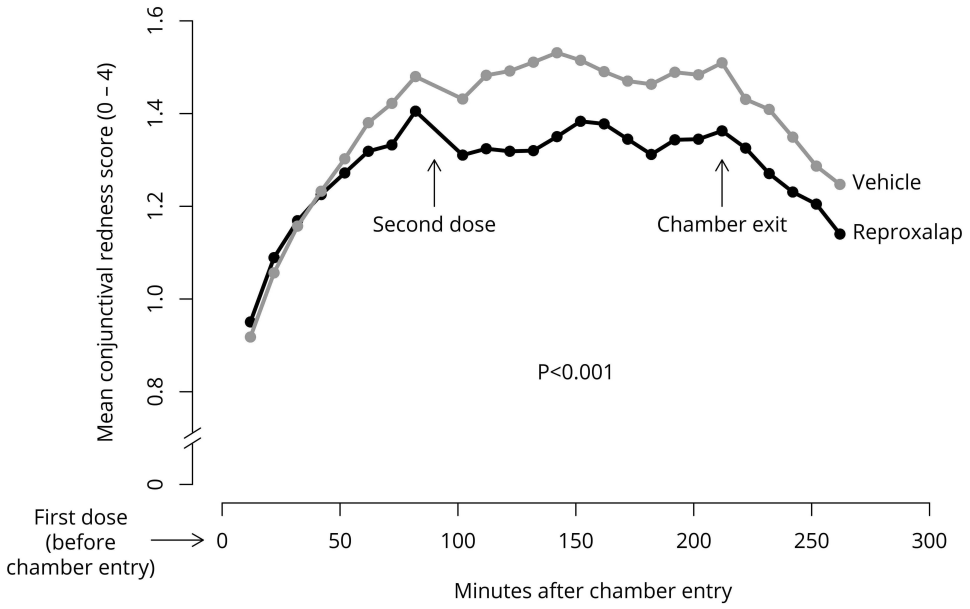


**Figure 3** Investigator-assessed conjunctival redness score. *P* value derived from MMRM analysis of change from baseline (just before chamber entry) over all time points in aggregate. MMRM = mixed-effect model for repeated measures.

## Safety and Tolerability

Reproxalap was considered safe and well tolerated. No clinically significant changes in visual acuity readings, fundus assessments, vital signs, or clinical laboratory parameters were observed with reproxalap. No serious or severe treatment-emergent adverse events (TEAEs) were reported, and no participants were discontinued or withdrew because of a TEAE. The most commonly reported TEAE was mild and transient installation site irritation, reported in 66 patients (69%) after reproxalap administration and in 4 patients (4%) after vehicle administration. Other TEAEs were infrequent and were reported by two or fewer patients following exposure to each treatment (lacrimation, eyelid swelling, pruritis, pain,

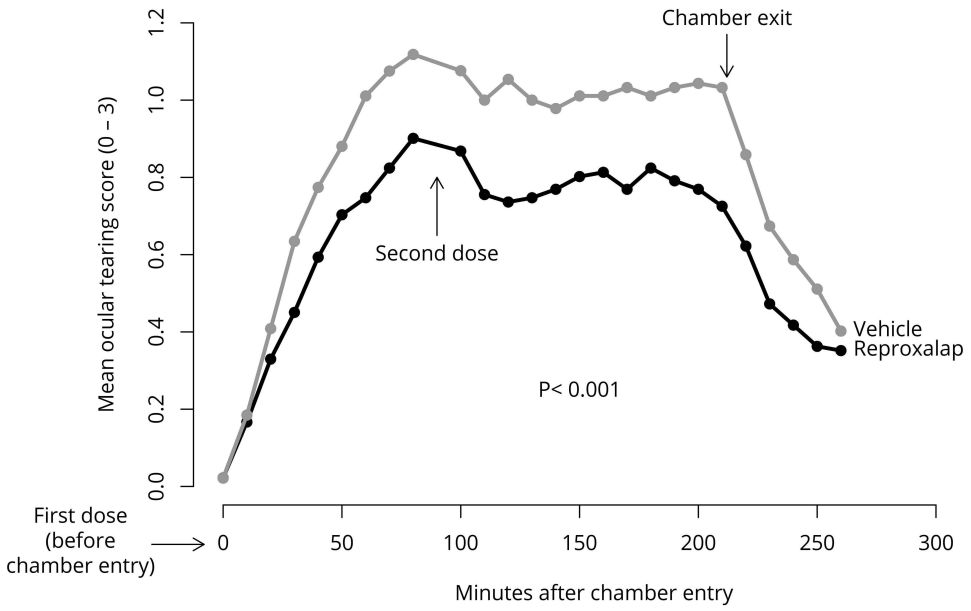


**Figure 4** Patient-reported ocular tearing score. *P* value derived from MMRM analysis of change from baseline (just prior to chamber entry) over all time points in aggregate. MMRM = mixed-effect model for repeated measures.

