Efficacy of N-acetyl aspartyl glutamic acid versus fluorometholone for treating allergic conjunctivitis in an environmental exposure chamber

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Abstract
Background: Topical mast cell stabilizers were previously shown to treat the signs and symptoms of seasonal and perennial allergic conjunctivitis safely and effectively in active and placebo-controlled trials. However, mast cell stabilizers have not been compared to topical corticosteroids for efficacy. We tested the non-inferiority of a topical mast cell stabilizer, N-acetyl aspartyl glutamic acid (4.9%, NAAGA), compared to fluorometholone (0.1%, FM) during controlled exposures to the airborne birch pollen allergen, Bet v 1, in an environmental exposure chamber (EEC).

Methods: This randomized, cross-over, investigator-blinded study included 24 patients with a history of birch pollen allergic conjunctivitis. Patients were randomized to 5 days of treatment with NAAGA, then FM (n = 12) or FM, then NAAGA (n = 12).

After each treatment, patients were exposed to a fixed airborne concentration of Bet v 1 in ALYATEC EEC. The primary endpoint was the amount of allergen required to trigger a conjunctival response (Abelson score ≥5). Groups were compared with a linear model for cross-over studies. Non-inferiority was assumed, when the lower bound of the risk ratio confidence interval (CI) was >0.5.

Results: At screening, the mean time-to-conjunctival response was 72.5 ± 35.9 min. NAAGA and FM extended the response time to 114.8 ± 55.0 and 116.6 ± 51.5 min respectively. The mean amounts of allergen required to trigger a conjunctival response were 1.165 ng after NAAGA and 1.193 ng after FM treatment. The risk ratio for the conjunctival response was 0.977 (95% CI: 0.812; 1.174), which indicated non-inferiority. Adverse events occurred less frequently with NAAGA (29.2%) than with FM (58.3%).

Conclusion: In patients with allergic conjunctivitis to birch pollen, NAAGA was non-inferior to FM in exposures to airborne Bet v 1. The EEC was a good model for simulating real-life airborne allergen exposure and for demonstrating the efficacy and safety of eye drops for treating allergic conjunctivitis.

Trial registration: Not registered.

Keywords
allergic conjunctivitis, birch allergen, environmental exposure chamber, fluorometholone, N-acetyl aspartyl glutamic acid
INTRODUCTION

The estimated prevalence of allergic conjunctivitis is 15%–20% in general populations of developed countries, and 40%–60% among individuals with allergies. The signs and symptoms of allergic conjunctivitis are mostly benign, but they significantly impact quality of life. Seasonal allergic conjunctivitis (SAC) occurs in 55% of allergic ocular pathologies, including perennial allergic conjunctivitis, vernal keratoconjunctivitis, lid skin involvement and keratitis. In central and northern Europe, SAC is most frequently triggered by birch pollen. SAC is a type I hypersensitivity response to airborne allergens, with mast cell degranulation and the release of histamine, prostaglandins, tryptases and leukotrienes. Histamine causes ocular pruritus, the main symptom of allergic conjunctivitis. Other symptoms include tearing, redness and swollen eyelids. Patients with SAC are commonly prescribed a local anti-allergy treatment that includes mast cell stabilizers and antihistamine eye drops to improve the symptoms and quality of life. The gold standard for establishing the efficacy and safety of an anti-allergy treatment is a randomized double-blind, placebo-controlled study. However, field studies are challenging because environmental humidity, air pressure and temperature are difficult to control. Moreover, during the pollen season, variations in pollen concentrations can lead to widely variable symptoms. Consequently, a large cohort is required to obtain significant results on treatment efficacy. The European Medicines Agency previously considered conjunctival provocation test (CPT) including environmental exposure chamber (EEC) as a useful study approach. These tools were validated for studying the pathology of allergies, including allergic conjunctivitis, and the efficacy of new therapeutics. EECs allow the simultaneous exposure of many patients to consistent levels of airborne allergens, in a temperature- and humidity-controlled environment. The ALYATEC EEC is a new generation chamber, validated for allergic asthma, rhinitis and conjunctivitis. Its capacity is 65 m², and it contains 20 seats. During allergen exposures, the numbers and sizes of airborne particles are continuously monitored to ensure a homogenous allergen distribution. The airborne allergen concentration delivered corresponds to the concentrations measured on high-pollen days during pollen season in the Strasbourg area. With the EEC model, we developed a new approach for studying anti-allergy treatments, which more closely resembled daily life exposure compared to the standard CPT test. In this study, we aimed to evaluate the efficacy of two different eye drop treatments given before individuals with birch pollen allergies were repeatedly exposed to the Bet v 1 allergen.
N-acetyl-aspartyl glutamic acid (NAAGA) is a mast cell membrane stabilizer with C3 convertase antagonist activity and anti-inflammatory properties. NAAGA eye drops are currently marketed in several countries; they were approved in 1984 for treating moderate forms of allergic conjunctivitis. The efficacy and safety of NAAGA eye drops were previously demonstrated in patients with allergic conjunctivitis, including SAC.\textsuperscript{2-14} SAC symptoms occur when pollen dissolves in the tear film, then crosses the conjunctiva.\textsuperscript{15} Consequently, we considered it important to adjust the conjunctival response, according to the tear renewal rate. Alternatively, the corticosteroid, fluorometholone, administered at 0.2% in eye drops, is recommended for short-term treatments of refractory SAC, with careful monitoring.\textsuperscript{6} However, NAAGA has not been compared to corticosteroids, like fluorometholone (FM), for efficacy in treating allergies.

