

Exploring the Effects of Omalizumab in Allergic Asthma

An Analysis of Biomarkers in the EXTRA Study

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Rationale: For many patients with asthma, allergic airway inflammation is primarily a Th2-weighted process; however, heterogeneity in patterns of inflammation suggests phenotypic distinctions exist that influence disease presentation and treatment effects.

Objectives: To assess the potential of fractional exhaled nitric oxide (F_{ENO}), peripheral blood eosinophil count, and serum periostin as biomarkers of Th2 inflammation and predictors of treatment effects of omalizumab.

Methods: The EXTRA omalizumab study enrolled patients (aged 12–75 yr) with uncontrolled severe persistent allergic asthma. Analyses were performed evaluating treatment effects in relation to F_{ENO}, blood eosinophils, and serum periostin at baseline. Patients were divided into low- and high-biomarker subgroups. Treatment effects were evaluated as number of protocol-defined asthma exacerbations during the 48-week treatment period (primary endpoint).

Measurements and Main Results: A total of 850 patients were enrolled. Data were available from 394 (46.4%), 797 (93.8%), and 534 (62.8%) patients for F_{ENO}, blood eosinophils, and serum periostin, respectively. After 48 weeks of omalizumab, reductions in protocol-defined exacerbations were greater in high versus low subgroups for all three biomarkers: F_{ENO}, 53% (95% confidence interval [CI], 37–70; *P* = 0.001) versus 16% (95% CI, –32 to 46; *P* = 0.45); eosinophils, 32% (95% CI, 11–48; *P* = 0.005) versus 9% (95% CI, –24 to 34; *P* = 0.54); and periostin, 30% (95% CI, –2 to 51; *P* = 0.07) versus 3% (95% CI, –43 to 32; *P* = 0.94).

Conclusions: The difference in exacerbation frequency between omalizumab and placebo was greatest in the three high-biomarker subgroups, probably associated with the greater risk for exacerbations in high subgroups. Additional studies are required to explore the value of these biomarkers in clinical practice.

Clinical trial registered with www.clinicaltrials.gov (NCT00314574).

Keywords: immunoglobulin E; eosinophil; fractional exhaled nitric oxide; periostin; severe asthma

Although inflammation is well established as an important target for treatment of patients with chronic asthma, there is increasing recognition of inflammatory and immunologic phenotypic distinctions that could influence treatment effects (1, 2). Varying responses to standard treatments are frequently observed in

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Because of the heterogeneity of response to therapy in asthma, an identification of markers that assess treatment effects of specific medications will improve the likelihood of treatment effectiveness and efficacy in individual patients. We explored the potential of three biomarkers of Th2-driven inflammation (fractional exhaled nitric oxide [F_{ENO}], peripheral blood eosinophils, and serum periostin) to serve as predictors of treatment effects to omalizumab using data from patients with severe allergic asthma enrolled in a large multicenter study evaluating the long-term efficacy and safety of omalizumab (EXTRA study); patients were divided into baseline low- and high-biomarker subgroups.

What This Study Adds to the Field

In this study, treatment effect of omalizumab (percent reduction in exacerbations) was greater in all three high baseline biomarker subgroups when compared with the low-biomarker subgroups, indicating that these patients may achieve greater benefit from omalizumab therapy. Additional studies are required to further explore the characteristics and potential prognostic effects of these biomarkers.

patients with asthma (3–5). A better understanding of the mechanisms underlying the heterogeneity of treatment responses in patients with asthma is critical to the development of personalized medicine. This approach aims to tailor treatment to the individual patient and could be facilitated by the identification of biomarkers that predict responses to therapy (6).

IgE is central to the pathophysiology of allergic asthma (7, 8). Airway inflammation is considered primarily an allergic, Th2-weighted disease in many patients with asthma, characterized by production of distinct cytokines including IL-4, IL-5, and IL-13 from Th2-type CD4⁺ cells (9–11), two of which (IL-4 and IL-13) contribute directly to IgE class switch, thereby increasing IgE levels (12). Unfortunately, levels of these cytokines are low and cannot be measured reliably in blood, whereas serum IgE itself, although readily measurable, has not been particularly predictive of response to anti-IgE therapy (13, 14). Furthermore, antigen-specific IgE has not been predictive of responses to omalizumab (15).

