

Intravenous anti-IL-13 mAb QAX576 for the treatment of eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) is a chronic allergic disease with limited treatment options.

Objective: We evaluated QAX576, an mAb against IL-13, in the treatment of patients with EoE.

Methods: Patients (18-50 years) with proton pump inhibitor-resistant esophageal eosinophilia received intravenous QAX576 (6 mg/kg) or placebo (2:1) at weeks 0, 4, and 8 and were followed for 6 months. The primary end point was the responder rate for a greater than 75% decrease in peak eosinophil counts at week 12. Efficacy was to be declared if the lower 90% confidence limit for the proportion of responders on QAX576 was 35% or greater. Secondary end points included changes in esophageal eosinophil counts, symptoms assessed by questionnaire scores, and quantification of a series of biomarkers.

Results: Twenty-three patients completed the study up to week 12, and 18 continued to the end of the study. For the proximal and distal esophageal biopsies combined, the responder rate was 12.5% (90% confidence limit, 1% to 43%) with placebo, compared to 40.0% (90% confidence limit, 22% to 61%) with QAX576. Although the primary end point was not met, the

mean esophageal eosinophil count decreased by 60% with QAX576 versus an increase of 23% with placebo ($P = .004$), and the decrease was sustained up to 6 months. There was a trend for improved symptoms, particularly dysphagia. QAX576 improved expression of EoE-relevant esophageal transcripts, including eotaxin-3, periostin, and markers of mast cells and barrier function, for up to 6 months after treatment. QAX576 was well tolerated.

Conclusions: QAX576 significantly improved intraepithelial esophageal eosinophil counts and dysregulated esophageal disease-related transcripts in adults with EoE in a sustained manner. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: QAX576, mAb, cytokine, T_H2 cells, IL-13, eosinophilic esophagitis, randomized clinical trial, targeted therapy, mast cell, barrier function, transcriptome

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Eosinophilic esophagitis (EoE) is an emerging chronic gastrointestinal disorder characterized by marked esophageal eosinophilia that is etiologically linked to immune hypersensitivity to dietary antigens and persists from childhood into adulthood.^{1,2} EoE is increasing in prevalence, with rates as high as 1:2500 children,^{3,4} and is now the leading cause for proton pump inhibitor-resistant dysphagia and food impaction in adults.^{5,6} EoE represents the most common of the eosinophilic gastrointestinal disorders, which collectively have substantial morbidity and are associated with the lowest quality of life compared with that seen in patients with a series of other chronic pediatric diseases.⁷ The high response rate to elimination diets, especially amino acid-based elemental diets, and the frequent recurrence of disease with food reintroduction imply that EoE is mediated by means of immune sensitization to foods, although skin testing is not typically helpful. Indeed, experimental EoE in mice can be induced by exposure to dietary or inhaled allergens⁸ or IL-13,^{9,10} a T_H2 -associated cytokine, through a variety of entry points, including the skin,¹¹ respiratory, and gastrointestinal tracts. Consistent with an allergic etiology, swallowed glucocorticoids are effective for the treatment of EoE and elicit local changes in esophageal gene expression,¹² but there are substantial rates of glucocorticoid resistance,¹³ relapse during therapy, or both, necessitating better treatment.¹⁴

In addition to being sufficient to induce EoE-like responses in mice,^{10,15} IL-13 is induced 16-fold in the esophagi of patients with EoE and can induce a series of EoE-related transcripts in esophageal epithelial cells *ex vivo*.¹⁶ Furthermore, mice deficient in IL-13 or the IL-13-signaling molecule signal transducer and activator of transcription 6 are protected against EoE features, including allergen-induced esophageal eosinophilia in murine models.¹¹ These data provided the rationale for this exploratory

Abbreviations used

CPA3: Carboxypeptidase A3
 DSG1: Desmoglein-1
 EGD: Esophagogastroduodenoscopy
 EoE: Eosinophilic esophagitis
 hpf: High-power field
 MDQ: Mayo Dysphagia Questionnaire
 PRO: Patient-reported outcome
 SAE: Serious adverse event

proof-of-concept study, which was designed to determine the safety and efficacy of neutralizing IL-13 in patients with EoE.

QAX576 is a highly potent and specific inhibitor of human IL-13 activity in cell-based *in vitro* assays. It completely inhibits eotaxin production from human lung fibroblasts induced by human recombinant IL-13, as well as the activity of human T cell-secreted IL-13, demonstrating its ability to neutralize native IL-13. We evaluated the effects of QAX576 in adults with active EoE. QAX576 significantly improved intraepithelial esophageal eosinophil counts and levels of esophageal disease-related transcripts in patients with EoE and demonstrated trends for improved symptoms.

METHODS**Study design**

An overview of the study is presented in [Fig 1, A](#). In brief, patients underwent a screening esophagogastroduodenoscopy (EGD) and baseline evaluations, followed by 2:1 randomization to the study medication or placebo. The study drug was administered at weeks 0, 4, and 8, and EGD was repeated 4 weeks after the last dose. The patients were followed up for 6 months. Responders who relapsed had the option to enter an open-label extension with 3 more monthly doses of QAX576. At the end of the study, EGD was repeated. A list of investigators and further details on study design are provided in [Appendix E1](#) and the [Methods section](#) in this article's Online Repository at www.jacionline.org.

Patients

Male or female patients (non-childbearing potential) aged between 18 and 50 years with active EoE were eligible to participate in the study. Required histologic findings on esophageal biopsy included peak eosinophil density of 24 cells or greater per high-power field (hp; $\times 400$ magnification) in the proximal or distal esophagus validated by a central laboratory pathology service (Quintiles Laboratories, Marietta, Ga). The inclusion and exclusion criteria are given in detail in the [Methods section](#) in this article's Online Repository.

Study treatments

Eligible patients were randomly assigned in a 2:1 ratio to receive either intravenous infusions of QAX576 (6 mg/kg) or placebo over 2 hours. The study medication was administered every 28 days for a total of 3 doses, after which patients were observed for an additional 6 months. Further details are provided in the [Methods section](#) in this article's Online Repository.

Efficacy: Histology

The primary end point measure was the proportion of patients with a 75% reduction in peak esophageal eosinophil counts in the proximal and distal esophageal biopsy specimens combined 4 weeks after the last dose of study medication (day 85) compared with baseline values. Efficacy was to be declared if the lower 90% confidence limit for the proportion of responders

receiving QAX576 was 35% or greater. The assessments were made primarily by 1 pathologist who was blind to the treatment assignment. Further details are provided in the [Methods section](#) in this article's Online Repository. Absolute and percentage changes in peak and mean eosinophil counts from baseline to day 85 were the key secondary end points.

Other secondary end points

Efficacy: Symptoms. Because an EoE-specific patient-reported outcome (PRO) instrument was not available at the time of this study, symptoms were captured by using the Mayo Dysphagia Questionnaire (MDQ) with a 2-week recall period¹⁷ because dysphagia is the predominant symptom in adults with EoE (see the [Methods section](#) and [Tables E1 and E2](#) in this article's Online Repository at www.jacionline.org).

Efficacy: Molecular. The EoE transcriptome before and after study medication (QAX576 and placebo) was compared by using the NanoString nCounter technology (NanoString Technologies, Seattle, Wash) for direct multiplexed measurement of gene expression. Biopsy tissue collected for RNA extraction was immersed in 1 mL of RNAlater. RNA was isolated with the Qiagen RNeasy Micro Kit (Qiagen, Hilden, Germany) and analyzed by the Biomarker Development group at Novartis (Basel, Switzerland). Further details of the transcriptomic methods are presented in the [Methods section](#) in this article's Online Repository.

Circulating protein biomarkers and histochemistry.

Quantitative determination of periostin was performed by means of liquid chromatography–mass spectrometry. Histochemical staining for collagen, periostin, and eotaxin was performed. The details of these methods are presented in the [Methods section](#) in this article's Online Repository.

Statistical methods

The primary variable was the proportion of responders, who were defined as subjects exhibiting a reduction of 75% or more in peak eosinophil count per hp (distal and proximal esophagus combined) from baseline to day 85.

Two-sided 90% CIs were calculated by using Agresti-Coull CIs for binomial proportions. Furthermore, between-treatment comparison with respect to the proportion of responders was assessed by using the Fisher exact test and associated *P* values.

Key secondary end points were the absolute and percentage change in peak and mean values from baseline to day 85. These were assessed by fitting an analysis of covariance model and adjusting for the baseline value. Two-sided 90% CIs of the treatment estimate and corresponding *P* values are presented. Treatment difference in clinical symptoms, as determined by using the MDQ, was analyzed with the Wilcoxon rank sum test. The molecular expression profile and transcript dysregulation were obtained by using NanoString and quantified by means of bioinformatics analysis (see the [Methods section](#) and [Table E3](#) in this article's Online Repository at www.jacionline.org).

Residuals were examined to ensure the models were appropriate. No α adjustments were performed because this was an exploratory study.

Sample-size calculations showed that with a minimum of 12 patients receiving QAX576, if at least 7 patients responded to treatment, then the lower CI would be greater than the desired 0.35 limit. This was regarded as sufficient evidence that QAX576 would have an effect in the treatment of patients with EoE. In addition, approximately 7 patients with EoE were to be included on placebo for the interpretation of safety and tolerability aspects in this study. In total, 21 subjects were to be randomized in this study at a 2:1 (QAX576/placebo) ratio to allow for dropouts.

RESULTS**Study population**

Twenty-five patients were randomized: 17 received QAX576, and 8 received placebo. Two patients receiving QAX576 were withdrawn after 1 dose, one because of a positive drug screen, and the other because of a non-drug-related serious adverse event (SAE). Twenty-three patients completed the day 85 assessments.