In this study, we compared the efficacies of 4.9% NAAGA and 0.1% fluorometholone in patients allergic to birch pollen that were exposed to birch allergen in ALYATEC EEC. After each of the two treatment periods, we investigated the amount of airborne birch pollen allergen required and the time required to elicit a conjunctival response during allergen exposure.

2 | MATERIALS & METHODS

This randomized, investigator-blinded, cross-over, non-inferiority, phase IV study was conducted between September and October 2017 (i.e. outside the pollen season) at the ALYATEC Research Center (Strasbourg University Hospital, France). We aimed to compare the efficacy and safety of two topical anti-allergic eye drops: 4.9% NAAGA (NAABAK\textsuperscript{®}, Laboratoires Théa) and 0.1% FM (FLUCON\textsuperscript{®}, Alcon Laboratory).

2.1 | Participants

Eligible individuals had mild asthma (GINA step 1 or 2) and an asthma control test result ≥20, according to the American Thoracic Society. Inclusion criteria were as follows: age 18–65 years; a history of moderate allergic conjunctivitis, according to the European Academy of Allergy and Clinical Immunology recommendations; disease persistence for at least 2 consecutive years; a positive skin prick test to birch pollen (papule diameter ≥6 mm larger than a negative control); birch-specific (Bet v 1) IgE production >0.1 kU/L and a positive unitary conjunctival allergen challenge test to birch allergen. Exclusion criteria were as follows: a personal or family history of glaucoma; ocular hypertension; eye surgery in the previous 6 months and other forms of allergic conjunctivitis or non-specific conjunctival hyperactivity.

The study protocol was approved by the local ethics committee (CCP Sud-Est IV, Lyon, France: approval number 17/038, 11 July 2017) before study initiation. All enrolled patients provided written informed consent. The study was conducted in accordance with the principles of Good Clinical Practice and the ethical principles of the Declaration of Helsinki. The study was registered in the EudraCT database, access number: 2017-001838-26.

2.2 | Study design and data collection during exposure in the EEC

The study comprised a screening period, a cross-over treatment period and an end-of-study visit (Figure 1).

The screening period included a screening visit, an individual CPT visit, and two allergen exposures (Expos 1 and 2) in ALYATEC EEC with a wash-out period of 7–14 days between visits (Figure 2). For the CPT, a diluted allergen extract was instilled in the eye at increasing concentrations for a rapid determination of the intensity and severity of the allergic eye response.