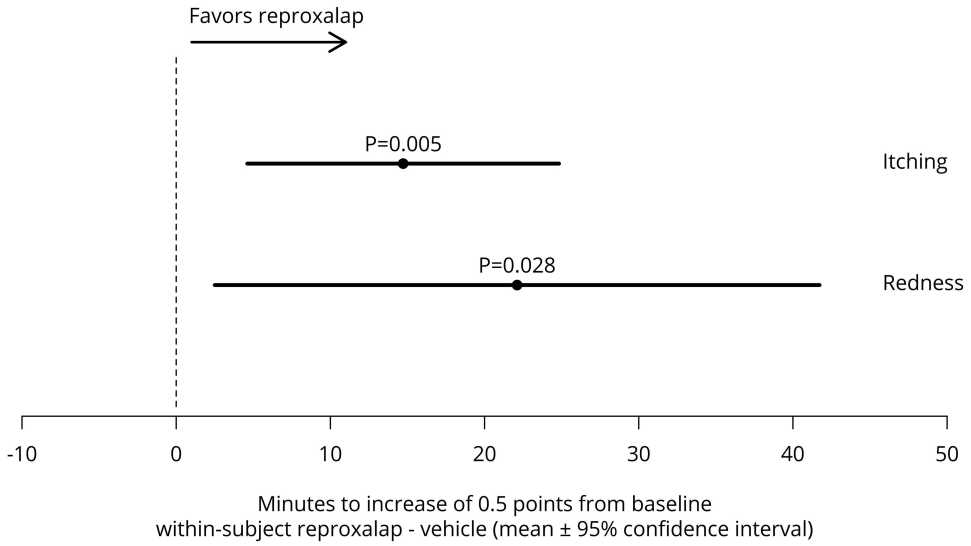


**Figure 5** Mean (± 95% CI) within-patient time to patient-reported ocular itching and investigator-assessed redness score of 0.5[9] (scale 0–4) for reproxalap minus vehicle during the allergen chamber. *P* values derived from 1-way *t* test versus 0 (no difference between reproxalap and vehicle).

hyperemia, asthenopia) except for transient and predominantly mild chemosis, which was observed in 8 patients (8.8%) following reproxalap treatment.

## Discussion

The INVIGORATE Trial demonstrated rapid and sustained activity of reproxalap in diminishing ocular symptoms and redness in patients with allergic conjunctivitis over a period of 3.5 hours in an allergen chamber (Figures 2–4; $p < 0.001$ for all assessments). The results are consistent with the activity of reproxalap reported in a field trial, a small proof-of-concept allergen chamber trial, and the Phase 3 ALLEVIATE conjunctival challenge trial in patients with allergic conjunctivitis.[7–9] Approximately half the patients with ocular itching, the principal symptom of allergic conjunctivitis, also report ocular dryness, the principal symptom of dry eye disease, suggesting a strong relationship between the two

conditions.[21] Therefore, the activity of reproxalap in allergic conjunctivitis may also be consistent with reported results in dry eye disease clinical trials.[11,12]

The clinical relevance analyses for INVIGORATE were exclusively within-patient and used an empirically derived clinical relevance threshold of 0.5 units (0–4 scale) for ocular itching and redness.[9] Although intra-chamber escalation to 0.5 units occurred in most patients with regard to ocular itching and in fewer patients with regard to ocular redness, time to response (Figure 5) and GEE responder proportion (Supplemental Figures 1 and 2) analyses, two distinct though possibly correlated statistical methods, statistically favored reproxalap over vehicle for ocular itching ($p = 0.005$ and $p = 0.003$, respectively) and redness ($p = 0.028$ and $p = 0.005$, respectively). The results suggest that the treatment group differences in changes observed in the allergen chamber were likely meaningful to individual patients. However, clinical utility analyses are limited by the notion that patients are generally not concerned with changes relative to vehicle, but rather changes relative to baseline. Because the change from baseline is larger than that of the change across treatment groups, it is possible that in a real-world treatment setting, patients with allergic conjunctivitis could recognize clinical improvement in ocular itching and redness after reproxalap administration.