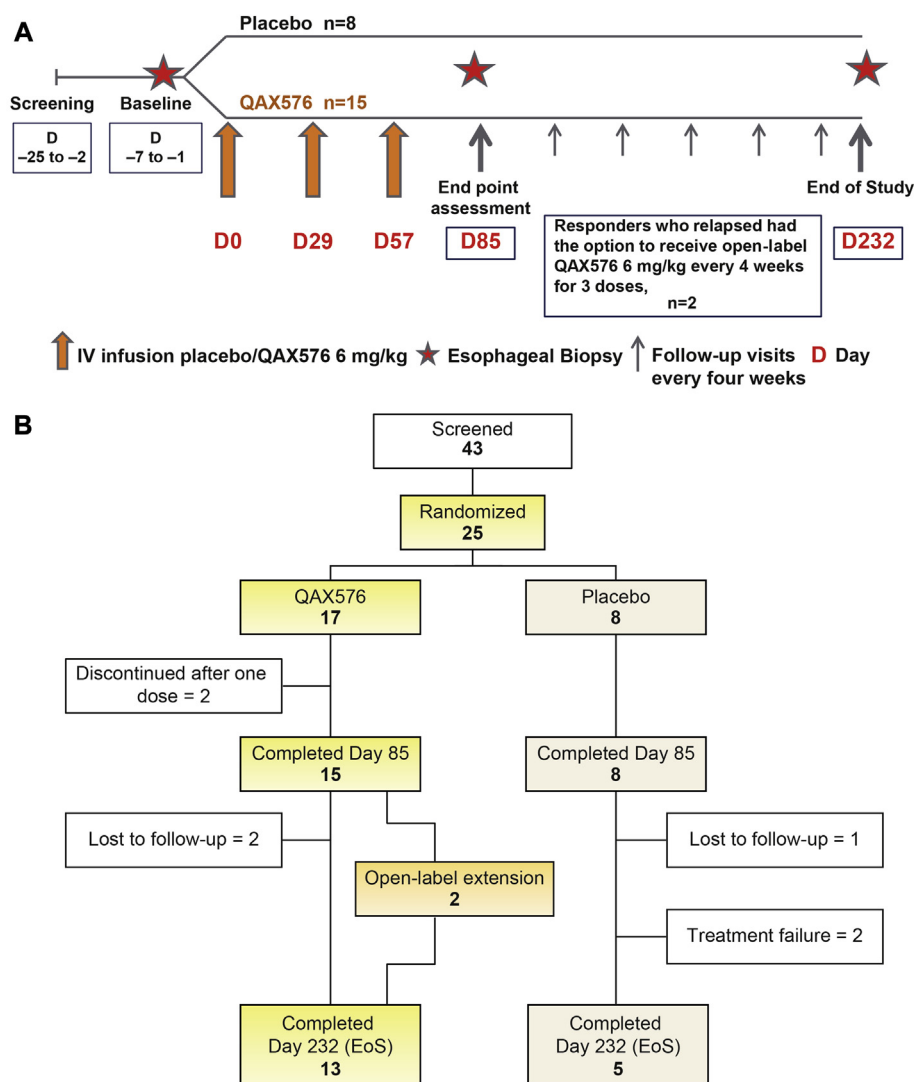


FIG 1. Study design and patient disposition. The overall study design (A) and patient disposition during the study (B) are presented. *EoS*, End of study.

Eighteen patients continued for an additional 5 months (ie, for 6 months after the last dose), with 2 receiving open-label study medication (Fig 1, B). The characteristics of the study patients are summarized in Table I. The placebo and QAX576 cohorts were similar in terms of baseline characteristics. The mean age of the patients was 30.3 ± 9.9 years (mean $\pm$ SD); 96% were male, and 96% were white. All patients had peak counts of 24 or more eosinophils/hpf in the esophageal biopsy specimens at baseline. For the population as a whole, peak eosinophil counts ranged from 38 to 200 eosinophils/hpf (mean, 89.8 eosinophils/hpf), and the mean eosinophil count ranged from 10 to 98 eosinophils/hpf (mean, 36.6 eosinophils/hpf). Dysphagia was the predominant symptom in 78% of patients, with 72% of patients reporting this as moderate or severe. Fifty-seven percent of the patients had previously been taking swallowed glucocorticoids, and 62% of these patients were reported as resistant to steroids. Seven subjects receiving placebo and 8 receiving QAX576 were taking proton pump inhibitors or H₂-receptor blockers during the study, but none started new treatment with these agents between days 1 and 85.

Efficacy: Histology

As the primary end point of the study, the proportion of responders was 40% (90% CI, 22% to 61%) with QAX576 and 13% (90% CI, 1% to 43%) with placebo. There was no significant difference between the 2 treatment groups with respect to the proportion of responders ($P = .3452$) based on a 2-sided Fisher exact test.

Eosinophil load (mean count of the proximal and distal esophagus) decreased by 60.0% in the QAX576-treated group compared with an increase of 23.3% in the placebo group. The statistical analysis of covariance indicated that the absolute difference between the QAX576 and placebo groups in the percentage change in mean eosinophil count was 87%. The 90% CI of the true difference ranged between 41% and 133% ($P = .004$). The individual changes in absolute eosinophil counts are shown in Fig 2, A and B, and the percentage changes from baseline are shown in Fig 2, C. Notably, the response was maintained up to the end of the study (ie, 6 months after the last dose of study drug) in the majority of the QAX576-treated patients (Fig 2, A and B).

TABLE I. Baseline characteristics

Subjects	QAX576, 6 mg/kg (n = 17)	Placebo (n = 8)	Total (n = 25)
Age (y)			
Mean (SD)	30.7 (9.58)	29.5 (11.22)	30.3 (9.92)
Range	18-45	18-48	18-48
Sex, no. (%)			
Male	16 (94.1)	8 (100.0)	24 (96.0)
Female	1 (5.9)	—	1 (4.0)
Race, no. (%)			
White	16 (94.1)	8 (100.0)	24 (96.0)
Black	1 (5.9)	—	1 (4.0)
Weight (kg)			
Mean (SD)	85.4 (20.7)	81.6 (19.8)	84.2 (20.1)
Height (cm)			
Mean (SD)	178.2 (6.7)	175.9 (8.1)	177.4 (7.1)
BMI (kg/m ²)			
Mean (SD)	26.8 (5.6)	26.2 (5.3)	26.6 (5.4)
Peak eosinophil count/hpf*			
Mean (range)	88.2 (48-200)	91.3 (38-130)	89.2 (38-200)
Mean eosinophil count/hpf*			
Mean (range)	35.4 (10-98)	39.1 (13-82)	36.6 (10-98)

*For proximal and distal esophageal biopsy specimens combined.

Efficacy: Symptoms

The “sum change in MDQ scores” for individual patients for the subset of questions relevant to EoE is shown in Fig E1 in this article’s Online Repository at www.jacionline.org. The estimated difference between treatments in the change in MDQ score was 1.8 ($P = .30$). There were trends for improved frequency and severity of dysphagia in the QAX576-treated patients (see Fig E2 in this article’s Online Repository at www.jacionline.org). Eight of the 10 symptom responders receiving QAX576 also had a decrease in esophageal eosinophil counts compared with 4 of the 5 symptom nonresponders.

Efficacy: Molecular

By using the day 85 transcriptome as the primary comparison point, there was a cluster of 29 EoE genes (of 91 screened) that exhibited a statistical significance between day 85 placebo-treated compared with QAX576-treated samples ($P < .05$, fold change >1.5). Overall expression profiles of these 29 significant genes are shown in Fig 3, A, by using averaged heat diagrams with gene symbols and dysregulation scales. The 2 groups of patients before therapy were equivalent. The QAX576 group showed a marked improvement compared with the placebo group at both day 85 and at the end of the study, demonstrating sustained molecular efficacy of QAX576 in the majority of the patients (see Fig E3 in this article’s Online Repository at www.jacionline.org). Of note, there was a universal improvement in the cardinal EoE genes, such as *CCL26* (eotaxin-3), *TNFAIP6* (tumor necrosis factor, alpha-induced protein 6), *CPA3* (carboxypeptidase A3; mast cell product), *ALOX15* (arachidonate 15-lipoxygenase), *SLC26A4* (solute carrier family 26, member 4), *LRRC31* (leucine rich repeat containing 31), and the epithelial chemokines *CXCL1/CXCL6* (chemokine [C-X-C motif] ligand 1/6), as well as genes implicated in tissue remodeling, such as *COL8A2* (collagen, type VIII, alpha 2), *POSTN*

(periostin), and several keratins. QAX576 also increased the levels of *DSG1* (desmoglein-1), a gene product markedly decreased in the esophagi of patients with EoE and shown to be involved in the impaired barrier function of the esophagus that is characteristic of EoE,¹⁸ corroborating the previous finding that IL-13 can directly regulate these genes in the esophageal cells *ex vivo*. As a representative summary of EoE signature reversal by IL-13 neutralization at day 85, there was expression reversal of a cluster of principle pathogenic EoE genes (Fig 3, B), namely eotaxin-3 (*CCL26*) for eosinophil chemotaxis, *POSTN* for tissue remodeling/fibrosis, *CPA3* for mastocytosis, and *DSG1* for epithelial barrier integrity.

By measuring the response (as determined by the change in mean eosinophil counts), we aimed to identify baseline transcripts that would predict responsiveness to QAX576. By using statistical screening ($P < .05$, fold change >1.5), 3 genes were found to be associated with subsequent anti-IL-13 responses, namely *CXCL1*, *CXCL6*, and *DHRS9* (dehydrogenase/reductase member 9), with 4.0-fold, 3.6-fold, and -1.8 -fold changes in nonresponders versus responders, respectively (Fig 3, C). Interestingly, both *CXCL1* and *CXCL6* are epithelium-derived chemokines that are associated with QAX576 efficacy (Fig 3, A) and used by a molecular diagnostic panel reported recently as well-recognized upregulated EoE genes.¹⁹

In light of all 29 unique genes associated with QAX576 efficacy at day 85, we carried out a comprehensive pathway analysis aiming to elucidate the protein-protein interaction network in the context of the existing literature.²⁰ As shown in Fig 3, D (and see Fig E4 in this article’s Online Repository at www.jacionline.org for higher resolution), it is notable that the interaction network consists of several key nodes serving as organization centers, namely *DDP4* (dipeptidyl-peptidase 4) for chemokine regulation, *B2M* (beta-2 microglobulin) for antigen presentation, *DSG1* for barrier integrity, *NTRK1* (neurotrophic tyrosine kinase, receptor, type 1) for epithelial differentiation, *KRT14/16* (keratin 14/16) for epithelial cell proliferation and inflammation, *CA2* (carbonic anhydrase II) for ion-channel/epithelial homeostasis, and *TNFAIP6/CXCL1/CXCL6* cluster for immunoinflammation. Indeed, most of these processes have been demonstrated to be critically involved with mounting allergic inflammation, particularly in the context of EoE, suggesting a broad efficacy range of anti-IL-13 intervention.