The response was evaluated with four conjunctival symptom scores: itching, redness, chemosis and tearing. Ocular itching was assessed by the patient on a 5-point scale: 0 = none, 1 = mild (intermittent tickling sensation), 2 = moderate (contiguous itching, but no desire to rub), 3 = severe (contiguous itching with a desire to rub) and 4 = very severe (incapacitating itch with an irresistible urge to rub). Ocular redness was assessed at the nasal and temporal areas of each eye by the study physician on a four-point scale: 0 = absent, 1 = mild, 2 = moderate and 3 = severe. A slit lamp was used to evaluate redness and chemosis. Tearing was assessed by the physician on a four-point scale: 0 = absent, 1 = mild (eyes feel slightly watery), 2 = moderate (blows nose occasionally) and 3 = severe (tears rolling down cheeks). Chemosis was assessed by the study physician on a three-point scale: 0 = absent, 1 = mild (detectable with a slit lamp, conjunctiva separated from sclera), 2 = moderate (visually evident, raised conjunctiva, particularly at the limbal area) and 3 = severe (conjunctival ballooning). The sum of the scores was calculated for the total ocular symptoms score (TOSS, range: 0–13), first described by Abelson.\textsuperscript{16} The historical severity of ocular symptoms was also assessed at screening with a visual analogue scale (VAS), where 0 = no symptoms and 10 = the worst imaginable severity.

When the Abelson score was <5 at baseline (Expos 1 and 2), the patient was withdrawn from the study. Eligible patients were randomly allocated at a 1:1 ratio to one of two cross-over treatments, each with two treatment periods, where the drugs were administered in a sequence of FM/NAAGA or NAAGA/FM.

After a wash-out period of 7–14 days, and before treatment, the baseline conjunctival response to airborne birch allergen was assessed on two consecutive exposures for up to 4 h each (Expos 1 and 2). Then, at 24 and 48 h after the last dose of Treatment 1, patients were again exposed to the allergen (Expos 3 and 4). Finally, at 24 and 48 h after the last dose of Treatment 2, patients were once again exposed to the allergen (Expos 5 and 6). All allergen exposures were conducted by nebulizing a solution of lyophilized extract, always from the same batch (100 IR; Stallergenes Greer\textsuperscript{®}), diluted in saline. During each exposure, the allergen was collected on fiberglass filters located close to the patient chairs. After each exposure, nebulized
Bet v 1 was quantified with a monoclonal antibody on an ELISA, according to the manufacturer’s protocol (Indoor Biotechnologies Inc., Charlottesville, VA). Consequently, a total dispersion of 60 ng/m$^3$ of airborne Bet v 1 was confirmed after each exposure.

All exposures lasted up to 4 h. Clinical assessments of the allergen conjunctival response were performed every 10 min during the first hour of exposure, then every 20 min, until a positive conjunctival response was observed. A positive conjunctival response was defined as a mean Abelson TOSS ≥5, averaged over both eyes.

The forced expiratory volume in 1 s (FEV$_1$) was carefully monitored with a portable spirometer, before, every 20 min during and after each allergen exposure. When the FEV$_1$ dropped by ≥20%, the patient was withdrawn from the EEC. Vital signs were monitored before and after each session. At the end of the second day of exposure, all patients were treated with antihistamine eye drops. One tablet of antihistamine was administered for rhinitis, and two puffs of short-acting beta 2 agonists were administered for asthma. When an immediate bronchial reaction occurred during an exposure, the patient remained under medical supervision for 6 h. Before leaving the clinical centre, patients were given a rescue medication kit that contained oral antihistamines to be used as needed.

### 2.3 Treatment administration

Each treatment was self-administered at home. One drop was applied three times/day for 5 consecutive days, followed by a 7- to 14-day wash-out period. During treatment, patients were required...
to keep a daily diary to confirm treatment adherence and report adverse events (AEs). Patients were contacted daily by the medical team to check compliance and record potential treatment-emergent AEs (TEAEs). At onsite visits, the pharmacist checked compliance by weighing each vial and comparing the before and after treatment weights. These data were collected in the study file. Compliance with study treatment was 100%. Investigators evaluated blinded data throughout the study to avoid bias when evaluating conjunctivitis. Only the pharmacist who allocated the subjects to the treatments was not blinded.

