Published in final edited form as:

Gastroenterology. 2014 August ; 147(2): 324–333.e5. doi:10.1053/j.gastro.2014.04.019.

Efficacy, Dose Reduction, and Resistance to High-dose Fluticasone in Patients with Eosinophilic Esophagitis

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Author Contributions: M.E.R. obtained funding and supervised the study. B.K.B., G.J.G., G.T.F., J.S., R.E.K., W.D.J., and M.O. facilitated recruitment, T.W. performed the Eosinophilic Esophagitis Diagnostic Panel experiments, E.S. processed study samples, M.H.C. provided pathological insight regarding the biopsy sections involved and reviewed specimens, C.M. and E.K. analyzed and interpreted the data, B.K.B., G.J.G., G.T.F., J.S., M.H.C., J.P.A., S.P., P.E.P. and M.E.R. were involved in the study concept and design. All authors were involved in writing the manuscript.

Disclosures: B.K.B., G.J.G., G.T.F., J.S., E.K., R.E.K., E.S., C.M., W.D.J., M.O., J.P.A., S.P., and P.E.P. have no conflicts of interest to disclose. M.E.R. serves as a consultant for Pluristem Pharmaceuticals, Novartis, and Immune Pharmaceuticals, and has an equity ownership in Immune Pharmaceuticals; M.E.R. has a royalty from reslizumab, a drug being developed by Teva Pharmaceuticals. None of these interests are directly related to the present study presented herein. M.E.R. and T.W. are co-inventors for a pending patent based on the Eosinophilic Esophagitis Diagnostic Panel (EDP) test described herein. M.H.C. is a consultant with Meritage Pharma, Receptos, Aptalis Pharma, and Novartis.

Transcript Profiling: The microarray data is not of sufficient scope that it should be included in a repository.

Writing Assistance: There was no writing assistance for this manuscript.

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Abstract

Background & Aims—We evaluated the efficacy and safety of high-dose swallowed fluticasone propionate (FP) and dose reduction in patients with eosinophilic esophagitis (EoE) and analyzed esophageal transcriptomes to identify mechanisms.

Methods—We conducted a randomized, multisite, double-blind, placebo-controlled trial of daily 1760 mcg FP in participants 3–30 years old with active EoE. Twenty-eight participants received FP and 14 received placebo. After 3 months, participants given FP who were in complete remission (CR) received 880 mcg FP daily, and participants in the FP or placebo groups who were not in CR continued or started, respectively, 1760 mcg FP daily for 3 additional months. The primary endpoint was histologic evidence for CR. Secondary endpoints were partial remission (PR), symptoms, compliance, esophageal gene expression, esophageal eosinophil count, and the relationship between clinical features and FP responsiveness.

Results—After 3 months, 65% of subjects given FP and no subjects given placebo were in CR ($P=.0001$); 12% of those given FP and 8% of those given placebo were in PR. In the FP group, 73% of subjects remained in CR and 20% were in PR after the daily dose was reduced by 50%. Extending FP therapy in FP-resistant participants did not induce remission. FP decreased heartburn severity ($P=.041$). Compliance, age, sex, atopic status, or anthropomorphic features were not associated with response to FP. Gene expression patterns in esophageal tissues of FP responders were similar to those of patients without EoE; there was evidence for heterogeneous steroid signaling in subjects that did not respond to FP.

Conclusions—Daily administration of a high dose of FP induces histologic remission in 65%–77% of patients with EoE after 3 months. A 50% dose reduction remained effective in 73%–93% of patients that initially responded to FP. Nonresponders had evidence of steroid resistance; histologic and molecular markers may predict resistance. Clinicaltrials.gov number: NCT00426283

Keywords

treatment; inflammation; dysphagia; steroids

Introduction

Eosinophilic esophagitis (EoE) is an emerging immune-mediated disease characterized by intense eosinophil infiltration of the esophageal mucosal epithelium that is refractory to acid-suppressive therapy and often associated with significant tissue remodeling.^{1–3} First described in the late 1970s, the incidence and prevalence of EoE has been on the rise. It is now a global health disease reported in every continent except Africa and has been shown to affect ~1:2,500 individuals.^{1–5} An immunological etiology for EoE is supported by the reversibility of the disease after dietary avoidance of specific foods,⁶ the reoccurrence of the disease upon re-introduction of the removed foods,⁷ the induction of the disease in mice by exposure to allergens,⁸ and genome-wide transcriptome analysis of esophageal tissue that implicates an interplay of innate and adaptive Th2 immunity.⁹ The disease has a strong hereditary component with a large sibling risk ratio ($\lambda_s \sim 80$),¹⁰ and early genetic analyses have identified susceptibility loci in regions containing candidate genes that are expressed in

epithelial cells and strongly implicated in regulating antigen recognition (*TSLP*, thymic stromal lymphopoietin) and inflammatory cell recruitment and activation (*CCL26*, eotaxin-3).¹⁰