Three potential markers of Th2-driven inflammation involved in inflammatory pathways downstream from these cytokines have been investigated previously: (1) fractional exhaled nitric oxide (F_{ENO}), (2) peripheral blood eosinophils, and (3) serum periostin. The measurement of F_{ENO}, which is generated by an enzyme (inducible nitric oxide synthase) induced by IL-13 (16), has been standardized for clinical use by the American Thoracic Society (ATS) (17). The presence of blood eosinophilia has long been

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associated with asthma (18), and there is evidence that there is a distinct phenotype (eosinophilic asthma) associated with Th2-driven inflammation (10). Serum periostin, which is also generated by airway epithelial cells in response to IL-13, was recently identified as a marker of Th2-related airway inflammation in patients with asthma (10, 19) and its presence has been associated with a greater FEV₁ improvement with anti-IL-13 therapy (20).

Omalizumab (Xolair) is a recombinant humanized monoclonal antibody that selectively binds to free IgE. It is indicated in the United States for adults and adolescents (≥ 12 yr of age) with moderate-to-severe persistent allergic (IgE-mediated) asthma whose symptoms are inadequately controlled with inhaled corticosteroids (ICS) (21). Although previous studies have demonstrated that omalizumab has important benefits for patients with moderate-to-severe uncontrolled allergic asthma (4, 22–30), not all patients with inadequately controlled asthma respond to omalizumab and predictors of response to this biologic agent are limited.

In these prespecified and *post hoc* analyses, we explored the potential of Th2 inflammatory biomarkers (F_{ENO}, blood eosinophils, and serum periostin) to serve as baseline predictors of therapeutic benefit of omalizumab treatment in patients with inadequately controlled severe allergic asthma who were enrolled in a large multicenter longitudinal study (the EXTRA study) (26).

METHODS

Patients

Full details of the patient population enrolled in this study were described previously (26). In short, patients were included in the study if they were 12–75 years of age and had a history of severe persistent allergic asthma for more than 1 year before screening. Patients whose asthma remained inadequately controlled despite treatment with high-dose ICS plus long-acting β_2 -agonist (LABA) were enrolled (*see* online supplement and Hanania and coworkers [26] for details).

Study Design

EXTRA was a prospective, multicenter, randomized, parallel-group, double-blind, placebo-controlled study. Full details of the study design have been published previously (26). In brief, after a 2- to 4-week run-in period, eligible patients were randomized in a 1:1 ratio to receive omalizumab or placebo (in addition to high-dose ICS and LABA, with or without additional controller medications) for 48 weeks. To account for baseline asthma controller medication, three strata were included in the randomization: M1 (ICS plus LABAs alone); M2 (ICS plus LABAs plus one or more additional asthma controller medications but excluding oral corticosteroids [OCS]); and M3 (ICS plus LABAs plus OCS). Omalizumab dose and dosing regimen were determined based on serum total IgE level (international units per milliliter) and body weight (kilograms) at screening, according to the US omalizumab dosing table (ensuring a minimum dose of 0.008 mg/kg/IgE [IU/ml] every 2 wk or 0.016 mg/kg/IgE [IU/ml] every 4 wk) (21). No modifications to the dose of omalizumab or concomitant asthma medications were allowed during the study.

Assessments and Variables

Primary and secondary outcomes measures from the EXTRA study have been reported previously (26). In the present study, three biomarkers (F_{ENO}, peripheral blood eosinophils, and serum periostin) were evaluated as predictors of treatment effects. The criteria for division of patients into subgroups with low and high levels of each biomarker at baseline were defined before evaluation of treatment effects, but were not prespecified in the study protocol. The median value for each biomarker across the subjects was used to provide an approximately similar distribution of data across subgroups. Evaluation of treatment effects within F_{ENO} subgroups was a prespecified subanalysis, and was conducted only at sites in the United States. Measurement of F_{ENO} was performed using a hand-held portable device (NIOX MINO; Aerocrine, Solna, Sweden) in accordance