2.4 Clinical outcomes

The primary outcome was the amount of allergen required to trigger the conjunctival response. All patients remained in the EEC until they showed a positive conjunctival response (Abelson score ≥5). We estimated the amount of allergen (ng) that affected the ocular surface by multiplying the tear film renewal rate (TRR, mm$^3$/min) by the airborne Bet v 1 concentration (60 ng/m$^3$) and by the mean time (min) required to induce the conjunctival response. The tear renewal rate (TRR) was calculated for the ocular surface as follows: ocular surface = $4\pi r^2/2 \times$ eye thickness, where $r = 7$ mm and eye thickness = 0.5 mm. Thus, the TRR was $154 \times 106$ mm$^3$/min.

The secondary efficacy parameters included the time required to trigger the conjunctival response in the EEC and the kinetics of the conjunctivitis symptoms during allergen exposure. The priming effect was defined as the difference in the times-to-conjunctival responses between Expo 1 and Expo 2. The persistence of the treatment effect was assessed as the times-to-conjunctival responses measured at 24 h post-treatment (Expos 3 and 5) compared to the times measured at 48-h post-treatment (Expos 4 and 6).

2.5 Statistical analysis

A power analysis estimated that a sample size of 24 subjects was required to achieve 90% power in detecting non-inferiority with a one-sided test, when the non-inferiority margin was 0.5 and the actual mean ratio (NAAGA/FM) was 1, with a significance level of 0.025.

Efficacy analyses were performed in the intent-to-treat (ITT) population, defined as all patients who received at least the first treatment, as planned in the randomized sequence and then had been exposed at least once to birch allergen in the chamber.

The primary efficacy endpoint was the amount of allergen required for a conjunctival response (Abelson score ≥5). Log-transformed values were estimated for statistical analysis with a linear mixed model. The difference in the least-squares means between treatment groups (NAAGA vs. FM) and the lower bound of its one-sided 97.5% confidence interval (CI) were exponentiated, which resulted in the geometric means of both the NAAGA:FM ratio and the lower bound of its one-sided 97.5% CI. Non-inferiority was assumed, when the lower bound of the one-sided 97.5% CI of the ratio was >0.5. Consequently, the 0.5 margin is unitless because it refers to a ratio.

The time required to trigger a positive conjunctival response was analysed in a Cox proportional hazard model for cross-over designs, with a period effect. The difference between the two treatment groups was estimated with the hazard ratio (NAAGA:FM) and a one-sided 95% CI. The upper bound of the hazard ratio CI was compared to the non-inferiority threshold, which was set at 2. An upper bound below this threshold indicated that, at any time, the risk of a conjunctival response in the study group was not greater than two-fold the risk of the reference group. The median survival (i.e. response) times predicted in the stratified Cox model and their 95% CIs were calculated as described previously.$^{17,18}$ The treatment priming effect and persistence were analysed with Cox proportional hazards models.

3 RESULTS

Among the 31 patients screened, 28 were included and underwent baseline exposures (Expos 1 and 2). Among these, 24 were randomized to one of the two treatment groups. All randomized patients underwent all six allergen exposures in the EEC and completed the end of the study visit.

3.1 Baseline characteristics

The two patient groups were not significantly different in patient characteristics (Table 1), including the mean diameter of the skin prick test, the amount of birch-specific IgE produced and amount of allergen required for a response during the CPT. All patients presented moderate-to-severe conjunctivitis based on the VAS. Co-sensitizations and co-morbidities were frequent: 79.2% of patients were poly-sensitized with mild and moderate-to-severe allergic rhinitis to grass and ash pollen, cat or dog allergens and house dust mites. Only 8.3% were mono-sensitized to birch pollen. In addition, 16 patients (66.7%) had mild, controlled concomitant asthma, consistent with the study inclusion criteria.

3.2 Allergen required to trigger the conjunctival response

The mean amounts of allergen needed to trigger a conjunctival response (Table 2) were 1.165 ng (95% CI: 0.958; 1.416) after NAAGA treatment, and 1.193 ng (95% CI: 0.981; 1.450) after FM treatment. The ratio of mean amounts (NAAGA/FM) was 0.977 (95% CI: 0.812; 1.174; Table 2). Non-inferiority was achieved at the two-sided alpha level of 0.05 because the lower bound (0.812) was above the non-inferiority margin (0.5).
3.3 | Baseline conjunctival response and priming effect

At baseline (before treatment), the time-to-conjunctival response during Expo 1 was 72.5 ± 35.9 min (median 63.5 min), which corresponded to 0.80 ng Bet v 1. During Expo 2, the response time was 58.7 ± 28.5 min (median 43.0 min), which corresponded to 0.63 ng Bet v 1. The Cox model showed a significant difference between Expos 1 and 2 (p = 0.0401), with a hazard ratio of 2.375 (95% CI: 1.040; 5.425), consistent with a priming effect (Figure 3).