The most effective medicine for the treatment of EoE is off-label use of topical steroids such as fluticasone propionate (FP) and budesonide.¹¹⁻¹⁵ Faubion et al. showed that swallowed FP was effective and safe for treating EoE in four pediatric patients.¹⁶ Konikoff et al. demonstrated complete remission in 50% of patients with EoE after a three-month treatment course of 880 mcg of daily swallowed FP compared to 9% in the placebo group.¹⁷ Potential theories for this apparent lack of response to FP in half of patients include poor compliance, the existence of a steroid-resistant phenotype, and inadequate FP dosing. It is notable that polymorphisms in the TGF- β gene are associated with steroid resistance in EoE.¹⁸ Additionally, responsiveness to FP has been reported to negatively correlate with younger patient age, smaller height, and lower weight.^{17, 19}

In vitro, cardinal components of the EoE transcriptome including IL-13-induced eotaxin-3 in esophageal epithelial cells are inhibited by glucocorticoids in a dose-dependent manner.²⁰ Notably, asthma has parallels with the type of inflammation seen in EoE, and higher dosages of glucocorticoids are more effective than lower dosages in patients with refractory asthma. Additionally, we have noted in clinical practice that several patients who did not respond to 880 mcg daily FP responded to a higher dose, 1760 mcg daily FP. Therefore, in this study, we aimed to determine the efficacy and safety of a high FP dosage (1760 mcg daily) in inducing remission, the effect of prolonging high-dose FP therapy in FP-resistant EoE, and the ability to maintain disease remission while reducing FP dosage (880 mcg daily) in FP-responsive EoE. Furthermore, we analyzed esophageal transcriptomes to identify mechanisms involved in remission status.

Methods

Participants

This study was initiated at Cincinnati Children's Hospital Medical Center (CCHMC) on December 12, 2006. Subsequently, The University of Utah, Colorado Children's Hospital, and The Children's Hospital of Philadelphia were added as study sites. All participants underwent allergy testing and either completed a 3-month elimination diet or refused such diet treatment. Participants were 3–30 years of age and required to have an upper endoscopy, esophagogastroduodenoscopy (EGD), that showed active EoE at the time of screening. Active EoE was defined as ≥ 24 eosinophils/ high-power field (HPF) in the proximal or distal esophagus while being treated with a proton-pump inhibitor (PPI) for at least two months or having a negative pH probe. Participants were both newly diagnosed and relapsed EoE patients. Potential participants were excluded if there was a history of poor tolerance to FP, inability to use a metered-dose inhaler (MDI), concurrent or recent use of systemic corticosteroids, comorbid eosinophilic disorders, or diagnosis of or at risk for diabetes (type I or type II). The study was approved by the institutional review board (IRB) at each institution, and assent and/or informed consent were obtained. The study was conducted in compliance with International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) guidelines, and the applicable regulatory requirements, including

the U.S. Code of Federal Regulations (45 CFR 46, 21 CFR 50, 21 CFR 56, 21 CFR 312). All authors had access to the study data and have reviewed and approved the final manuscript. Participants were instructed not to change PPI dosage and/or diet therapy during the study.

Study Design

This 6-month study was a double-blind, placebo-controlled, randomized trial to determine the safety and efficacy of daily high-dose (1760 mcg) FP in participants with EoE. Participants who met inclusion criteria were randomly assigned to either high-dose FP, 1760 mcg divided into two daily 880-mcg doses, or placebo for 3 months (Phase 1). Two-thirds of the participants were assigned to the FP group. The placebo was generously provided by GlaxoSmithKline. Randomization was performed by members of the CCHMC investigational pharmacy. The randomization scheme was generated by using the site Randomization.com (<http://www.randomization.com>). Participants were assigned to a single treatment by using the method of randomly permuted blocks. The allocation sequence was only known by the CCHMC pharmacist, and the site pharmacist was only made aware of individual assignments after enrollment/assignment. To maintain the blind, only one staff member, in addition to the pharmacy staff, at each site was unblinded. This individual was unblinded to the individual participant randomization after the first 3 months in order to stratify each participant for the following 3 months. After all participants completed the study and the database was finalized, team members were unblinded for data analysis purposes.