Biomarkers

There was no relationship between blood eosinophil counts and QAX576 treatment. Baseline and day 85 serum periostin levels were available for 14 subjects receiving QAX576 and 8 subjects receiving placebo. At baseline, the mean periostin level for the placebo group was 79.8 ng/mL (range, 42.7-151.0 ng/mL), and that for the QAX576-treated group was 91.4 ng/mL (range, 34.5-241.3 ng/mL). In both treatment groups there was a modest (not statistically significant) increase in periostin levels with treatment, which gradually decreased toward baseline values by the end of the study. There was no clear correlation of the baseline periostin levels or the change from baseline in periostin levels to eosinophil counts on the esophageal biopsy specimens at day 85. Of the subjects in the QAX576-treated group, those who had baseline periostin levels of greater than the median value (64.1 ng/mL) had a 71.4% response rate (in terms of a 75% reduction in the peak eosinophil count for the distal and proximal esophageal biopsy specimens combined) in contrast to a 14.3% response rate in

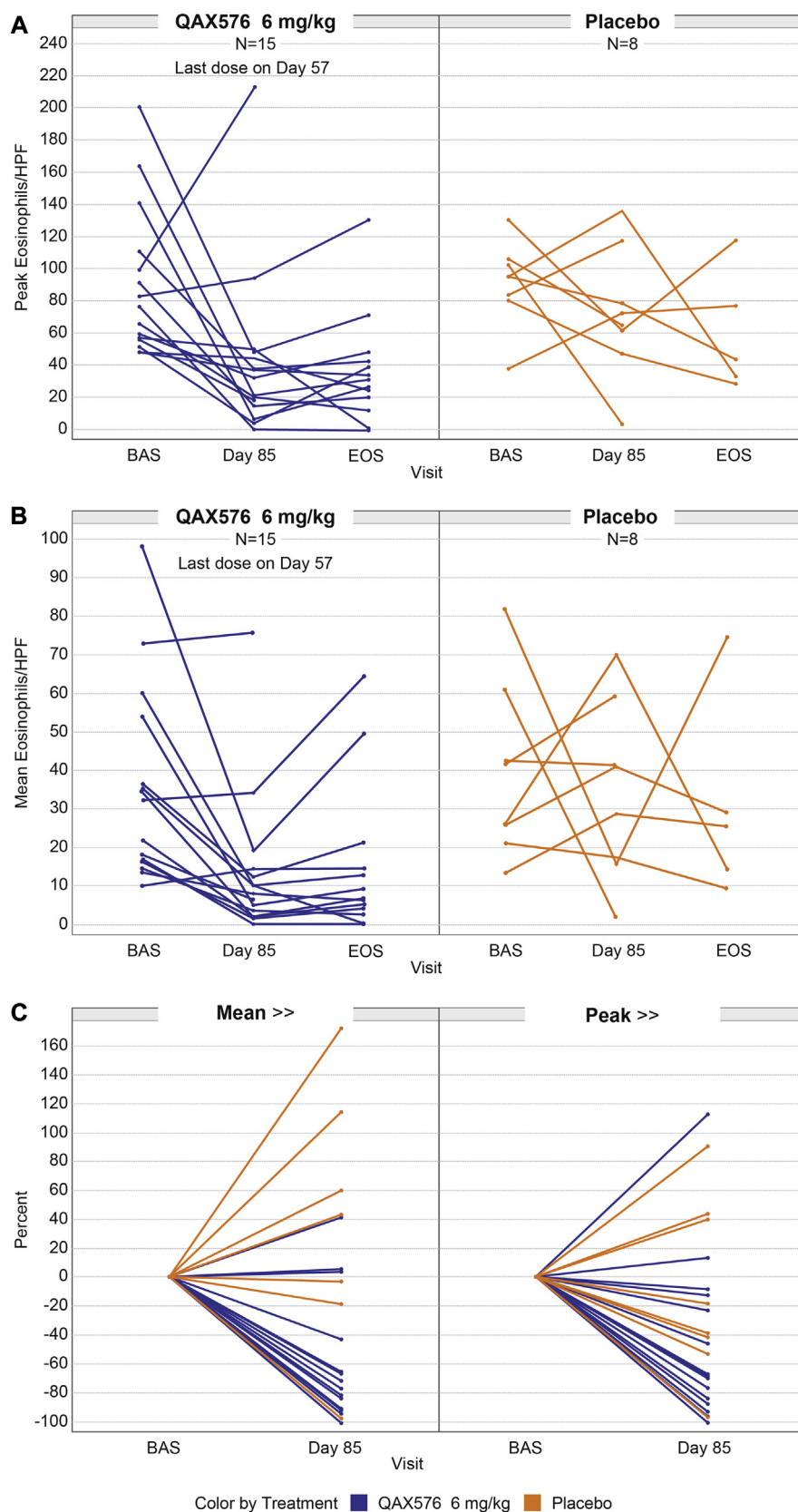


FIG 2. Changes in eosinophil counts during the study. Individual changes in peak eosinophil counts (**A**), mean eosinophil counts (**B**), and percentage change in eosinophil counts (**C**) from baseline to the end of the study (proximal and distal esophagus combined) are shown. *BAS*, Baseline; *EOS*, end of study.

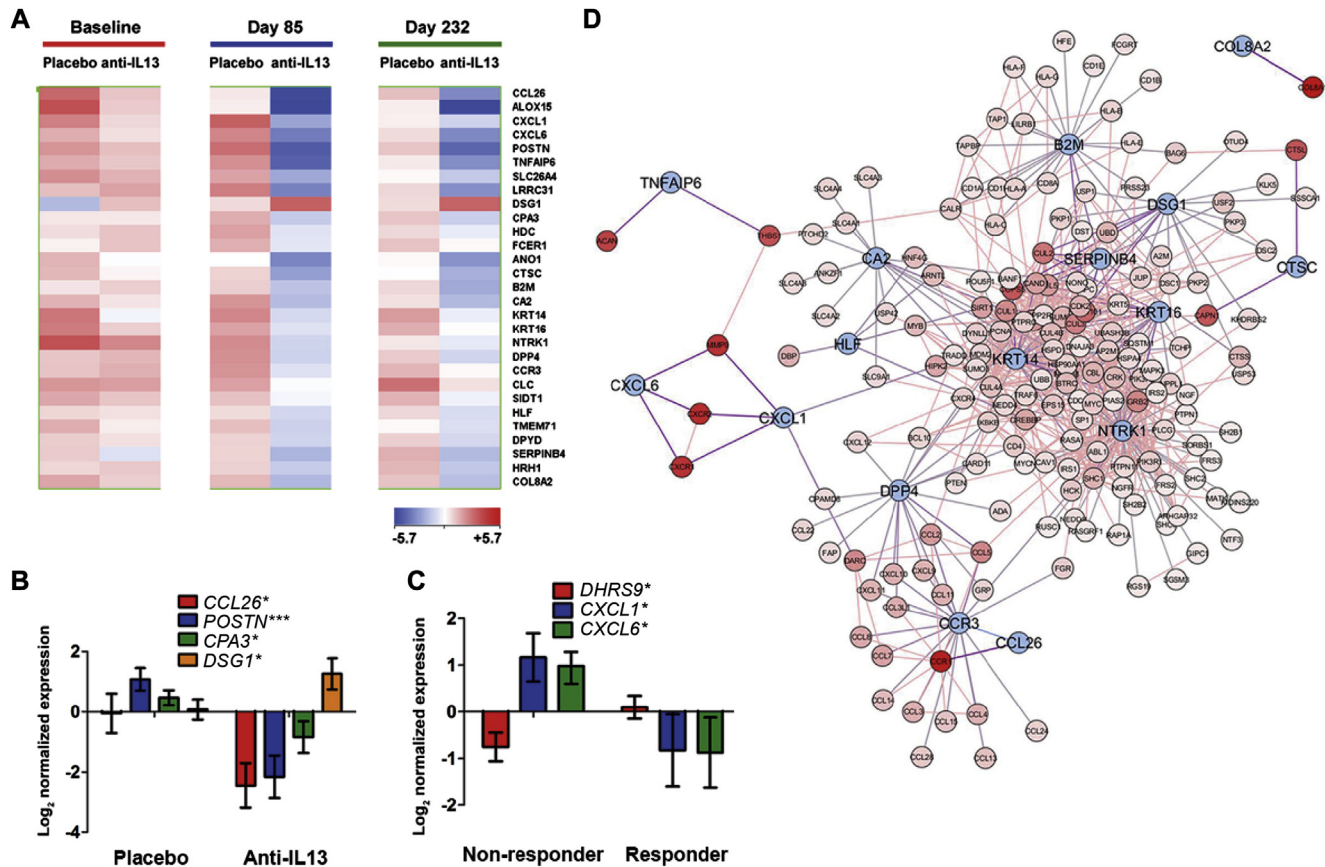


FIG 3. Molecular efficacy of anti-IL-13/QAX576 intervention. Biopsy specimens taken from the esophagus were subjected to NanoString-based transcriptome assay. **A**, Among the 91 known EoE genes screened, the 29 transcripts shown in the heat diagram were significantly different when comparing day 85 placebo-treated and QAX576-treated samples ($P < .05$, fold change > 1.5 , 2-tailed t test). Data are from the normalized median value and the average value of all patients in the same cohort. The expression patterns at the end of the study are also shown. **B**, Among the 29 genes in Fig 3, A, 4 representative genes cardinal for EoE pathogenesis were chosen to establish a bidirectional bar chart illustrating the effect of anti-IL-13 at day 85. Results for placebo versus anti-IL-13 (mean $\pm$ SEM) are shown. $*P < .05$ and $***P < .001$, 2-tailed t test. Difference in the Log₂ median-normalized ratio indicates fold change. **C**, Biopsy RNA samples in the QAX576 group were screened at baseline on the basis of their subsequent responses to QAX576 in terms of mean esophageal eosinophil counts. Three significant genes (mean $\pm$ SEM: $P < .05$, fold change > 1.5 , 2-tailed t test, responders vs nonresponders) are shown in the bar chart, demonstrating the prediction power at baseline to subsequent response to anti-IL-13. A heat diagram is exhibited at the top panel to illustrate the fold change. **D**, Using the 29 genes in Fig 3, A, as input, a comprehensive pathway analysis was performed, focusing on protein-protein interactions. The network demonstrates several communication centers (shown as blue nodes) contributing to most of the organization/regulating functions (**D**). The different levels of red indicate the statistical strength of the interaction; the darker red is more significant.

subjects with periostin levels of less than the median ($P = .10$, Fisher exact test). There was no trend discernible with serum levels of eotaxin-1 and eotaxin-3, which were largely less than the limit of quantification. Tissue eotaxin-3 protein levels decreased in all the subjects treated with QAX576 (see Table E4 in this article's Online Repository at www.jacionline.org).