with ATS guidelines (31). At each visit, F_{ENO} measurements were taken at least twice, and the mean value was recorded. The median of the biomarker was predefined as the cut-off for determining low and high subgroups (<19.5 ppb; ≥ 19.5 ppb). A *post hoc* analysis with an alternative cut-off for defining low- and high-F_{ENO} subgroups (<24 ppb; ≥ 24 ppb) was also performed based on the recently published ATS guidelines (17). Evaluation of treatment effects within peripheral blood eosinophil subgroups was also a preplanned subanalysis. Peripheral blood eosinophil counts at baseline were obtained from standard complete blood counts, and patients were divided into two predefined groups based on baseline median low ($<260/\mu\text{l}$) and high ($\geq 260/\mu\text{l}$) eosinophil counts. Serum periostin levels were assessed as a *post hoc* analysis, and were measured using an Elecsys periostin immunoassay (in development, not commercially available) on a cobase 601 analyzer (Roche Diagnostics, Indianapolis, IN). As for previous studies, the median of the biomarker was predefined as the cut-off for determining low and high subgroups (<50 ng/ml; ≥ 50 ng/ml) (20).

Treatment effect was evaluated within each biomarker subgroup as the number of observed protocol-defined exacerbations (defined as worsening asthma symptoms requiring treatment with systemic corticosteroids for ≥ 3 d; for patients receiving long-term OCS, an exacerbation was an increase of ≥ 20 mg in the average daily OCS dose); this was used to calculate protocol-defined exacerbation rates reported during the 48-week treatment period. Analyses were also conducted to assess time to first protocol-defined exacerbation and secondary efficacy endpoints of total asthma symptom score, albuterol use, asthma quality of life questionnaire (AQLQ), and % predicted FEV₁ by low and high subgroups of each of the three biomarkers (F_{ENO}, peripheral blood eosinophils, and periostin).

Safety was evaluated by recording and monitoring the frequency and severity of treatment-emergent adverse events (AE) and serious AEs.

Statistical Analysis

Efficacy analyses were performed in the modified intent-to-treat (mITT) population and safety analyses were conducted in all patients who received the study drug, as described previously (26). Poisson regression with adjustment for overdispersion was used to estimate the rate of protocol-defined asthma exacerbations over 48 weeks. This method accounts for differential time on the study and allowed adjustment for the following covariates: dosing schedule (every 2 or 4 wk); prior exacerbation status (≤ 1 exacerbation, >1 exacerbation in the previous year); and concomitant asthma medications at baseline (M1, M2, and M3 subgroups). A separate Poisson regression model was run for each of the biomarker-low and -high subgroups to provide the estimated exacerbation rates in the placebo group and omalizumab group. The Poisson regression model also provided an estimate for the relative reduction in the exacerbation rate (omalizumab vs. placebo), which was assessed as one minus the exacerbation rate ratio. The *P* values for testing the relative reduction equal to 0, indicating no benefit from omalizumab, are provided, as are the confidence intervals (CI) of the relative reduction for each of the low and high subgroups.

Exacerbation rates in the low- and high-biomarker subgroups in the placebo arm were used to assess the prognostic effect of a biomarker, whereby a difference between exacerbations in the low and high subgroups implies a prognostic effect. The predictive effect of a biomarker was assessed by comparing the relative reduction (omalizumab vs. placebo) between the low- and high-biomarker subgroups; a difference in relative reduction between the biomarker-high and -low subgroup implies a predictive effect.

Time to first protocol-defined asthma exacerbation was estimated using Kaplan-Meier methodology. The hazard ratio (omalizumab vs. placebo) for exacerbations was estimated using the Cox proportional hazards model with the following covariates: dosing schedule (every 2 or 4 wk); prior exacerbation status (≤ 1 exacerbation, >1 exacerbation in the previous year); and concomitant asthma medications at baseline (M1, M2, and M3 subgroups). A hazard ratio less than 1 indicates a lower risk of developing an exacerbation in the omalizumab group.

Secondary endpoints of change from baseline at Week 48 in total asthma symptom score, albuterol use, AQLQ, and FEV₁ were assessed using an analysis of covariance model including terms for dosing schedule (every 2 or 4 wk) and concomitant asthma medications (M1, M2, and M3 subgroups) at baseline as covariates. A separate analysis of

covariance model was run for each of the biomarker-low and -high subgroups.