After 3 months, participants had an EGD. Participants assigned to FP who were in CR, ≤ 1 eosinophil/HPF in the proximal and distal esophagus (FP responders), were assigned to receive half the dosage of FP (one daily 880-mcg dose) for 3 additional months (Phase 2). FP-treated participants who had >1 eosinophil/HPF after 3 months (FP non-responders) were assigned to continue receiving the high dose of FP (1760 mcg, two daily 880-mcg doses) for 3 additional months. Placebo-treated participants who had ≤ 1 eosinophil/HPF at month 3 were assigned to receive high-dose FP (1760 mcg, two daily 880-mcg doses) for 3 additional months. Placebo-treated participants who had ≥ 1 eosinophil/HPF after 3 months were to discontinue placebo treatment and the study. Participants completed a semi-validated EoE symptom score,²¹ which included both frequency and severity questions, at the beginning of the study and after 3 months (Phase 1) and 6 months (Phase 2). See Supplementary Data for full Methods.

Molecular Gene Expression Profiling by EoE Diagnostic Panel

We have chosen 94 representative EoE genes as the molecular foundation of the EoE Diagnostic Panel (EDP).²² Among these 94 genes, 77 serve as definitive diagnostic and treatment assessment genes. RNA extracted from fresh distal biopsies at CCHMC was reverse transcribed into cDNA, which was subjected to Taqman quantitative polymerase chain reaction (qPCR) amplification of the 94 genes using a 7900HT qPCR amplification system (Applied Biosciences).

Statistical Analysis

Demographic and participant characteristic data were summarized within each treatment group using frequency and percentage for categorical data and mean and standard deviation or median and interquartile range for continuous data. Treatment groups were compared using Fisher's Exact test for categorical data and the Wilcoxon Rank Sum test for continuous data.

Analysis of 3-Month Data

On the basis of Konikoff et al.,¹⁷ it was estimated that the study participants would have a 75% response rate to 1760 mcg daily FP and a 10% response rate to daily placebo. Under these assumptions, a Fischer's exact test with a 0.050 two-sided significance level would have a 95% power to detect the difference between the FP and placebo group if at least 39 subjects completed the study (26 FP, 13 placebo). The primary endpoint for this study was remission at 3 months (categorical outcome) and was summarized using frequency and percentage, which were compared between treatment groups using Fisher's Exact test. At the interim analysis, the test was conducted at $\alpha = 0.01$, and at the final analysis, the test was conducted at $\alpha = 0.04$, in order to maintain the overall study Type I error at 5%.

For symptom data, the change from screening score (continuous outcome) was compared between treatment groups using the Wilcoxon Rank Sum test. Changes in eosinophil levels were analyzed separately by location: distal, proximal, and maximum of distal and proximal.

To investigate the effect of demographic and participant characteristics on remission, logistic regression models were conducted with the following covariates separately, including the interaction of the covariate and treatment: age, weight, height, BMI Z-score, atopic status, and compliance. Similar analyses were conducted for the percent change in eosinophil counts since screening using analysis of covariance models.

FP responsiveness analysis included participants randomized to the FP treatment group. To determine whether participant characteristics were predictive of achieving remission, logistic regression models were conducted separately for each of the following predictors: age, weight, height, BMI Z-score, race, ethnicity, atopic status, eosinophil level at the time of screening, and compliance. Analyses of the change in eosinophil counts were conducted using regression analysis.

P values <0.05 were considered significant for all tests except for the primary endpoint at the interim analysis and at the end of the study. All statistical tests were completed using SAS 9.3 (Cary, NC).

Analysis of 6-Month

The percentage of participants in remission at the end of the study and the change in eosinophil counts from baseline were summarized for the treatment groups resulting from the initial randomization and subsequent treatment strategy for Phase 2. No statistical comparisons across groups were conducted.

Results

Participant Characteristics

Fifty-one participants were screened for the study; 9 were excluded (7 did not meet inclusion criteria, 2 declined to participate), and 42 were randomized (Figure 1). Of the 42 randomized, 28 received FP and 14 received placebo. Thirty-six participants completed Phase 1 of the study ($n = 17, 11, 5,$ and 3 from CCHMC, The University of Utah, Colorado Children's Hospital, and The Children's Hospital of Philadelphia, respectively). Five participants withdrew from the FP group during Phase 1 — 2 due to prohibited medications, 2 due to loss to follow-up, and 1 due to an AE (absence seizure that was deemed unlikely to be related to FP). One participant from the placebo group was lost to follow-up during Phase 1. During Phase 2, 2 participants withdrew from the study for unknown reasons. Thus, 34 participants completed Phase 1 and 2 of the study. Baseline participant characteristics were similar between the FP and placebo groups. The participants of both groups were primarily male, white (race), atopic, and were on PPI at time of the screening visit (Table 1).