Safety

QAX576 was generally well tolerated. The most common adverse events were cough and reflux symptoms (Table II), but no specific adverse event appeared to be consistently more common in the QAX576 group than in the placebo group. Two SAEs were reported, one in the placebo group and one in the QAX576 group; neither was considered to be related to the study medication. The SAE that was reported in the QAX576-treated patient was an

asymptomatic cyst-like lesion in the right calf that predated enrollment in the study, but upon subsequent investigations, it turned out to be a spindle cell sarcoma. This patient was withdrawn after the first dose of QAX576.

DISCUSSION

We report the first study with QAX576, a fully human anti-IL-13 mAb, for the treatment of EoE. Although a preliminary phase II study, it demonstrates that (1) QAX576 reduces the intraepithelial eosinophil count in the esophagus, as measured by the mean value; (2) the effect is sustained for an additional 6 months after the third monthly infusion of the drug in the majority of subjects; (3) QAX576 improves the expression of a large set of esophageal transcripts involved in EoE, including eotaxin-3, periostin, markers of tissue remodeling, and mast

TABLE II. Adverse events (all subjects) listed by frequency (number [percentage]) of subjects

	QAX576, 6 mg/kg (n = 17), no. (%)	Placebo (n = 8), no. (%)
Subjects with AE(s)	10 (58.8)	5 (62.5)
Cough	4 (23.5)	1 (12.5)
Nasal congestion	3 (17.6)	2 (25.0)
Oropharyngeal pain	3 (17.6)	2 (25.0)
Gastroesophageal reflux disease	4 (23.5)	0 (0.0)
Headache	3 (17.6)	1 (12.5)
Nausea	3 (17.6)	1 (12.5)
Chills	2 (11.8)	1 (12.5)
Contusion	2 (11.8)	1 (12.5)
Vomiting	2 (11.8)	1 (12.5)
Arthralgia	1 (5.9)	1 (12.5)
Back pain	1 (5.9)	1 (12.5)
Dermatitis	2 (11.8)	0 (0.0)
Dizziness	1 (5.9)	1 (12.5)
Fatigue	2 (11.8)	0 (0.0)
Ligament sprain	1 (5.9)	1 (12.5)
Nasal mucosal discoloration	2 (11.8)	0 (0.0)
Pyrexia	2 (11.8)	0 (0.0)
Sinus congestion	2 (11.8)	0 (0.0)
Sinusitis	1 (5.9)	1 (12.5)
Tonsillolith	0 (0.0)	2 (25.0)

AE, Adverse event.

cell-specific markers, and changes in impaired barrier function genes; (4) the changes in gene expression were present in nearly all patients, regardless of their remission status, and were sustained for 6 months after the last QAX576 dose; and (5) QAX576 was well tolerated. In addition, there were trends for improved clinical symptoms, particularly in the severity of dysphagia, and trends that showed the association of high levels of serum periostin with drug responsiveness. Periostin can directly enhance eosinophil adhesion,^{21,22} as well as increase keratinocyte production of thymic stromal lymphopoietin,²³ a potent T_H2-skewing cytokine that has been genetically linked to EoE susceptibility.^{24,25} It is notable that high serum periostin levels have been shown to be predictive of responsiveness of asthmatic patients to biological therapies²⁶ and to track with lung eosinophil levels,²⁷ highlighting the IL-13–periostin–eosinophil axis. It is interesting to note that although the transcriptome was assessed in esophageal mucosal biopsy specimens, which contain eosinophils and other inflammatory cells that express many of the genes evaluated, the ability of QAX576 to alter genes involved in diverse functions (eg, remodeling and epithelial barrier), as well as specific cellular signatures (eg, mast cells), highlights the likely pleiotropic effect that IL-13 has on multiple cell types, which is consistent with prior whole-tissue transcriptome analysis.²⁸ These preliminary findings merit replication in a larger and more generalized study population because the current study size was small and consisted mostly of young white male subjects in whom conventional intervention (diet or topical steroids) had largely failed. These future studies should also incorporate the lessons learned (eg, selecting patients based on biomarkers) from other biological agents for the treatment of other allergic diseases.²⁹

Although the primary end point of this study was not met, it is important to note that the threshold for this change in eosinophil counts was empirically chosen at a 75% reduction of the peak eosinophil level. The threshold of esophageal eosinophil counts

has not been established. In fact, after the study was initiated, it has been proposed that mean eosinophil values might more likely reflect the “eosinophil load” compared with peak values.³⁰ We are encouraged that the observed change in eosinophil counts was associated with notable trends for improved clinical outcomes, even using a PRO instrument that is not specific for EoE.

To date, early clinical trials with targeted therapy for EoE, including anti-IgE,³¹ anti-IL-5,^{32,33} and chemoattractant receptor-homologous molecule expressed on T_H2 cells antagonists,³⁴ have shown only modest beneficial effects, with substantial patient heterogeneity. Even recent studies with swallowed glucocorticoids have revealed substantial patient heterogeneity, with some patients being completely steroid resistant.¹³ Nevertheless, our findings demonstrate that the EoE transcriptome is markedly reversed by anti-IL-13 in the large majority of the patients (see Fig E3), regardless of the clinicopathologic improvement observed. This establishes that (1) IL-13 is more or less uniformly operational in patients with EoE; (2) the effected genes are downstream from IL-13; (3) the cardinal eosinophil chemokine eotaxin-3,¹⁶ the remodeling and T_H2-promoting gene periostin,²¹ and *ALOX12* and *ALOX15* are IL-13 dependent *in vivo* and promote tissue remodeling^{35,36}; (4) expression of *CPA3*, which is a marker of the esophageal mast cells involved in EoE,²⁸ is markedly downregulated by QAX576; and (5) anti-IL-13 has the potential to improve barrier integrity because values of *DSG1* and epithelial differentiation genes were improved after therapy. The observed long-term effects of QAX576 (6 months after the last dose), including those on expression of genes involved in tissue remodeling, might argue for a potential additive effect and/or advantage over long-term treatment compared with other therapeutics, including corticosteroids. Although these findings are encouraging and informative in terms of the molecular action of IL-13, they were not accompanied by comparable generalized clinicopathologic improvements, calling attention to better understanding of the relationship between these findings and/or more effective use of QAX576 (eg, in patient subgroups with adjuvant therapy). It is important to note that the majority of patients enrolled in this study were refractory to conventional therapy (diet, glucocorticoids, or both) and therefore might represent a difficult-to-treat group. Finally, the combined patient heterogeneity and small sample size undoubtedly reduced the ability to prove some of the observed trends.

Taken together, we have provided proof of principle that QAX576 significantly improves intraepithelial esophageal eosinophil counts and dysregulated esophageal disease–related transcripts in adults with EoE. These results, combined with the observed trends for improved clinical responses, highlight the importance of further examining the effect of QAX576 and other related biological therapeutics for the treatment of EoE by using a PRO that is specific for EoE,³⁷ which is currently being developed. This study also demonstrates that a specific anti-IL-13 antibody can alter the biology of a human T_H2-driven disease at the molecular level in a relevant tissue, which provides confidence in the anti-IL-13 approach treatment of other T_H2-driven diseases.

Sample management, sample analysis, and technical assistance were provided by Martin Letzkus, Urs Affentranger, Aurélie Seguin, Tiziana Valensise, Stephan Bek, Junli Yu, and Shenglin Ma of the Biomarker Development Group of Novartis Institutes for Biomedical Research. Editorial

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Clinical implications: QAX576 improved esophageal eosinophil counts and expression of genetic markers of mastocytosis, eosinophil chemotaxis, tissue remodeling, and barrier function; these findings have implications for disease modification in patients with EoE and similar T_H2 -driven diseases.

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Online Repository

Supplement to: Rothenberg ME, Wen T, Greenberg A, et al. Intravenous anti-IL-13 monoclonal antibody QAX576 for the treatment of eosinophilic esophagitis.

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Appendix 4

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Appendix 5

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Table E2. An example for arriving at the sum change in the MDQ score

Table E3. Nanostring codeset probe sequences for genes reported in this study

Table E4. IHC staining scoring for Eotaxin-3 expression in esophageal biopsies

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28
29

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37
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Appendix 2: Methods

Study design

The study was conducted in compliance with the International Conference on Harmonization and the Principles of Good Clinical Practice, at four sites in the United States. The study protocol was reviewed and approved by each site's institutional review board. The first patient was enrolled in December 2009, and the last patient completed the study in January 2012.