To explore alternative cut-offs in addition to the prespecified cut-off (medians), subpopulation treatment effect pattern plots (STEPP) plots were generated, based on methodology developed by Lazar and co-workers (32). This method evaluated treatment-effect heterogeneity across overlapping intervals of continuous biomarkers. Specifically, the plot provided reduction in exacerbations (vs. placebo) for subgroups of patients with biomarker values within a range of seven possible cut-offs corresponding to the 20th, 30th, 40th, 50th, 60th, 70th, and 80th percentiles of biomarker values. Each subgroup contains 40% of patients with biomarker values around the selected cut-off (i.e., 20% with biomarker values below the selected cut-off, and 20% with values equal to or above the selected cut-off).

RESULTS

The disposition of the 850 patients (omalizumab, $n = 427$; placebo, $n = 423$) enrolled in the EXTRA study has been described previously (26). FE_{NO} and serum periostin measurements at baseline were available in 394 (46.4%) and 534 (62.8%) patients, respectively (Figure 1). Data on peripheral blood eosinophil counts at baseline were available for 797 (93.8%) patients (mITT population).

Overall, patient demographic and baseline characteristics were well balanced between the two treatment groups, and were similar between the total EXTRA study population and those patients with biomarker data available. However, there were a higher percentage of females receiving placebo in the overall study and across all biomarker subgroups (see Table E1 in the online supplement).

Between the biomarker-low and -high subgroups (Table 1), an imbalance was observed for mean total IgE, which was higher in the biomarker-high versus biomarker-low subgroups, and mean weight, which was lower in the serum periostin-high versus periostin-low group. Mild correlations were observed between the three biomarkers (Spearman correlation coefficient ranged from 0.35–0.38 for each pair of biomarkers). Therefore, in general, patients who were in one high-biomarker subgroup had elevated baseline values for the other two biomarkers, although the cut-off-defined subgroups for each biomarker did not completely overlap.

Efficacy

The results of the primary and secondary efficacy analyses for the mITT population have been reported previously (26).

Evaluation of Treatment Effects within Biomarker Subgroups

FE_{NO} . Exacerbation rates in the FE_{NO} -low and -high subgroups were 0.71 and 1.07, respectively, in the placebo group and 0.60 and 0.50, respectively, in the omalizumab group. The omalizumab group demonstrated a 53% (95% CI, 37–70; $P = 0.001$) reduction in exacerbations versus the placebo group in the high (≥ 19.5 ppb) FE_{NO} subgroup, compared with a 16% (95% CI, –32 to 46; $P = 0.45$) reduction in the low (< 19.5 ppb) FE_{NO} subgroup after 48-weeks of treatment, suggesting a greater benefit of omalizumab over placebo for patients with higher levels of FE_{NO} at baseline (Figure 2).