Interim Analysis

Twenty-six participants were included in the interim analysis (17 FP, 9 placebo). The percentage of CR was significantly higher in the FP group (71%) than in the placebo group (0%) ($P = 0.0007$). On the basis of these results, we closed recruitment. Participants who were already enrolled in the trial were allowed to complete the trial.

Efficacy

In Phase 1, the percentage of CR (both proximal and distal peak eosinophil count ≤ 1 eosinophil/HPF) was significantly higher in the FP group (15/23, 65%) than in the placebo group (0/13, 0%; $P = 0.0001$). Responsiveness was also evaluated using different threshold values, as reported by Konikoff.¹⁷ The values comprised a peak eosinophil count of ≤ 6 or ≤ 14 eosinophils/HPF, a mean (of proximal and distal peak values) eosinophil count of ≤ 1 or ≤ 2 eosinophils/HPF, and decreases of $\geq 90\%$ or $\geq 95\%$ of peak eosinophil count values. On the basis of these criteria, 73%, 77%, 63%, 68%, 75%, or 70% remission was observed after FP, respectively (Figure 2a). In the placebo group, one participant had partial remission (≤ 14 eosinophils/HPF). None of the placebo participants met the criteria for remission in any other category (Figure 2a). Responsiveness was also evaluated individually for the proximal or distal esophagus, by peak $\leq 1, 6$ or 14 eosinophils, and decreases of $\geq 90\%$ or $\geq 95\%$ of peak eosinophil count values. In the proximal esophagus (Figure 2b), the FP group had 73%, 77%, 82%, 81% and 81%, while the placebo group had 0%, 15%, 15%, 8% and 0%, respectively. In the distal esophagus, the FP group had 59%, 73%, 77%, 70% and 65%. The placebo group had remission rates of 8%, 15%, 23%, 8%, and 8% respectively. The mean, proximal only, and distal only peak values had similar efficacy (Figures 2b, 2c).

At the end of Phase 2, FP Phase 1 non-responders who had continued on high-dose FP in Phase 2 had EoE that remained largely FP resistant (Supplementary Figure 1a). One participant who was in PR after Phase 1 and continued on high-dose FP in Phase 2 was in PR after the 3 additional months of Phase 2. Sixty-two percent of the participants from the Phase 1 placebo group who initiated high-dose FP in Phase 2 were in CR after 3 months,

and 69% were in remission using different threshold values of response as reported by Konikoff (Supplementary Figure 1a).¹⁷ Of the FP Phase 1 responders, who had their dose halved in Phase 2, 73% were in CR (Figure 3), and 80% were in remission using different threshold values of response (Supplementary Figure 1a).¹⁷ These same thresholds were used to individually evaluate remission in the distal or proximal esophagus (Supplementary Figures 1b,c).

A responder analysis as a function of age, height, weight, BMI Z-score, compliance, atopic status, and screening esophageal eosinophil count revealed that none of these variables correlated with response (Supplementary Tables 1 and 2). Participants with a new diagnosis of EoE and participants with long-standing EoE at the time of enrollment had 70% and 61.5% complete response, respectively.

Safety

Vital signs and lab results were stable throughout the study (data not shown). AEs were documented from the time of consent until a 30-day follow-up interview. AEs were reported according to the MedDRA System Organ Class (Table 2). One participant reported oral thrush at the 3-month study visit, which had spontaneously resolved by the follow-up phone call visit at the end of Phase 2. That participant was in the high-dose FP group during Phase 1 and remained on high-dose FP during Phase 2. No statistically significant differences in AEs were observed between the high-dose FP and placebo groups during Phase 1. Instances of decreased cortisol (n = 8) (Supplementary Table 3), increased cortisol (n = 1), and elevated glucose (n = 1) were reported as AEs. For decreased cortisol, 7/8 events occurred after a participant was on high-dose FP, and 1/8 occurred at screening before randomization. Out of the 7 that occurred after randomization, 5/7 occurred initially during Phase 1 while the participant was on high-dose FP, and 2/7 occurred during Phase 2 in participants who went from placebo to high-dose FP. For the 5 events of decreased cortisol occurring during Phase 1, 1 resolved before the 3-month visit, 3 resolved during Phase 2 after FP dose reduction, and 1 did not resolve after dose reduction. The decreased cortisol events in Phase 2 did not resolve before the end of study. While the decreased cortisol events between the FP and placebo groups during Phase 1 were not significant (P = 0.15), there was a trend towards decreased cortisol in participants on high-dose FP. All decreased cortisol AEs were from one study site only (CCHMC), and most were measured via saliva (71%, 5/7). CCHMC was the only study site to collect cortisol via saliva. No participant was reported to exhibit clinical signs of adrenal insufficiency or glucocorticoid toxicity.