Inclusion criteria as per study protocol

1. Male and female subjects with symptomatic eosinophilic esophagitis aged 18 to 50 years.
2. Female subjects were required to be:
 - Surgically sterilized at least 6 months before study participation (documentation of sterilization to be provided);
 - OR
 - Postmenopausal (no regular bleeding for at least 2 years) status must be confirmed by a plasma FSH level of >40 IU/L at screening or baseline.

Note: A pregnancy test was to be performed on all female subjects regardless of reported reproductive status at specified time points throughout the study.
3. Histological findings on esophageal biopsy to include peak eosinophil density ≥ 24 per HPF (400x) in the proximal or distal esophagus validated by a central laboratory pathologist (Quintiles Laboratory).
4. Elimination diet must either:
 - Not be indicated following allergy evaluation including skin prick testing with multiple food antigens. The type of allergy testing will be determined at the

discretion of clinical staff allergists.

OR

- Have been refused to be followed by subjects.

OR

- Have undergone a minimum of 3 months of elimination diet as indicated by skin prick testing without detectable resolution by repeat endoscopy with biopsies, demonstrating persistent EoE or subject refusal to follow elimination diet.

5. Subjects treated with a proton pump inhibitor (PPI) must have been on treatment for at least 2 months before enrollment. The PPI must be used before endoscopy to rule out the possibility of GERD in the proximal or distal esophagus.

6. Failure of histological improvement is defined as eosinophil density ≥ 24 per HPF (400x) after 2 months treatment with a PPI or documented by prior endoscopy.

OR

Lack of complete disappearance of symptoms.

7. Subjects had to be able to communicate well with the investigator, to understand and comply with the requirements of the study and also to understand and sign the written informed consent.

Exclusion criteria as per study protocol

1. Subjects have received systemic corticosteroid therapy, by any route of administration, within 3 months before dosing or have received topical corticosteroids (swallowed aerosolized fluticasone or budesonide) within 2 months before dosing.

2. Comorbid eosinophilic disorders (other than atopic dermatitis not requiring chronic steroid therapy).

3. History of exposure to human therapeutic antibody, immunoglobulin, or other plasma product within 6 months of dosing (e.g., Xolair®).
4. History of clinical schistosomiasis, or stool examination positive for ova or parasites or travel within the preceding 6 months to an area with endemic schistosomiasis, including but not limited to Southeast and Southwest Asia, South America, and Africa. Travel to these areas was not to be planned for at least 6 months after the last dose.
5. Subjects who have not had a trial of PPI or prior allergy testing/elimination diet and who did not fulfill entry criteria after PPI or initiation of elimination diet therapy.
6. Participation in any clinical intervention with any drug administration within 4 weeks before initial dosing or longer if required by local regulations, and for any other limitation of participation based on local regulations.
7. Donation or loss of 400 mL or more of blood within 8 weeks before initial dosing, or longer if required by local regulation.
8. A past medical history of clinically significant ECG abnormalities. An abnormal ECG is defined as PR >220 ms, QRS complex >120 ms, QTcB >430 ms, or any significant morphological changes, other than non-specific T-wave changes.
9. Any surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the subject in case of participation in the study. The investigator was to make this determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following: major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection.

10. History of immunodeficiency diseases, including a positive HIV (ELISA and Western blot) test result.

11. Positive Hepatitis B surface antigen (HBsAg) or Hepatitis C test result.

12. History of drug or alcohol abuse within the 12 months before dosing, or evidence of such abuse as indicated by the laboratory assays conducted during the screening.

13. Subjects that took acetaminophen (paracetamol) chronically, that is, more than 1 g/day for more than 3 out of 7 days, or more than 2 g/day for more than 1 out of 7 days (added as part of Amendment 5).

Study treatments

Patients were instructed to maintain their baseline diet throughout the study. Patients already on PPIs, nasal, or inhaled steroids were allowed to continue these throughout the study. Responder patients who relapsed were allowed to go into an open-label study, where they continued to receive study medication monthly for three more doses.

Randomization was performed by Novartis. Each study site's unblinded pharmacist received the "Master Randomization List" as well as instructions for requesting a randomization number from Novartis. The randomization was performed by using a "Request & Response" exchange system by an e-mail between the study site's unblinded pharmacist and Novartis. Patients, investigators, and study personnel were blinded to treatment assignment throughout the study.

Assessments

Efficacy—histology

Subjects had a standard endoscopy, and up to six biopsy specimens were obtained from the proximal and distal thirds of the esophagus. Two biopsies from the proximal and two from the distal esophagus were cut serially in 5- μ m sections, and stained with H&E and used for eosinophil counts. The eosinophils in the most densely infiltrated area were counted in five consecutive HPFs, using an American Optical Microstar Model #1982 microscope at a magnification of 400x. The counts were read blind, without any knowledge of treatment allocation or visit status. Data were expressed as the maximum (peak) and the mean eosinophil count per HPF for the 10 counts from each location (i.e., proximal and distal esophagus), and for the 20 counts from the proximal and distal esophagus combined. Responders were defined by a reduction in the peak eosinophil counts per HPF by 75% or more at Day 85, compared with the baseline counts.

Efficacy—symptoms

A subset of questions from the MDQ was used to derive the “sum change in MDQ scores” from baseline to Day 85. The specific questions used to derive this and the further details of deriving this score are given in Tables S1 and S2 in Appendix 5. On this basis, a symptom responder would show a positive score.

Efficacy—transcriptomics, circulating protein biomarkers and histochemistry

Transcriptomics

The EoE transcriptome before and after study medication (QAX576 and placebo) was compared using the NanoString nCounter technology (NanoString Technologies, Inc., Seattle, WA, USA) for direct multiplexed measurement of gene expression. Biopsy tissue collected for RNA extraction was immersed in 1 mL RNAlater. RNA was isolated using the Qiagen RNeasy Micro Kit and analyzed by the Biomarker Development group at Novartis, Basel. Samples were processed with the nCounter Prep Station and Digital Analyzer of NanoString Technologies. The custom-designed nCounter Gene Expression CodeSet contained probes for 91 EoE-related genes, nine candidate reference genes for normalization, and two gender control genes. Probe sequences for genes reported in this study are shown in Supplementary Table S3. 250 ng of RNA was hybridized with the CodeSet at 65°C for 16 h. Post-hybridization processing procedures were carried out as recommended by NanoString Technologies. Cartridges were scanned at a resolution of 1155 fields of view. Gene expression barcode counts were analyzed using the nSolver Analysis software v1.0 (NanoString Technologies). Raw NanoString barcode counts for each gene were subjected to two subsequent normalization steps. First, a technical lane-to-lane normalization was performed using the geometric mean of counts obtained for six spike-in artificial positive control probe sets enclosed in the CodeSet. Second, a biological, mRNA content-related normalization (overriding the preceding technical normalization) was performed using the geometric mean of the technically normalized counts of the three reference genes (*ALAS1*, *CLTC*, and *GAPDH*).

Circulating protein biomarkers

Eotaxin-1 and eotaxin-3 were assayed by a sandwich ELISA technique using commercial kits (Human CCL26/Eotaxin-3 Immunoassay Cat No.: Dcc260; Lot No.: 286565; Human CCL11/Eotaxin-1 Immunoassay Cat No.: STX00; Lot No.: 287254; Vendor: R&D system).

Histochemistry

Immunohistochemical staining for periostin was performed on 4- μ m formalin fixed paraffin embedded tissue sections using Ventana Discovery XT Staining Module. Following deparaffinization, antigen retrieval and incubation with blocking reagents, tissue sections were incubated with the rabbit anti-human periostin pAb (AB14041, 1:2000) for 60 minutes in an automated slide-staining device. IHC staining for eotaxin-3 was similarly performed with the goat anti-human eotaxin-3/ccl26 pAb (AF635, 1:100).

Appendix 3: Statistical methods

No formal statistical hypothesis was tested in this exploratory proof of the concept study. Subjects were analyzed according to treatment received. All subjects that received at least one dose of the study drug and had evaluable biopsy data were included in primary analysis sets. The samples size determination was based on being able to show that the lower 90% confidence limit for the proportion of responders in the QAX576-treated group is at least 35%. If seven out of 12 (58%) subjects treated with QAX576 responded, the lower 90% confidence limit would be 36%. In total, 21 subjects were to be randomized in this study in 2:1 (QAX576: placebo) ratio in order to have at least 18 subjects complete Day 85 assessments.

To identify differentially expressed transcripts at baseline vs. Day 85, the normalized gene expression data/profiles (expression data acquired by Nanostring) were imported into the software of GeneSpring GX 12.5 by corresponding format. The proximal and distal data from each subject were analyzed collectively. Two primary comparison points, namely placebo-treated at D85 and QAX576-treated at D85, were selected due to the biological and pharmaceutical significance. Two-tailed student t-test was used to identify transcripts being significantly modulated by QAX576 with a P value of <0.05 deemed as statistically significant. A fold-change filter of 1.5 in either direction was also used on top of the P value screening to yield biologically significant transcripts.

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221 **Appendix 5**

222 Tables

Table E1. Method for calculating the sum change in the MDQ score

MDQ question #	Question	Score range		Possible maximum change from baseline to Day 85
		Min	Max	
1	In the past 2 weeks, have you had trouble swallowing, not associated with other cold symptoms (such as strep throat or mono)?	1	2	±1
2	How would you rate the severity of your trouble swallowing over the past 2 weeks?	1	5	±4
4	How often have you had difficulty swallowing in the past 2 weeks?	1	8	±7
9	In the past 2 weeks, have you ever had solid food (not including medications) stick in your swallowing tube for over 5 minutes?	1	2	±1
10	Has it been painful to swallow in the past 2 weeks?	1	2	±1
13	In the past 2 weeks, how frequently have you noticed heartburn?	1	4	±3
14	How would you rate the severity of your heartburn over the past 2 weeks?	1	5	±4
16	In the past 2 weeks, has your heartburn awakened you at night?	1	2	±1
20	In the past 2 weeks, how frequently have you noticed acid regurgitation?	1	4	±3
21	How would you rate the severity of your acid regurgitation over the past 2 weeks?	1	5	±4
		10	44	±29
The change in the score for the subset of 10 questions from the MDQ listed above was used to ascertain the effect of QAX576 on the frequency and severity of the symptoms most relevant to EoE. Responses to some questions (e.g., yes/no) allowed for a change of ±1, whereas others responses allowed for a change of up to ±7. An improvement in symptoms was thus scored with a positive value and a worsening with a negative value, and a no change in symptoms was scored 0. An illustration of the scoring to arrive at the “sum change in MDQ” is shown in Supplementary Table S2. On this scoring system, a positive value for the sum change would indicate an improvement in symptoms.				