3.4 | Post-treatment time-to-conjunctival response

The mean times-to-conjunctival responses were 114.8 ± 55.0 min after NAAGA treatment, and 116.6 ± 51.5 min after FM treatment. The cumulative probabilities of remaining in the exposure chamber over time were based on the survival curves estimated with the Cox model (Figure 4). The estimated hazard ratio was 0.730 (two-sided 95% CI: 0.309; 1.725). The upper bound of the two-sided 95% CI (1.725) was <2, and thus, non-inferiority was concluded. Based on this model, the predicted median times to the responses were 122 min (95% CI: 101; 143) after NAAGA treatment, and 102 min (95% CI: 82; 123) after FM treatment.

3.5 | Kinetics of Abelson score

The kinetics of the response were assessed over time with the Abelson TOSS (redness, pruritus, tearing and chemosis) (Figure 5). In both treatment groups, symptoms appeared in the following chronological order: pruritus, redness, tearing and chemosis. The TOSS values were not significantly different between groups.
Persistence of treatment effect

The persistence of the treatment effect for each drug was assessed by comparing the conjunctival responses measured in the EEC at 24 and 48 h post-treatment. Response times were compared between Expos 3 and 4 (at 24 and 48 h after the last dose instillation for Treatment 1) and between Expos 5 and 6 (at 24 and 48 h after the last dose instillation for Treatment 2). For FM, the mean times-to-conjunctival responses were 117 ± 52 min at 24 h and 86 ± 50 min at 48 h post-treatment. Based on the Cox model, these times were significantly different (p = 0.0138); the hazard ratio was 3.167 (95% CI: 1.265–7.929). Similarly, for NAAGA, the mean times-to-conjunctival responses were 115 ± 55 min at 24 h and 78 ± 45 min at 48 h post-treatment. Again, the times were significantly different (p = 0.0017); the hazard ratio was 5.500 (95% CI: 1.895–15.960). These data were consistent with the absence of a persistent treatment effect for both FM and NAAGA.

3.7 | Safety

Treatment-emergent AEs occurred less frequently with NAAGA treatment (29.2%) than with FM treatment (58.3%; Table 3). All TEAEs were mild in both groups, except one patient who reported a moderate headache with the NAAGA treatment.

4 | DISCUSSION

EECs are useful for evaluating the efficacy of anti-allergic medications, particularly when the emphasis is on the onset and duration of these drugs. In this study, we showed that 4.9% NAAGA was non-inf erior to 0.1% FM in patients with birch allergies. The allergen challenge was performed in an ALYATEC EEC, according to current guideline recommendations and based on a previous validation of dose standardizations. The exposures were continuously monitored, and the airborne allergen concentration was reproducible, with an inter-test coefficient of variation <30%.

Currently, we lack consensus on which clinical end-point should be used to determine the efficacy of anti-allergy drugs tested in allergen chambers. In this study, patients were carefully selected based on Abelson scores, a method validated by the FDA. The scores were measured during both an individual CPT.

| TABLE 2 Amount of allergen and exposure time required to achieve a positive conjunctival response |
| --- |
| **Amount of allergen (ng)** |
| **Descriptive means ± SD** |
| FM | 1.32 ± 0.58 |
| NAAGA | 1.30 ± 0.63 |
| **Estimated mean (two-sided 95% CI)** |
| FM | 1.193 (0.981; 1.450) |
| NAAGA | 1.165 (0.958; 1.416) |
| **Ratio (NAAGA/FM) (one-sided 97.5% CI)** |
| 0.977 (0.812; inf) |
| **Time-to-conjunctival response (min)** |
| **Median survival time (two-sided 95% CI)** |
| FM | 102 (82, 123) |
| NAAGA | 122 (101; 143) |
| **Hazard ratio (NAAGA/FM)** |
| 0.730 (0.309; 1.725) |

Abbreviations: FM, fluorometholone; NAAGA, N-acetyl aspartyl glutamic acid.