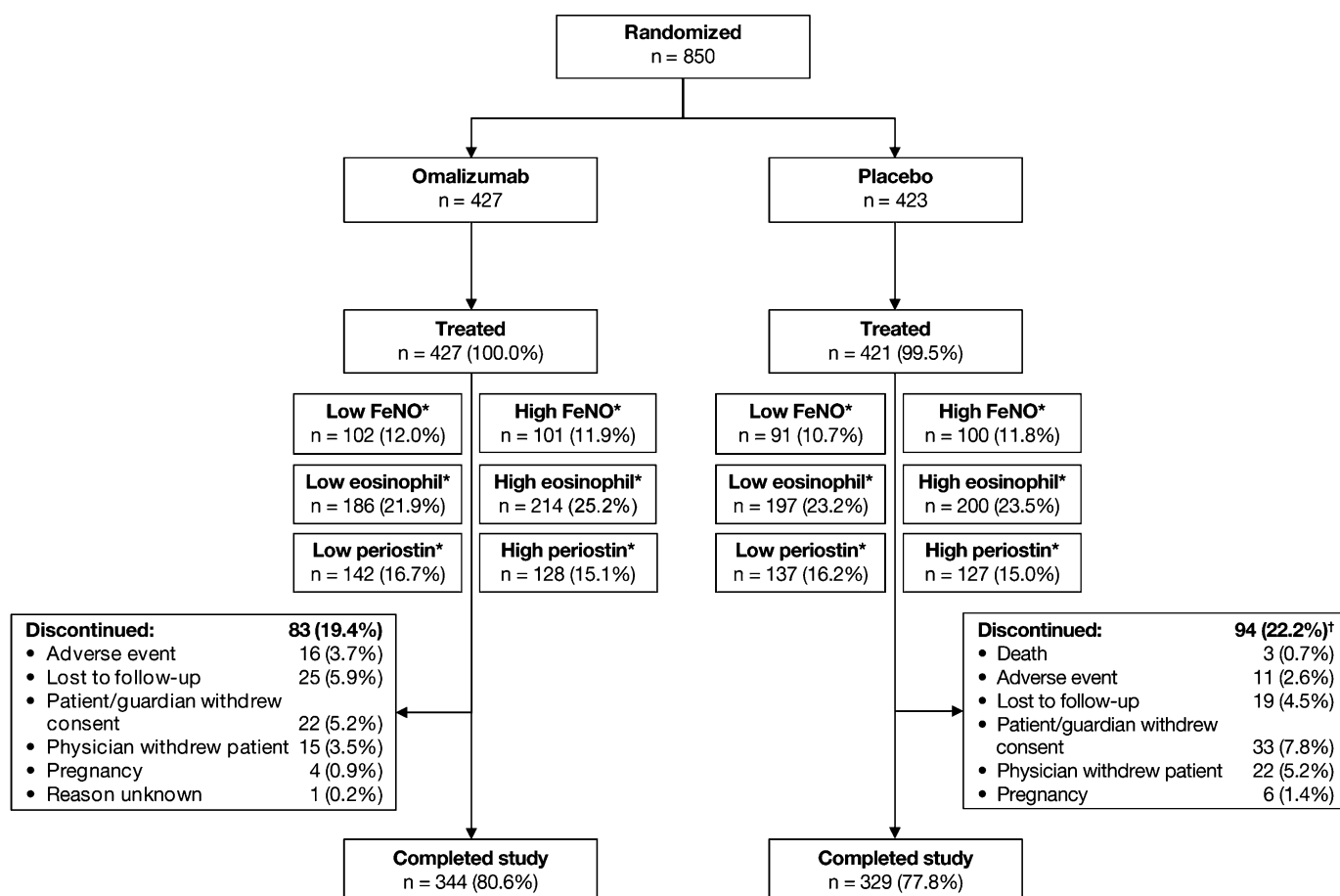


Figure 1. Patient disposition. *Low and high levels of each biomarker at baseline. †Includes two patients in the placebo group who were randomized but did not receive allocated treatment (no data available post baseline). FE_{NO} = fractional exhaled nitric oxide.

TABLE 1. PATIENT DEMOGRAPHICS AND BASELINE CHARACTERISTICS FOR THE SUBGROUPS OF PATIENTS WITH LOW OR HIGH BASELINE PERIPHERAL BLOOD EOSINOPHIL COUNTS, F_{ENO} , AND SERUM PERIOSTIN (FOR COMBINED OMALIZUMAB AND PLACEBO PATIENT POPULATIONS)