Table E2. An example for arriving at the sum change in the MDQ score

Question	Response		Score
	Baseline	Day 85	
In the past 2 weeks, have you had trouble swallowing?	Yes	Yes	0
How would you rate the severity of your trouble swallowing?	Severe	Mild	2
How often have you had difficulty swallowing?	With every meal	Less than once a week	2
Have you ever had solid food stick in your swallowing tube for over 5 minutes?	No	No	0
Has it been painful to swallow in the past 2 weeks?	Yes	No	1
In the past 2 weeks, how frequently have you noticed heartburn?	Less than once a week	About once a week	-1
How would you rate severity of your heartburn over the past 2 weeks?	Moderate	Mild	1
In the past 2 weeks, has your heartburn awakened you at night?	About once a week	Less than once a week	1
In the past 2 weeks, how frequently you noticed acid regurgitation?	About once a week	Less than once a week	1
How do you rate severity of your acid regurgitation over the past 2 weeks?	Mild	Mild	0
Sum change in MDQ			7

Table E3. Nanostring codeset probe sequences for genes reported in this study

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>ADAMDEC1</i>	GTGGATCTTTTTCCCCAGGTTAGAACT GTGCCATCTCAGGAAGTTGTCAA	CCCACACGTCGATTGTTGAAGCTAATCCC GCTGAGAAGCTGAGCATGGTC
<i>ALAS1</i>	AGTGTTCAGAAATGATGTCCATTTTT GGCATGACTCCATCCCGATCCCC	GAGAACTCGTGCTGGCGATGTACCCTCCA ACACAACCAAAGGCTTTGCCA
<i>ALOX15</i>	TCGTAGGGCATGTCCAGCTTTGCATTTC CGGATCTCAATTTCTTATCCAG	CCCACTGTTTTCCACCACGCTGGGCCGC AGGTAC
<i>B2M</i>	CACGGAGCGAGACATCTCGGCCGAAT GCTGTCAGCTT	CAGGCCAGAAAGAGAGAGTAGCGCGAGC ACAGCTAAGGC
<i>BATF</i>	TTGAGCATCTTCTTAATACTCTTCTG CATTGGGCCTGCAACCCA	TATTTAGAAAAATAACCGTTCCCTGCCC GCCTTGCTCTGTGCCATGGGAC
<i>BCL2A1</i>	GGCAATGTGCTGAGAATGCTCACTGAG CTTGACTGAGTTATGACACATGA	CTGGTGGAGAGCAAAGTCTTGAGCTGGC TCACCTTGAAGCTGTTGA
<i>CA2</i>	CCTTCAAAAAAATACCTAGAACGGCCA GTCCATCAGGTTGCTGCACAGCT	GGAATCCAGCACATCAACAACCTTTCTGA AGGCCCGGTTTAGCGCTGCCAA
<i>CCL11</i>	TTTGGAGTTGGAGATTTTTGGTCCAGA TACTTCATGGAATCCTGCACCCA	GGCAACACTCAGGCTCTGGTTTGGTTTC AAAAATGGTGATTATTTATGGC
<i>CCL18</i>	ACACTGATTGATCCATGCATTGAATGT ACGAAGAGTTGAAGGGAAAGGGG	AGATTTGTCATTTGATACATATGGCACA ATGTCTGCTGAGAAAGCTAATC
<i>CCL24</i>	GGGATGATGTGGTGGGCACAGACACCA AGGAACAGAAGGCTGGTTACTAT	TGGAAACAAAGAACATGCAGCAGGGAGA GGGGATGACCACAGAGCCCGTA
<i>CCL26</i>	AAGGGGCTTGTGGCTGTATTGGAAGCA GCAGGTCTTGGATATG	TGTTACTGGTGAATTCATAGCTTCGCAC CCAGGTCCAGGG
<i>CCL5</i>	AAGAGTTGATGTACTCCCGAACCCATT TCTTCTCTGGGTTGGCACACACT	AAATTTGTGTAAGTTCAGGTTCAAGGAC TCTCCATCCTAGCTCATCTCCA
<i>CCR3</i>	TGTGGAAATGCCTCCAGCTATATACTG TATCCTCTGGGTAAAGAGCACTG	GGCCATAACGAGCAGAGGGAGAACGAGA CAGAAGATGGTCATTCTCAGAG

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>CDH1</i>	CAGGCACCTGACCCTTGTACGTGGTGG GATTGAAGATCGGAGGATTATCG	ATCAGCATCAGTCACTTTCAGTGTGGTG ATTACGACGTTAGCCTCGTTCT
<i>CDH2</i>	GAGGAAAAGGTCCCCTGGAGTTTTCTG GCAAGTTGATTGGAGGGATGACC	CCGCAGTGAAAGGTTTTTATCTCTATCA GACCTGATCCTGACAAGCTCTT
<i>CDH26</i>	TTTCATTTACCCTCAAAGCTAGGCTGC CAGTGGAAGCTAAGAATAACACA	TATTACCTGCTGTTCCCAAGTGGCTGGGT AATCTATGGGTTATAT
<i>CEACAM7</i>	CTCATGTTTTTAGGAAAGTTTTGTCTG TTCTGGGATCTACCTGCTTC	GTTAAAGCAGAAAGGACTTTCCTTACTGT GCTGACTCAGACAGCCTGGACT
<i>CF1 (IF)</i>	CAGACATAAGTCACATTGTTGGCATCC ATACAGACTAAGGGGCTCCAGA	CACCTGGGAACTCTGGTTTTCCACAGTTT TCCCCCAACTCACAACACCC
<i>CH25H</i>	ACAGTTCACAGACGCTCATATACTGCG TTGCCAGCGCGAACGAGGACGAG	GTGGCACCCGAGCAGTGTGACGTTTCATC ATGTCGAAGAAGCCCAAAGAAA
<i>CLC</i>	TTGTCACAGTAGAACCAGTAGACAAAAG AGGCAGCCTCTGTGTATGGCAGC	CACCTGCAGATATGGTTCATTCAAGAAA CAGGCAAGTGGTCGCCCTTTGA
<i>CLDN7</i>	TCGCGGCACAGGGAGTAGGATACGCCG GGAGGGTGGTTCCAGACAAAATT	TCTTGTACCAAACTACACACAAATCGA CCCCTCCAGTGAAGCGATGGCC
<i>CLEC7A</i>	CTTTCTCTGAAACAACAGCTATCCTGG TATTGCTTTGAGAGTCGAAGTGT	TCCCAAAATTACAGCAATGAGGCGCCAA GGAGGAGATGCAGCACACGATC
<i>CLTC</i>	CAGACTCCATAGTCAGGGTACTGAAGC CAATGTTTGCTGGGTTGATACCC	TACCACCTGGGCCTGCTCTCCTACTTTTT CTCTAATGCAGATGAATTTGT
<i>COL1A1</i>	GCCCTGCGGCACAAGGGATTGACACGC GTTCCCCAAATCCGATGTTTCTG	GTCTTTCAGCAACACAGTTACACAAGGA ACAGAACAGTCTCTCCCGCCCA
<i>COL1A2</i>	TTTAGCACCAGGTTGACCAGCAGCACC AGCAGGACCAGCAAATCCATTGG	GGACCAACAACACCGTTTTTCACCCTTAGG CCCTTTGGCTCCTCTTTCTCC
<i>COL3A1</i>	GACTGACCAAGATGGGAACATCCTCCT TCAACAGCTTCCTGTTGTGCCAA	AGACACATATTTGGCATGGTTCTGGCTT CCAGACATCTCTATCCGCATAG

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>COL5A2</i>	TGCGCATGGTTTCAATCTGACTACTGA GTGACTTCAGGGTAGCATGAACC	CGTGCGGGCTGGGTGCTTTTTTCGAGCCAT CGGGGC
<i>COL8A2</i>	TCTGTGAAATAGCTCCCGTTTTTTTTTC TTGGGACCCTACTTAGTTC	AACCACCCCTCCTGAAAGGTTTGAGGAA TGAAATTTGGCAGAAGCTCAGC
<i>CPA3</i>	TTCTCACTAACTCGGAAATCCACCATC ATATTAGCAGCTACGTGGTGGGT	CATAGTGCATTTTATTTTGATCCAAGGC AGACTGGATGGCTTGGGATTCC
<i>CTSC</i>	TTCTGAAGGTACACCACTACTTTTTTT TCTTGTGGTCCCATAACCGAGCA	TGATGGTGAAATGGCCAGAATTGCCAAG GTCATCATATGCTGTATCCAGC
<i>CTSS</i>	TCTGAGTCGATGCCCTTGTTATCAATG ATGTACTGGAAAGCCGTTGTCAT	TTGAGTCATATTGACATTTCTGATCCAT GGCTTTGTAGGGATAGGAAGCG
<i>CXCL1</i>	CTCTATCACAGTGGCTGGCATGTTGCA GGCTCCTCAGAAATATTAACATA	TTCACAATGATCTCATTGGCCATTTGCTT GGATCCGCCAGC
<i>CXCL2</i>	GGCCATTTTCTTGGAATCCTCAGCCTC TATCACAG	GTTACAAAATAGACACACATACATTTCC CTGCCGTCACATTGATCTCACT
<i>CXCL6</i>	GCTCCGCTGAAGACTGGGCAATTTTAT GATGCATGGTCTTTTTTGTACT	ACCAACCCTTCCTTCTTATTCTTACTGGG TCCAGGGATCTCCAGAAAAC
<i>DHRS9</i>	CCACCAGCGATGGTGGGACTCCCTTTT GCAATCCAAATAAGCACGATCTA	CCATTCCAGCCCTCCTTTTCAAAGAAAAC TTGAGCAGGGACCCTGGGATA
<i>DPP4</i>	AAATCCACTCCAACATCGACCAGGGCT TTGGAGATCTGAGCTGACTGCTG	CTGCTAGCTATTCCATGGTCTTCATCAGT ATACCACATTGCCTGG
<i>DPYD</i>	CCACCTTCCTTTGCAGCTCTTGCGATGC TCACAATATCAGTGACATTTGG	TTAATCCCATCAGACCTGAGACAGTGTT GGTGGCTGTAACGCCATTGGCA
<i>DSG1</i>	GTCACTACCACATTTGAAGACTCTCTA GGATGATGGATAGTCAGAGAGGT	GCATACTCAGACTACCTATCATGCCGGA AGTTGGTTGGATTACTCTTTCT
<i>EDNRA</i>	GTCATGAGGGTGTAGAAGATCGCAGTG CACACCAAGGGCATACAGAAATA	CACTGAGGGCAATTCTCAAGCTGCCATTC CTTCTGTTCAACATCTCACAA