*From a linear mixed model adjusted for a fixed treatment, the period, sequence effects and a random patient effect.

*Non-inferiority was assumed, when the lower bound of the one-sided 97.5% CI (0.812) was above the non-inferiority margin (0.5).

*From a Cox model with a period effect.

*Non-inferiority can be assumed at the two-sided alpha level of 0.05 because the upper bound (1.725) is below the non-inferiority margin (2.0).
and a baseline allergen exposure in the EEC. Nevertheless, standardized diagnostic criteria for patient selection and a consensus on quantifiable primary outcomes are needed for future studies in allergic conjunctivitis.

Here, we combined evaluations of safety and efficacy based on recommendations from more advanced methodologies. The amount of allergen needed to induce a conjunctival response was chosen as the primary end-point to demonstrate drug efficacy. This measurement was more objective than the efficacy evaluated in field studies, where the amounts of pollen are unknown. Moreover, allergen chamber studies can largely avoid confounding factors.

Drug efficacy was also evaluated by quantifying the time to a conjunctival response to Bet v 1. Re-challenging patients at 24-h intervals showed that the risk of conjunctival symptoms increased with time, consistent with a priming effect that lowered the allergen threshold for subsequent symptoms. This priming effect is well known; it was previously observed with ragweed and grass pollen tested in exposure chambers. We performed the allergen challenge at 24 h after the last treatment instillation. This study design differed from other studies that investigated treatment efficacy, where the topical treatment was administered either just before or just after the allergen challenge. We selected a 24 h time interval based on the sustained relief and the long onset observed with corticosteroid treatments. Consequently, we demonstrated that both NAAGA and FM significantly increased the time to symptom onset.

**FIGURE 5** Abelson total ocular symptom scores assessed in the EEC after treatment. Mean symptom scores were assessed every 10 or 20 min. Assessments started at 24 h after the last dose of (A) NAAGA treatment or (B) FM treatment and continued until a conjunctival response occurred (mean Abelson score ≥5). The numbers of patients who remained in the EEC are indicated above each time-point.

**TABLE 3** Treatment-emergent adverse events (TEAEs)

| All TEAEs | FM (N = 24) | NAAGA (N = 24) |
|-----------|-------------|----------------|
|           | NAE | NP | %  | NAE | NP | %  |
| Eye irritation | 3  | 3  | 12.5 | -  | -  | -  |
| Eye pruritus | 7  | 7  | 29.2 | 6  | 5  | 20.8 |
| Ocular hyperaemia | -  | -  | -  | 2  | 1  | 4.2  |
| Vision blurred | 2  | 2  | 8.3 | -  | -  | -  |
| Xerophthalmia | 1  | 1  | 4.2 | -  | -  | -  |
| Upper abdominal pain | 1  | 1  | 4.2 | -  | -  | -  |
| Conjunctivitis | -  | -  | -  | 2  | 2  | 8.3  |
| Hordeolum | 1  | 1  | 4.2 | 3  | 2  | 8.3  |
| Dysgeusia | 2  | 2  | 8.3 | -  | -  | -  |
| Headache | 1  | 1  | 4.2 | 2  | 1  | 4.2  |
| Asthma | 1  | 1  | 4.2 | -  | -  | -  |
| Cough | 2  | 1  | 4.2 | -  | -  | -  |

Abbreviations: %, NP/N × 100; FM, fluorometholone; NAAGA, N-acetyl aspartyl glutamic acid; NAE, number of adverse events; NP, number of patients with at least one adverse event.
The kinetics of symptoms is an important issue. Previous studies, with grass or ragweed pollen extracts, showed that nasal and/or ocular symptoms increased over the first 2 h.\textsuperscript{24,26,27} In those studies, the conjunctival response was based on only itching and tearing. In the present study, in addition to tearing and itching, we included redness and chemosis assessed by the study physician with a portable slit lamp. We found that these symptoms appeared chronologically, first itching and redness occurred, then tearing and finally, chemosis. This finding was consistent with those previously reported.\textsuperscript{6,9,16}