	Low F_{ENO} Subgroup (<19.5 ppb) (n = 193)	High F_{ENO} Subgroup (≥ 19.5 ppb) (n = 201)	Low Eosinophil Subgroup ($<260/\mu L$) (n = 383)	High Eosinophil Subgroup ($\geq 260/\mu L$) (n = 414)	Low Periostin Subgroup (<50 ng/ml) (n = 279)	High Periostin Subgroup (≥ 50 ng/ml) (n = 255)
Mean (SD) age, yr	45 (13)	43 (15)	45 (14)	44 (15)	46 (12)	44 (16)
Mean body weight (SD), kg	90 (22)	85 (20)	90 (21)	85 (21)	93 (21)	82 (19)
Sex, n (%)						
Female	130 (67)	128 (64)	242 (63)	279 (67)	181 (65)	168 (66)
Race, n (%)						
White	155 (80)	138 (69)	284 (74)	318 (77)	214 (77)	183 (72)
Mean total serum IgE (SD), IU/ml	169 (126)	197 (152)	156 (123)	196 (140)	152 (120)	200 (144)
Mean prebronchodilator FEV ₁ (SD), % predicted	66 (14)	64 (16)	67 (15)	64 (15)	65 (13)	64 (16)
Hospitalized for asthma, n (%)*	23 (12)	23 (11)	36 (9)	50 (12)	28 (10)	38 (15)
Mean (SD) number of exacerbations*	2 (1)	2 (3)	2 (1)	2 (2)	2 (2)	2 (3)
Mean (SD) total asthma symptom scores	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Mean (SD) number of albuterol per day, puff	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)
Mean (SD) AQLQ	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)
Mean (SD) F_{ENO} , ppb	12 (4)	45 (32)	20 (14)	37 (33)	21 (18)	37 (34)
Mean eosinophil count, per μL	264 (208)	445 (376)	152 (58)	535 (336)	257 (169)	444 (390)
Mean (SD) periostin, ng/ml	49 (15)	60 (24)	48 (14)	58 (20)	41 (6)	67 (18)
Concomitant asthma medications, n (%)						
M1 subgroup	76 (40)	71 (35)	148 (39)	146 (35)	73 (35)	82 (32)
M2 subgroup	93 (48)	94 (47)	194 (51)	177 (43)	137 (49)	119 (47)
M3 subgroup	24 (12)	36 (18)	41 (11)	91 (22)	45 (16)	54 (21)

Definition of abbreviations: F_{ENO} = fractional exhaled nitric oxide; ICS = inhaled corticosteroids; LABA = long-acting β_2 -agonist; M1 = ICS plus LABAs alone; M2 = ICS plus LABAs plus one or more additional asthma controller medications (excluding OCS); M3 = ICS plus LABAs plus OCS; OCS = oral corticosteroids.

*During the past 12 months.

Peripheral blood eosinophils. Exacerbation rates in the eosinophil-low and -high subgroups were 0.72 and 1.03, respectively, in the placebo group and 0.65 and 0.70, respectively, in the omalizumab group. A greater percentage reduction in protocol-defined exacerbations during the 48-week treatment period was observed in patients with high baseline eosinophil counts ($\geq 260/\mu L$) than in patients with low eosinophil counts ($<260/\mu L$) (32%, 95% CI, 11–48 [$P = 0.005$] vs. 9%, 95% CI, –24 to 34 [$P = 0.54$]), suggesting patients with higher baseline eosinophil counts

received a greater benefit from omalizumab therapy than those with lower counts (Figure 2).

Serum periostin. Exacerbation rates in the periostin-low and -high subgroups were 0.72 and 0.93, respectively, in the placebo group and 0.73 and 0.66, respectively, in the omalizumab group. The protocol-defined exacerbation rate reduced post-treatment by 30% (95% CI, –2 to 51; $P = 0.07$) in patients with high (≥ 50 ng/ml) baseline periostin levels, and by 3% (95% CI, –43 to 32; $P = 0.94$) in patients with low (<50 ng/ml) baseline periostin levels (Figure 2).