Reproxalap is a new chemical entity directed toward the novel pharmaceutical target of RASP, thereby effecting a unique pharmacology distinct from the single-drug, single-protein pharmacology that makes up nearly all of the current therapeutic landscape. Although much of the activity of RASP has been attributed to activation of classic proinflammatory mediators,[2,3] RASP theoretically bind any protein with accessible amine and thiol moieties,[1] and thus likely modulate the activity of proteins not directly associated with inflammation. Therefore, the rapid activity of reproxalap in the allergen chamber may not exclusively represent immune-modulating activity. For example, RASP have been shown to mediate nociceptive transient receptor potential (TRP) receptor family and vasomotor function,[22,23] which could account in part for the symptomatic and redness activity of reproxalap, respectively. However, acute RASP modulation is unlikely to explain long-term activity observed in noninfectious anterior uveitis and dry eye disease clinical trials lasting weeks,[10,12] allowing for the possibility that the activity of reproxalap may include distinct acute non–immune-modulating and long-term immune-modulating mechanisms of action.

The drop comfort of reproxalap more than one hour after instillation was previously characterized,[24] and in most patients, includes mild instillation-associated irritation, followed by soothing sensations. This is consistent with the INVIGORATE results in which symptom responder rates were superior to those of vehicle, yet more than half the patients reported initial, mild transient irritation. Because TRP receptors modulate nociception and soothing sensations and are intimately regulated by RASP,[22] TRP receptor modulation may explain the post-instillation experience observed in INVIGORATE and other trials of reproxalap. If reproxalap does modulate the TRP family, then the significant number of patients who do not report post-instillation irritation (approximately 30%) is puzzling and may be a result of lack of reporting, neurobiological corneal sensation differences from patient to patient, or the presence of mild forms of diseases in which sensation is diminished, such as neurotrophic keratitis. However, post hoc analyses indicated that ocular itching and redness were not different across patients who reported post-instillation irritation versus those who did not (data not shown).

Surprisingly, given the significant incidence of allergic conjunctivitis, new mechanisms of action for treatment have not been introduced in decades. Although topical antihistamines are considered standard of care for allergic conjunctivitis, the most common reports submitted to the US Food and Drug Administration Adverse Event Reporting System database for topical antihistamine monotherapy (olopatadine, ketotifen, alcaftadine, epinastine, and bepotastine) are "treatment failure" (45%) and "drug ineffective" (17%),[25] which may partially explain why polypharmacy is common in allergic conjunctivitis.[14] Histamine is rapidly metabolized and likely peaks soon after allergen exposure, thereby limiting the duration of activity of antihistamines.[17] Furthermore, many non-histaminic mediators likely contribute to the clinical presentation of ocular allergy.[6] Because of toxicity concerns,[15] corticosteroids can only be safely used on a short-term basis. Therefore, novel treatments are needed. The data reported herein suggest that use of the novel RASP modulator reproxalap may be an effective treatment for the symptoms and signs of allergic conjunctivitis. By extension, based on the activity reported herein, RASP modulation may represent a potential therapeutic approach for other allergic diseases.

Additional studies on the mechanism of reproxalap and the pathophysiologic activity of RASP in allergy are warranted. Limitations of the current clinical trial include the use of a single site (the allergen chamber), though the number of validated allergen chambers available for clinical testing worldwide is limited. Exposure was restricted to a single allergen (ragweed) considered representative of seasonal allergy, and it is theoretically possible that exposure to perineal allergens could yield

different results. Due to dilution of allergen exposure and potential clearance of allergen from the ocular surface, any ophthalmic solution or suspension will likely demonstrate at least some activity in allergen chamber trials, as was evidenced by the intra-chamber dose of vehicle. Further, although post-instillation site irritation was transient and generally resolved prior to chamber entry, thereby precluding the need for additional measures to prevent unblinding of technicians in the chamber, patients may have noticed a difference in irritation between vehicle and reproxalap administration, which could have affected patient blinding and symptom reporting. However, instillation site irritation is common with topical ocular drugs and represents an unavoidable consequence of using ophthalmic solutions in clinical trials. Had irritation persisted after chamber entry, itching and redness scores may have been higher following reproxalap administration than with vehicle administration, in contrast to the results. Future trials may include active controls, such as antihistamines, although comparison to antihistamines is of limited clinical relevance since topical antihistamines are now available without prescription and many patients seeking medical assistance have already failed antihistamine therapy. Finally, consensus recommendations regarding the utility of allergen chambers in drug development have been summarized,[26] and include the importance of confirmatory field trials and other challenge models, as have been described with reproxalap.[7,9]

## Data Sharing Statement

Due to commercial and legal restrictions, supporting data is not available.

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## Disclosure

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