The main limitations of this study were inherent to the EEC model: the study was mono-centric, we selected patients with a seasonal allergy to a specific birch allergen extract, and measurements were conducted outside the pollen season. Nevertheless, a previous study demonstrated a strong clinical correlation between exposure to natural pollen and a controlled exposure to a pollen antigen in an environmental chamber.\textsuperscript{25} In the present study, we selected a Bet v 1 concentration (60 ng/m\textsuperscript{3}) that corresponded to the airborne concentrations found during the pollen season (up to 6000 pollen grains/m\textsuperscript{3}). Of note, the last treatment instillation was applied the night before the first exposure (Expos 3 and 5). It might have been interesting to investigate the effect of applying the treatment just before entering the chamber; that is, immediately before exposure. An immediate treatment might have more effectively prevented the appearance of symptoms.

Another study limitation was that both treatments had been on the market for many years; therefore, re-packaging the vials was not possible. The degree of bias introduced by this limitation remained unknown.

In conclusion, this study demonstrated that 4.9% NAAGA was as effective as 0.1% FM eye drops for limiting the risk of a conjunctival response in an EEC. Moreover, we found that NAAGA had a lower rate of TEAEs than FM. These findings supported the use of NAAGA eye drops for treating allergic conjunctivitis. We also showed that the ALYATEC EEC was a good model for assessing the efficacy of treatments for allergic conjunctivitis.

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CONFLICTS OF INTEREST
FdB is the medical expert, co-founder and shareholder of ALYATEC. FdB received grants from STALLERGENES GREER, CHIESI and personal fees from ALK ABELLO, MUNDIPHARMA, NOVARTIS and REGENERON. FdB is also a member of the Boards at STALLERGENES GREER, NOVARTIS, ALK ABELLO, MUNDIPHARMA, MEDAPHRMA, BOEHRINGER, ASTRAZENECA and CALOR. ND, IC and AG are ALYATEC employees and have no other conflict of interest to report. TB received personal fees from ALCON, DENSMORE, SANTEN, THEA, ALLERGAN, DOMPE and HORUS.

AUTHOR CONTRIBUTION
FdB was the coordinating investigator of the study and contributed to the study protocol elaboration and statistical analysis plan. AG contributed to data analysis and medical writing. ND contributed to the study protocol elaboration, project management, medical writing and editorial assistance. IC was co-investigator of the study and contributed to patient supervision, data monitoring and analysis. TB participated in this study as an expert ophthalmologist and advised in the evaluation of the patients. All authors contributed to the analysis of the study results and reviewed the manuscript.