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>EML5</i>	CAGCCAAAGGTTTAGTAGGATGGACAG CAAGTGCCCAAAGTTCACCTTCA	ATGATCTACTAGGCTCCATATCCTGACCG AACGATCATCACTTCCAGTCA
<i>FCER1 (MS4A2)</i>	TCCACAGATTGTGAGTGACACAGCACT ACCAAGTCCCAGAATGGTGAGAA	TCATAAACACGATCCTCTGGAACCTTGT TTCCTTTGAGTTCTTCCCCAGC
<i>FGF9</i>	CCCATCCAAGCCTCCATCATACATTAT GCATGCGACAAAGTTTGGCAACA	CAGCCCTCCAGGAGGAGAAGCCTTTAAG TGCAGAACAAAATCAGCATATT
<i>FGG</i>	GGATCATCGCCAAAATCAAAGCCATCA AAGGCATCTCCAGCATCCCCACC	CCCAGGTACTGAACTGCATGCCATTATG GGATGTGAAAAACTTGTCACTA
<i>FLG</i>	CCTTGTCATATGTTTTTCTGCTTGAC TTCTGGATCCTGACTGCCACGG	CGACCCTGAGTGCCTAGAGCCATCTCCTG ATTGTT
<i>FMO2</i>	TTGATGGCTCCACAGAGTAGACGACTT GGGACATCATCATTTAGTACAGG	CCTCAAAGATGGCAGAAGTTTCTGTGAG CTCTTTCCTGCTAGATTTTACC
<i>FZD10</i>	GTTGAGGCGTTCGTAAAAGTAGCAGGC GATCACACAGGTGGCCGG	TTGTTTCATTTTGCACTTGTGCTGCGCCGC CAGGATCTTCCAGTAATCCAT
<i>GAPDH</i>	GCTCGGCTGGCGACGCAAAAGAAGATG CGGCTGACTGTGGAACAGGAGGA	CGACCTTCACCTTCCCCATGGTGTCTGAG CGATGTG
<i>GATA3</i>	ACCCTCAGCACAGGCTGCAGGAATAGG GACAAGACAGATG	TCGCTGACATGCACGCTGGTAGCTCATAC ACTGCT
<i>GUSB</i>	AGATTCTAGGTGGGACGCAGGCTCGTT GGCCACAGA	TCCAAGGATTTGGTGTGAGCGATCACCA TCTTCAAGTAGTAGCCAGC
<i>HAS2</i>	CGGGACCCTTTTCTGTGAAGTTGTTCT TCCAGATATAAGTGGCTGATTTG	CAATTGCGTTACGTGTTGCGAGCTTTCT TTATGTGACTCATCTGTCTCAC
<i>HDC</i>	TTCTGTGAGACAATTAGGACCCTTTAG ACGAAAAACCACCAGGCCAAGGT	GCCGGGATGAGGAAGAGACGGCCAGCTT TAGCTATTTCCCTTTAACACATT
<i>HLF</i>	CCAAAATGGGAGTCAGTTGTGTTTATG CCTCTGGTAAAGTGCTGATGTCA	CACACACATGAGCACAAGCACATATACA CGCACACACACACAGATGCACA

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>HPRT1</i>	TGAGCACACAGAGGGCTACAATGTGAT GGCCTCCCATCTCCTTCATCACA	CAGTGCTTTGATGTAATCCAGCAGGTCA GCAAAGAATTTATAGCCCCCT
<i>HRH1</i>	ACTCCATGTCAGCTGTTCTGCTACTCA AACATATCATTTTGAGCTGCCAC	AAAAATGCACTTGGTTCCTTCAAAGCGT CCCCTTCCGTAGGTGCACGGGA
<i>IFNG</i>	CTGGCTCAGATTGCAGGCATATTTTCA AACCGGCAGTAACTGGATAGTAT	ATCAGGGTCACCTGACACATTCAAGTTC TGTCTGACATGCCATTAAAGCA
<i>IL13</i>	CCTAACCTCCTTCCCGCCTACCCAAGA CATTTTTGACATCAGAAAGAAA	TGAAGACGGGCAGCACAGGCTGAGGTCT AAGCTAAGGAATTTTACCCCTC
<i>IL13RA1</i>	TTTCCTCCTTGGTTTGCTTCTCATAGA TGTCGTACTTCTTCCAGTGCAGA	CCATCACTGAGAGGCTTTCTTCAGGTTTT CTATCAGCACTACAGAGTCGG
<i>IL13RA2</i>	AAAGCGTGTGTATCTTCGCTTCAATGC CCTTGTTAAGATCAAACCATCT	TTCTGCCCAGGAACCTTGAACCTTCTGATC CATTTGTGCATTGCCATGGTA
<i>IL4</i>	TGCTTGTGCCTGTGGAAGTGTGTGCA GTCGCACC	CCAGAGGTTCTGTGCGAGCCGTTTCAGG AATCGGATCAGC
<i>IL4RA</i>	ATCTGCCGGGTCGTTTTCACTCCAAAT GTTGACTGCATAGGTGAGATGAT	GCTGCGATGCGGAGGGAGGGTTCTAGGT AGGTCACGTTATAGATTCTGAA
<i>IL5</i>	AAGCAGTGCCAAGGTCTCTTTCACCAA TGCACTTGTGGGAATTTCTGTGG	ACAGGAATCCTCAGAGTCTCATTGGCTA TCAGCAGAGTTCGATGAGTAGA
<i>IVL</i>	GTTACAGATGAGACGGGCCACCTAGC GGACCCGAAATAAGTGGAGC	CCCACCTCACCCATTAAAGAGACATGT AGAGGGACAGAGTCAA
<i>KIAA1199</i>	GATCTATAGGTGTCAAACCGGTCAGAA TGGATGACTGTGCCTGATTTGGG	CATCGGGCACCGGTTCAAATACTGGACC AGACGTTCACTCTCTTTCTTG
<i>KRT14</i>	TCCTCAATGGTCTTGAAGTAGGGACTG TAGTCTTTGATCTCAG	TGTCCACTGTGGCTGTGAGAATCTTGTT CCTCAGG
<i>KRT16</i>	GATGCTTGCTGGGAGGAAAGGTGGGCA TCCTCGCCCTC	AAGAGGACGAGGAGGAGGTGAAGACCTC GCGGGAAGAATAGGATTGGCCA

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>KRTAP3-2</i>	ATCTGGTCTACCTTAACATCTGGCAAA CTACTGAGGCAAGTGAATACTGA	TTCTTTTTCCTCCCATTAATAACCAAGG TAAGTTCTTCATATCTGGGTC
<i>LOXL4</i>	TCAAGCAGGAATGGTGGCCTTTCCAG GTTCCATCCTTAATAAAGTAC	GAAGACCATGGGGTTTATTCACCTTGA GCAAGAGCAAATCAGAGCCCCA
<i>LRRC31</i>	TCTTCCAAGTCTGGGAGAAAAGGCAGC AAGGCAACCATTCTTTTCATGTC	TGATGGAAAGGAGGGTTCCACCTACAAA ACCATTCCAGGAGATATCCAGT
<i>MKI67</i>	CTGATGGCATTAGATTCTGCACGCTA AGAGTTCTCCCTCTACATCTG	GTCTTTCTCTTCACCTACTGATGGTTTAG GCGTGTGCATGGCTTTGCCTG
<i>MMP12</i>	AATGCCACGTATGTCATCAGCAGAGAG GCGAAATGTGTTGATGTCAACAT	TCAGGATTTGGCAAGCGTTGGTTCTCTT TTGGGTCTCCATACAGGGACTG
<i>MMP2</i>	AATGCTGATTAGCTGTAGAGCTGAAGG CACGGCTGCCAGG	CACCGGGAGGAGCCACTCTCTGGAATCTT AAATTACCAGGTAGGAGTGAG
<i>MMP9</i>	AGATGTTACAGTTGCAGGCATCGTCCA CCGGACTCAAAGGCACAG	CCCATCCTTGAACAAATACAGCTGGTTCC CAATCTCCGCGATGGCGTCGA
<i>NTRK1</i>	CTCTGGAGCCAGCACAGCCGGGCGGTT GATCCCAAACTTGTTT	CACCCAATGTCATGAAATGCAGGGACAT GGCCAGCCCATC
<i>POSTN</i>	TTGGTGGGAGCAAAGAGTGTGAAGTGA CCGTCTCTTCCAAGGGC	CTCCCATGATCCTTTCTAGGACACCTCGT GGAAGTTTCTCAAAAGCCTCA
<i>PPIA</i>	AGCCACCATGCCAGTTGCTGCCTACA TTTTCAATATTCC	CAGGTGGTCTTCTGCCTCAGGTAATACA TTACAGACAGTG
<i>RASGRP1</i>	CAGGTTTCCATCTGCATCAAAAGATTG AATGCAGCTGTCAATGAGATCGT	ACAATTTCGGTGCATGGTCAGCATGACTT GCAACAGTTGGTTACTTCGACA
<i>RPS4Y1</i>	CTTATCTCTCTCTTCAGCAACAGTAAG TCGAATGCCCTTTCCCTGGGCA	AGATATGCTGCTACTGCAATTTAGCCAC TGCTCTGTTTGGTGGCCAGCCT
<i>SERPINB4</i>	GAGGTCTGCATCCCCATTGAAGATATT CACCATTCCCATGGTTCTCAACG	TAGGACTTTAGATACTGAGAGACCGTGG CTCCAGGTCATGCCTGA