Symptom Severity and Frequency

No statistically significant differences were found between the FP and placebo groups for total frequency or total severity of symptoms from the time of screening to month 3, the end of Phase 1. Early satiety and abdominal pain were the most frequent symptom at screening in the FP group (71% and 71%, respectively), whereas dysphagia was the most frequent symptom in the placebo group (69%). At month 3, abdominal pain was the most frequent symptom in the FP group (61%), and heartburn was the most frequent in the placebo group (58%). The change in heartburn severity from screening Butz 17 to month 3 was statistically significant between the FP and placebo groups (P = 0.041) due to a decrease in heartburn

severity in 8 participants in the FP group compared to 0 participants in the placebo group. Of note, 50% of placebo participants and 41% of FP participants reported “none” relative to heartburn severity at screening. No other significant differences were found between the treatment groups in screening frequency or severity changes for individual symptoms in Phase 1. This study was not designed nor powered to statistically compare age groups, but additional conclusions are summarized in Supplementary Table 4.

Molecular Gene Expression Profiling

A concurrent distal esophageal biopsy was subjected to molecular expression signature analysis based on representative EoE genes.^{9, 23} The EDP has been shown to possess high sensitivity and specificity with only a single biopsy; in contrast, histology requires 5-6 biopsies to reach >95% sensitivity.²⁴ Thus, in addition to providing key molecular insight into the action of steroids in the esophagus, this study provided an opportunity to examine the value of the EDP in a prospective, controlled trial. In the format of a heat diagram, the 77 definitive diagnostic gene expression profiles from each group at screening and the end of each of the two trial phases were juxtaposed (Figure 4A) with known EoE and control cases shown as references. Bidirectional gene signature changes can be readily observed from the heat diagram (yellow for upregulation, blue for downregulation). Phase 1 participants receiving the placebo did not have signature reversal, as bidirectional genes were still dysregulated similar to at the time of screening ($P = 0.16$, two-tailed student t-test). In contrast, a large portion of the participants receiving FP in Phase 1 exhibited a normalized signature compared to the dysregulated screening and placebo signatures ($P < 0.0001$ for both, two-tailed student t-tests), albeit there was still modestly dysregulated gene expression. Notably, the 6 FP participants with histologic PR or no remission also had a partial reversal with a signature different from the placebo group. After the placebo group received FP in Phase 2, their transcriptomes were dramatically normalized. However, there were still a few molecular non-responders whose signatures failed to normalize upon FP treatment, consistent with the histologic findings. These findings are likely attributable to individual FP sensitivity or the suboptimal effect of the FP dose.

The EDP is associated with an EoE score algorithm reflecting disease status and severity in a quantifiable number — the EoE score, based on a core set of 77 diagnostic genes. The EoE score was calculated for each subgroup (Figure 4B) and demonstrated that FP responders had quantifiable improvement in the transcriptome that was not observed to the same degree in participants with FP-resistant EoE. Notably, although the EoE score normalized in FP complete responders, it was still significantly different from the EoE score of healthy controls (NL vs. FP-CR, Figure 4B; Supplementary Table 5). ROC analysis indicated a sensitivity of 92% and specificity of 92% for the EDP to distinguish patients with active disease vs complete remission. Using the reported Dx-77 scoring, an analysis of active EoE samples (placebo treated and initial screening) vs. samples from patients in complete remission (Phase 1 & 2), revealed a sensitivity of 96% and specificity of 80%. Finally, the EDP score inversely correlated with the esophageal eosinophil count (Spearman $R = -0.82$; $P < 0.0001$).

We also aimed to identify esophageal gene expressions that might predict FP responsiveness by statistically screening the pre-FP treatment distal biopsy samples of the FP responder and non-responder cohorts. A total of 15 genes (Figure 4C, $P < 0.05$, fold change >2.0) on the EDP were identified that had a tendency to predict subsequent FP efficacy on the basis of CR criteria (≥ 1 eosinophil/HPF in the distal esophagus).