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REFERENCES
1. Leonard A, Bogacka E, Fauquert JL, et al. Ocular allergy: recognizing and diagnosing hypersensitivity disorders of the ocular surface. Allergy. 2012;67:1327-1337.
2. Virchow JC, Kay S, Demoly P, et al. Impact of ocular symptoms on quality of life (QoL) work productivity and resource utilisation in allergic rhinitis subjets – an observational, cross-sectional study in four countries in Europe. J Med Econ. 2011;14:305-314.
3. Leonard A, Piliego F, Castegnaro A, et al. Allergic conjunctivitis: a cross-sectional study. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2015;45:1118-1125.
4. Spieksma FTM. The Proceedings of the 7th International Palynological Congress (Part I) Pollinosis in Europe: New observations and developments. Rev Palaeobot Palynol. 1990;64:35-40.
5. D’Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. Allergy. 2007;62:976-990.
6. Abelson MB, Shetty S, Korchak M, et al. Advances in pharmacotherapy for allergic conjunctivitis. Expert Opin Pharmacother. 2015;16:1219-1231.
7. Committee for Medicinal Products for Human Use, European Medicines Agency. Guideline on the clinical development of medicinal products for the treatment of allergic rhinoconjunctivitis. EMEA/EPWP/2455/02. 21 October 2004.
8. Rösner-Fries K, Kaul S, Vieths S, et al. Environmental exposure chambers in allergen immunotherapy trials: Current status and clinical validation needs. J Allergy Clin Immunol. 2015;135:636-643.
9. Gherasim A, Fauquert J-L, Domis N, Siomboing X, de Blay F. Birch allergen challenges in allergic conjunctivitis using standard conjunctival allergen challenge and environmental exposure chamber. J Transl Allergy. 2021;11(6):e12053. doi:10.1002/ct2.12053.
10. Khayath N, Doyen V, Gherasim A, et al. Validation of Strasbourg environmental exposure chamber (EEC) ALYATEC in mite allergic subjects with asthma. J Asthma. 2020;57(2):140-148.
11. Gherasim A, Jacob A, Schoettel F, et al. Efficacy of air cleaners in asthmaics allergic to cat in ALYATEC® environmental exposure chamber. Clin Exp Allergy. 2020;50(2):160-169.
12. Denis D, Bloch-Michel E, Verin P, et al. Treatment of common ocular allergic disorders; a comparison of lodoxamide and NAAGA. Br J Ophthalmol. 1998;82:1135-1138.
13. Leonard A, Bremond-Gignac D, Bortolutti M, et al. Clinical and biological efficacy of preservative-free NAAGA eye-drops versus levocabastine eye drops in vernal keratoconjunctivitis subjects. Br J Ophthalmol. 2007;91:1662-1666.
14. Gündüz K, Üçakhan O, Budak K, et al. Efficacy of lodoxamide 0.1% versus N-acetyl aspartyl glutamic acid 6% opthalmic solutions in patients with vernal keratoconjunctivitis. Ophthalmic Res. 1996;28:80-87.
15. Fauquet JL, Jedrzejczak-Czechowicz M, Rondon C, et al. Interest Group on Ocular Allergy (IGOA) from the European Academy of Allergy and Clinical Immunology. Conjunctival allergen provocation test: guidelines for daily practice. Allergy. 2017;72:43-54.

16. Abelson MB, Chambers WA, Smith LM. Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. Arch Ophthalmol. 1990;108:84-88.

17. France LA, Lewis JA, Kay R. The analysis of failure time data in crossover studies. Stat Med. 1991;10:1099-1113.

18. Brookmeyer R, Crowley J. A confidence interval for the median survival time. Biometrics. 1982;38:29-41.

19. Day JH, Horak F, Briscoe MP, et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. Clin Exp Allergy Rev. 2006;6:31-59.

20. Pfaar O, Calderon MA, Andrews CP, et al. Allergen exposure chambers: harmonizing current concepts and projecting the needs for the future - an EAACI Position Paper. Allergy. 2017;72:1035-1042.

21. Mantelli F, Lambiase A, Bonini S, et al. Clinical trials in allergic conjunctivitis: a systematic review. Allergy. 2011;66(7):919-924.

22. Ellis AK, Ratz JD, Day AG, et al. Factors that affect the allergic rhinitis response to ragweed allergen exposure. Ann Allergy Asthma Immunol. 2010;104:293-298.

23. Patel D, Garadi R, Brubaker M, et al. Onset and duration of action of nasal sprays in seasonal allergic rhinitis patients: olopatadine hydrochloride versus mometasone furoate monohydrate. Allergy Asthma Proc. 2007;28:592-599.

24. Jacobs RL, Ramirez DA, Rather CG, et al. Redness response phenotypes of allergic conjunctivitis in an allergen challenge chamber. Ann Allergy Asthma Immunol. 2017;118:86-93.

25. Badorrek P, Dick M, Hecker H, et al. Anti-allergic drug testing in an environmental challenge chamber is suitable both in and out of the relevant pollen season. Ann Allergy Asthma Immunol. 2011;106:336-341.

26. Horak F, Berger UE, Menapace R, et al. Dose-dependent protection by azelastine eye drops against pollen-induced allergic conjunctivitis. A double-blind, placebo-controlled study. Arzneimittelforschung. 1998;48:379-384.

27. Horak F, Stübner P, Zieglmayer R, et al. Onset and duration of action of ketotifen 0.025% and emedastine 0.05% in seasonal allergic conjunctivitis: efficacy after repeated pollen challenges in the Vienna challenge chamber. Clin Drug Invest. 2003;23:329-337.

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