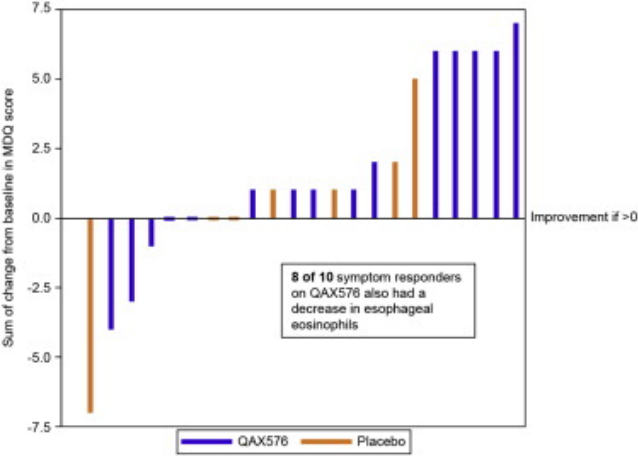
Gene identifier	Capture probe sequence	Reporter probe sequence
<i>SERPINE1</i>	GGTAGGTCATGCAGCCCCCTTGCATTTCTGCTCCTAAATCTATTGAACACA	CAATGCCGGCTGTGAGTCACCCTGTAATTGGGGAAAGTTCTGTCCT
<i>SH2D1B</i>	ATGCCTGCAGAAGTGGGTCATCAGTGA GAACTGTTACTCATCTCTTCAAC	CACAACCAGGAGAGTCTGAATTGTCCAC TAGAGTTTGGTGCTCTGGACCT
<i>SIDT1</i>	ATACGTCATTTTTTCCAAACACCACTC CAAGGACGGTGACCATGATGACC	GGCTAGCGAGGCCAGAACGTGGATTGCA GAGAAGATGACCCAGAACC
<i>SLC26A4</i>	TCTCAGGACCACAGTCAACAGTCCAAA TATAAGGCCAGCTAGTAAACCGA	ATATCTGTGCTAGGGATGCTTCCAAGGC CATTCCAAGAAGGAACTGAAC
<i>SPP1</i>	CTGACTCGTTTCATAACTGTCTTCCC ACGGCTGTCCCAATCAGAAGGCG	TTGTGGCTGTGGGTTTCAGCACTCTGGT CATCCAG
<i>SPRR2C</i>	ATGGCACCCAAAGAGAAGCTCTGAAAAG GCCGGTACAGCTGAGGACTTCCT	GCTCATGCCAGGTGACAGACAGAGACAG AAGACATCAAAATAATTCTTTG
<i>SPRR2D</i>	AAGGAGAAAGAAGCTCCCTGTGCATCC ATGGAAGGCTTTGGTGAGAAGAT	AGAAAGGGAATCTTTTGCTGTACAGAT CATCACAGGCAGGCCACAGGTT
<i>SPRR3</i>	TGCTCAGGGATCATCTGCTGTGCAGAG AAGGTCGAGGTG	TAAGCTCCTGAATTAAGCAGAAAAGTCTT AGTGGCTTTTCTGGTCTTCAGC
<i>STAT6</i>	CCCCAGTGAGCGAATGGACAGGTCTTT GGCAGAGAATGGCTGGATGTTCT	GGCTTCTTGGGATAGAGATTTTGTAGCT GAGCAAGATCCCGGATTCGGTC
<i>TBET (TBX21)</i>	CTTTAGTTTCCCAAATGAAACTTCCTG GCGCATCCAGTGCGCTCCTGTGT	AGCACAATCATCTGGGTCACATTGTTGG ACGCCCCCTTGTTGTTTGTGAG
<i>TFRC</i>	GTCTGGACAGGTATATTAGGCAATCCT GATGACCGAGATGGTGGAACCTG	AGTCTCCTTCCATATTCCCAAACAGCTTT TCTGCAGCAGCTCTGGAGATT
<i>TGFB1</i>	ACGGGTTTCAGGTACCGCTTCTCGGAGC TCTGATGTGTTGAAGAACATATA	CACTTTTAACTTGAGCCTCAGCAGACGC AGCTCTGCCCCGGGAGAGCAAC
<i>TMEM16A (ANO1)</i>	TCGTGCTCCGGGTTTTGCTGTGAAAA AGGAATCCTTATCAGACAAGTCA	GCTGTACTTGGCCTTTGTACACGTCGTTC TCTTCAAGATCTCATAGACAA

Gene identifier	Capture probe sequence	Reporter probe sequence
<i>TMEM71</i>	CAGTTGAGATATTCCGGTACATCTTGGG AAGGCCGCTTGCTCAAACCTTCAC	CCAGCATATTCTCTTTCCAACCTGGAAGA ACTTGCTACTGGTGTGACAT
<i>TNC</i>	CAGTGGTCCCTCCAATTTATGCCCGTT TGCGCCTGCCTTCAAGATTTCTG	CTTTGGTAAAATCCTTTCTCGCTCTGGG CCGCCTTATTCTCTCTCACC
<i>TNFAIP6</i>	CTGGCTGCCTCTAGCTGCTTGTAAGTT GCGAGATGG	CTCTGCCCTTAGCCATCCATCCAGCAGCA CAGACATGAAATCCAATTTTTT
<i>TNFSF13B</i>	ACGTTTTACAAAATCCTTACTGCCCCCT TTGAATTGTGTCCTTCCTCCAAG	TTAATGTCCTAGAGATGTGGGCACTTGC TCCATAGTAAATTATGGATGCC
<i>TPSAB1</i>	GGTATTTTGCCTCACAAATGTGGTTTT CCATTATGGGGACCTTCACCTGC	GACGATGCGGACGTCGTCTCCCGTGTAG GCGCCAAGGT
<i>TUBB</i>	AAAAGGACCTGAGCGAACAGAGTCCAT GGTCCCAGGTTCTAGATCCAC	CCTGCCCCAGACTGACCAAATACAAAGT TGTCTGGTCTAAAGATCTGGCC
<i>UBC</i>	CACTTCGAGAGTGATGGTCTTACCAGT CAGGGTCTTCACGAAGATCTGCA	TCCTTGTCTTGGATCTTTGCCTTGACATT CTCAATGGTGTCACTCGGCTC
<i>UPK1B</i>	TGCTTCAGGAAGAGGTTGGGTGTGAAA AAGTCTTGTTGTGTTGCTGCTGT	ACTGGTCATCATTGTTTGGAGGGCTGTT GTTTTGGTACCTCTCTAGCATC
<i>VCAM1</i>	GGACATAGATGGGCATTTCTTTCCAAG TTCAATACATTACAGGAAGTCTG	TACTGTTCTTCAGGCAGCAAGTTTTACT TTGACTTCTTGCTCACAGCAAG
<i>VIM</i>	CTGAAAGATTGCAGGGTGTTTTCGGCT TCCTCTCTCTGAAGCATCTCCTC	CTTTGCGTTCAAGGTCAAGACGTGCCAG AGACGCATTGTCAACATCCTGT
<i>XIST</i>	CAATTCATTACAGATAGGTTTAGCTCAT GCAATGCACATGACTTCCTCTGC	TACTGCCTCCCGATACAACAATCACGCAA AGCTCCTAACAAGCCCCAAAT

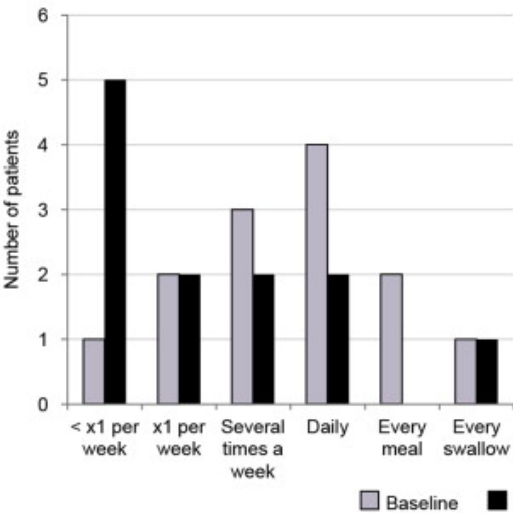
Table E4. IHC staining scoring for Eotaxin-3 expression in esophageal biopsies*

Treatment	Subject ID	Esophagus proximal		Esophagus distal	
		Baseline	Day 85	Baseline	Day 85
Placebo	0001_05109	2	3	2	2
	0001_05111	2	3	3	2
	0001_05121	No sample	1	No sample	3
	0002_05203	4	1	3	1
	0002_05204	No sample	No sample	3	2
	0005_05514	2	2	3	3
	0005_05518	No sample	3	No sample	3
	0005_05523	No sample	2	No sample	3
QAX576 6 mg/kg	0001_05108	4	1	3	1
	0001_05110	0	1	2	1
	0001_05116	2	2	2	1
	0001_05117	4	3	4	3
	0001_05122	No sample	2	No sample	3
	0002_05202	1	1	3	1
	0005_05506	2	3	3	1
	0005_05507	4	2	4	2
	0005_05512	4	1	3	2
	0005_05519	No sample	4	No sample	2
	0005_05520	No sample	2	No sample	2
	0005_06501	4	2	2	1
	0005_06515	No sample	1	No sample	3

*Staining evaluated as; 0=negative), 1=weak positive, 2=medium positive, 3=strongly positive), 4=very strongly positive, according to their staining intensity and staining area.



Frequency of Dysphagia



Severity of Dysphagia