Discussion

This study demonstrates that high-dose FP (1760 mcg daily) induces histologic CR in 65% of EoE participants and at least partial remission in 77% with barely any placebo effect. Further, after achieving remission, reducing the dose of FP from 1760 to 880 mcg daily results in 93% of EoE participants maintaining CR or PR. This finding supports dose reduction after CR is achieved with high-dose FP. Of note, the total dose is halved for the 1760 mcg to 880 mcg group, as well as administration of 4 puffs twice daily to 2 puffs twice daily. For EoE that does not respond to high-dose FP in 3 months, extending the timeframe for high-dose FP to 6 months does not increase remission status. Therefore, 3 months is an adequate timeframe to evaluate histologic response following introduction of FP therapy, and we suggest that that 3-month evaluation be considered in clinical practice following initiation of FP. Our study provides definitive evidence for substantial rates of steroid resistance in EoE (approximately 25%), even at the relatively high doses and prolonged exposure durations associated with our study. High-dose FP responders were not found to be different from non-responders in age, height, weight, BMI z-score, compliance, or atopic status. Notably, at the higher dose (1760 mcg) administered in this study, there was no affect of these phenotypic markers on responsiveness, strongly suggesting steroid resistance rather than inadequate dosage or delivery format. It is important to point out that we cannot directly conclude that there is a 15% difference in the response rate previously reported in our prior study with FP at 880 mcg¹⁷, as the 880 vs 1760 mcg doses were not directly compared in the same study; it remains possible that at least some of these patients would have responded to the lower dosage. Nevertheless, we feel the observed response rate to 1760 mcg of fluticasone suggests that starting at this higher dosage and then stepping down is warranted but further clinical trials are required before this can become a recommendation.

Molecular gene expression profiling represents the next generation of EoE diagnosis, and the representative EoE gene array, the EDP, was developed for this purpose.²² As a parallel study, we utilized the EDP to monitor the FP intervention efficiency by assessing the EoE signature and activity and steroid exposure, all of which can be quantified into a readily comprehended score by associated EDP algorithms.²² We showed that in those cases in which FP achieved histologic CR, the molecular signature also normalized. Yet, the EDP's EoE score from FP complete responders was still significantly different from the EoE score of healthy controls (Figure 4B), providing evidence for the persistence of molecular abnormalities even in patients with histologic CR and a potential molecular explanation for disease relapse.

Meanwhile, those cases in which FP failed to elicit histologic CR were associated with a partial EoE-like intermediate transcriptome. Therefore, FP was effective in reversing the

pathogenic gene expression signatures to a certain extent but failed to elicit CR. These findings indicate that these FP non-responders were indeed exposed to FP. The partial EoE-like intermediate signature is consistent with some participants having steroid resistance rather than non-compliance, which is in accord with the Responder Analysis (Supplementary Table 1). Indeed, participant compliance with medication intake did not correlate with the participant transcriptome changes on the basis of the data available (data not shown). The signature normalization in most of the participants who received high-dose FP in Phase 1 and high-dose FP in Phase 2 highlights the bona fide efficacy of FP intervention in EoE. Notably, this report is the first to demonstrate the value of the EDP in measuring disease activity in a controlled, prospective clinical trial.

Predicative medicine aims to select the optimal form of treatment on a patient-by-patient basis, which has been shown to be successful in some other gastrointestinal disorders, such as ulcerative colitis.²³ Therefore, we also screened the pre-FP treatment samples for their subsequent FP responsiveness and identified 15 genes exhibiting a tendency for FP efficacy predictions. Of note, we were limited by the sample size and did not have a power high enough to apply a false-positive correction filter on this result, as such, these findings should be considered preliminary. Further validating studies on these candidate genes would be of significant interest.

While AE occurrences were not significantly different between high-dose FP and placebo, the small sample size results in low power for detecting signals. CCHMC was the only site with low cortisol values, and most, but not all, were measured in saliva samples. CCHMC was the only site that included salivary cortisol measurements. Salivary cortisol measurements have limitations as the reference ranges have not been agreed upon and the cortisol may not be stable during the period before participants deliver the samples. At the same time, salivary measures free cortisol levels whereas serum measures free and bound cortisol; since free is the active form, it remains possible that salivary cortisol is a more sensitive measurement under these conditions.²⁴ Additionally, salivary levels are more stable compared with the diurnal variation seen in the blood (which peaks in the AM); as such, it may be easier to discern lower levels in the saliva.²⁵ Long-term topical steroid therapy (0.5 mg daily of budesonide in adults) has been reported to be well tolerated and effective in maintaining disease remission, although a rise in eosinophil levels during the 50 week observation period has been reported to occur.¹⁵ Further assessment of the effect of FP on the adrenal corticoid axis is warranted in future studies, especially since follow-up of the low cortisol values were not examined in this study.

The high-dose FP group had improved heartburn severity at the 3-month visit, whereas the placebo group remained the same. The frequency and severity for all other symptoms was similar between the groups. At the time of study initiation, a validated EoE symptom score measure was not available, which is a limitation to this component of our study. Although we observed no significant differences in changes in symptom frequency and severity, with the exception of heartburn severity, between treatment groups, participants were not required to have symptoms at study entry, and the study was not powered to detect differences in symptom frequency or severity. Nevertheless, we observed an improvement in heartburn severity in the FP group compared with the placebo group in Phase 1. All

participants had PPI-confirmed EoE and were instructed not to alter any medicine or diet during the trial. Therefore, it is unlikely that the reduction in heartburn severity in the FP group was due to PPI use. It is notable that symptom reduction is often less impressive than histological response. There are several explanations for this finding, including the possibility of insufficient duration of therapy, fixed fibrosis (although in our study this was not present), and the usage of non-validated and/or insufficient patient reported outcome metrics, as recently discussed.²⁶

Besides FP, budesonide has been shown to improve dysphagia^{13, 15, 18} and to significantly reduce esophageal eosinophils,^{14, 15} as compared to placebo, with complete remission (CR) of 35%-72% or 0-11% after budesonide or placebo, respectively. Dellon et al. conducted a randomized trial comparing nebulized budesonide and viscous topical budesonide (as an oral slurry) in EoE and demonstrated that symptoms improved in all groups;²⁷ however, the esophageal eosinophil reduction was greater with viscous topical administration, supporting the view that a topical mechanism was operational.²⁷ Indeed, swallowed glucocorticoids induce de-novo gene expression in the esophagus, suggesting a local effect, particularly on the epithelium.²⁸ While FP and budesonide are the most highly studied glucocorticoids for the treatment of EoE, a number of other steroids have been recently examined. Swallowed ciclesonide has also been shown to improve symptoms and histology but only in a small set of patients in an open-label study (n = 8).^{29,30} Bergquist et al. conducted a pilot study with topical mometasone furoate in 31 patients with EoE. Esophageal eosinophil levels were not assessed, but dysphagia and health-related quality of life improved after treatment.³¹

In conclusion, high-dose FP is effective in inducing histologic remission in EoE in 65% - 77% of participants following 3 months of therapy, with the range dependent upon the degree of histologic improvement. In FP responders, reducing the dose in half results in CR and PR in 73 and 20% of participants, respectively. In FP non-responders, extending high-dose treatment for an additional 3 months provided no benefit. Evidence for bona-fide steroid resistance is present at both histologic and molecular levels, further substantiating the need for the development of additional therapeutic interventions for this emerging medical disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Marshall Plaut, M.D., and Joy Laurienzo, R.N., B.S.N., for their oversight of the study progress and Dr. Daniel Rotrosen for his support of the study and review of the manuscript.

Grant Support: This work has been supported by the National Institute of Allergy and Infectious Diseases grant U01AI088806 to M.E.R., a GlaxoSmithKline grant (109928); and the National Center for Research Resources and the National Center for Advancing Translational Sciences of the National Institutes of Health (NIH) through grant 8 UL1 TR000077-05. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Abbreviations

AE	adverse event
BMI	body mass index
CCHMC	Cincinnati Children's Hospital Medical Center
CBC	complete blood count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CR	complete remission
EDP	Eosinophilic Esophagitis Diagnostic Panel
EGD	esophagogastroduodenoscopy (i.e. upper endoscopy)
EoE	eosinophilic esophagitis
FP	fluticasone propionate

HPF	high-power field
ICH-GCP	International Conference on Harmonization Good Clinical Practice E6
IRB	institutional review board
MedDRA	Medical Dictionary for Regulatory Activities
MDI	metered-dose inhaler
PPI	proton-pump inhibitor
PR	partial remission
qPCR	quantitative polymerase chain reaction

Figure 1. Flow diagram of study participants

Fifty-one participants were screened for the study; 9 were excluded, and 42 were randomized. Of the 42 randomized, 28 received FP and 14 received placebo. Thirty-six participants completed Phase 1, and 34 participants completed Phase 1 and 2 of the study.

Figure 2. Responsiveness in Phase 1

Response was defined as a peak count of ≤ 1 eosinophil/HPF in both the proximal and A) distal esophagus, B) proximal only, and C) distal only. Additional definitions were calculated on the basis of peak counts of ≤ 6 and ≤ 14 eosinophils/ high-power field (HPF), mean counts of ≤ 1 and ≤ 2 eosinophils/HPF, and decreases of $\geq 90\%$ and $\geq 95\%$ in eosinophil levels. * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$. In A, $P=0.0$ for the peak ≤ 6 eosinophils/HPF, In B, $P=0.0$ for the peak ≤ 1 eosinophil/HPF.

Figure 3. Effect of dose reduction on Phase 1 FP responders

Fluticasone propionate (FP) responders had their dosage of FP reduced from 1760 mcg daily to 880 mcg daily in an open-label study and had a repeat esophagogastroduodenoscopy (EGD) after 3 months. Each solid line represents a unique patient. The dashed lines represent the 15 eosinophils/HPF threshold for diagnosing EoE by consensus recommendations and 1 eosinophil/HPF for CR.

Figure 4. Esophageal transcriptome analysis

Total RNA from separately acquired biopsies was subjected to Eosinophilic Esophagitis (EoE) Diagnostic Panel (EDP) signature analysis. A) The heat diagram depicts the changes after fluticasone propionate (FP) exposure in the bidirectional gene dysregulation in each group with reference, historical EoE and normal (NL) cohorts (n = 15 and 14, respectively) and the samples at screening (no FP exposure) on the left. At the end of Phase 1 and 2, expression signatures from FP non-responders (NR), partial responders (PR), and complete responders (CR) are shown in the heat diagram (yellow, increase expression; blue, decreased expression). B) On the basis of the expression levels of the 77 diagnostic genes and a dimensionality reduction algorithm, an EoE score reflecting the EoE disease activity was calculated at the end of Phase 1 (ns, non-significant, **P < 0.001, ***P < 0.0001, two-tailed student t-test). C) To assess the prediction value of the EDP on FP responsiveness, samples at screening (i.e. pre-FP exposure) and the placebo samples at the end of Phase 1 (i.e. pre-FP exposure) were screened by bioinformatics in the context of their subsequent FP responsiveness on the basis of the CR criteria of <2 eosinophils/HPF in the distal esophagus, where the biopsies were acquired. The 15-gene cluster listed exhibits a potential for FP efficacy prediction with P < 0.05 and fold change >2.0.

Table 1Baseline participant characteristics^a

Variable	Fluticasone	Placebo
Gender, male/female (% male)	22/6 (79%)	13/1 (93%)
White race	25 (89%)	14 (100%)
Mean age at entry [years, (range)]	12.2 (3.54-26.90)	13.5(4.10-29.80)
Height [cm, (SD)]	141.3 (29)	148.9 (23)
Weight [kg, (SD)]	43.4 (25)	47.5 (24)
Atopic status	21 (78%)	14 (100%)
Proton pump inhibitor at screening	20 (71%)	10 (71%)
Peak eosinophils/HPF proximal esophagus [SD, (range)]	53.5 (62, 0-235)	87.1 (108, 1-343)
Peak eosinophils/HPF distal esophagus [SD, (range)]	56.3 (40, 1-155)	97.7 (98, 30-296)
Mean % blood eosinophils [SD, (range)]	5.5 (3)	7.8 (6)
Mean absolute blood eosinophils [K/mm ³ , (SD)]	0.35 (0.2)	0.53 (0.4)

^aP > 0.05 for all comparisons; ^awhen data was available;

HPF, high-power field; SD, standard deviation

Table 2

Number of participants experiencing adverse events in Phase 1

MedDRA System Organ Class	Control (n = 14)	Fluticasone (n = 28)	Fisher's Exact P Value
Eye disorders	2 (14.3%)	0 (0.0%)	0.11
Gastrointestinal disorders	2 (14.3%)	7 (25.0%)	0.69
General disorders and administration site conditions <i>Chest pain</i>	1 (7.1%)	1 (3.6%)	1.00
Immune system disorders	0 (0.0%)	2 (7.1%)	0.54
Infections and infestations	3 (21.4%)	3 (10.7%)	0.38
Injury, poisoning, and procedural complications <i>Scrapes and cuts</i>	0 (0.0%)	1 (3.6%)	1.00
Investigations <i>Abnormal lab values</i>	1 (7.1%)	5 (17.9%)	0.64
Nervous system disorders	0 (0.0%)	4 (14.3%)	0.28
Respiratory, thoracic, and mediastinal disorders	3 (21.4%)	3 (10.7%)	0.38
Skin and subcutaneous tissue disorders	1 (7.1%)	0 (0.0%)	0.33