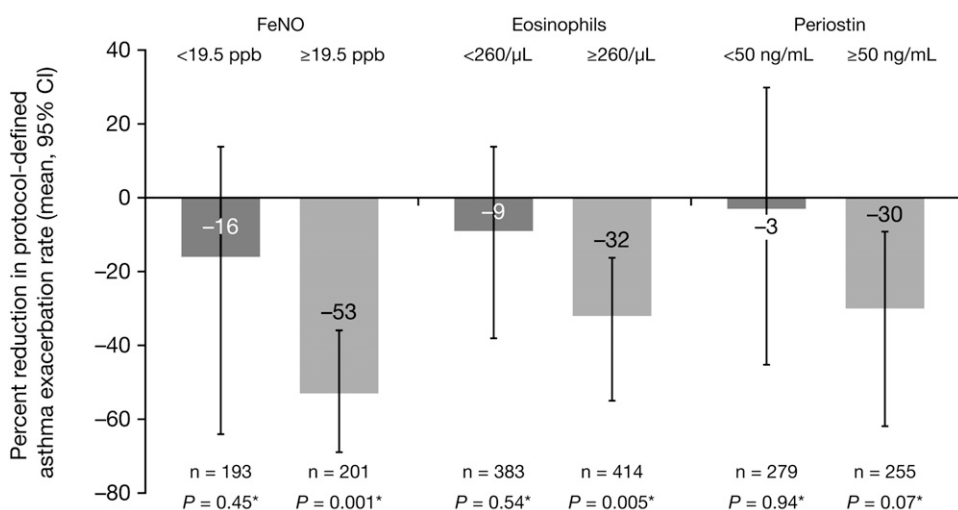


Figure 2. Mean percent reduction (95% CI) in protocol-defined asthma exacerbation rate in the low- and high-biomarker subgroups (baseline fractional exhaled nitric oxide [F_{ENO}], peripheral blood eosinophils, and serum periostin). *Exacerbation reduction P values; omalizumab versus placebo in each biomarker subgroup. CI = confidence interval.

Exacerbation rates						
	Low FeNO at baseline	High FeNO at baseline	Low eosinophils at baseline	High eosinophils at baseline	Low periostin at baseline	High periostin at baseline
Omalizumab	0.60	0.50	0.65	0.70	0.73	0.66
Placebo	0.71	1.07	0.72	1.03	0.72	0.93

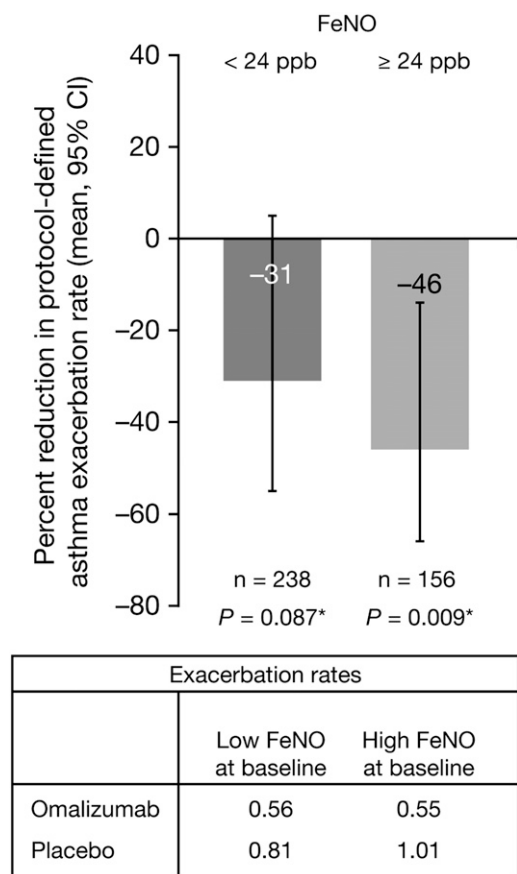


Figure 3. Post hoc analysis of mean percent reduction (95% CI) in protocol-defined asthma exacerbation rate in the low- and high-biomarker subgroups using a baseline fractional exhaled nitric oxide ($FeNO$) cut-off of 24 ppb. *Exacerbation reduction P values; omalizumab versus placebo in each biomarker subgroup. CI = confidence interval.

In each of the biomarker-high subgroups, a substantial reduction from placebo in the protocol-defined exacerbation rate for patients on omalizumab was observed. Conversely, no significant differences versus placebo were observed in any of the low-biomarker subgroups, suggesting a greater benefit of omalizumab over placebo for patients in the high-biomarker subgroups. Possible prognostic effects were observed in patients with high $FeNO$, peripheral blood eosinophil counts, and periostin levels at baseline, as demonstrated by higher exacerbation rates in the biomarker-high placebo subgroups than the biomarker-low placebo subgroups.

In the overall EXTRA study population, it was found that the treatment effect of omalizumab was smallest in the M3 subgroup (26). In an additional analysis, we did not find that the biomarkers had stronger predictive values in the subset of the M1+M2 population (see Figure E1).

Evaluation of Patterns of Treatment Effect for Varying Levels of Each Biomarker

To explore alternative biomarker cut-offs to those predefined (peripheral blood eosinophils and $FeNO$) or defined as part of the post hoc analysis (serum periostin), a STEPP analysis was performed for each biomarker subgroup. These analyses suggest that the predefined median cut-off (260/ μ L) seems to be appropriate for peripheral blood eosinophils, whereas slightly higher cut-offs (e.g., around the 60th percentiles, 24 ppb and 53 ng/mL, respectively) may be preferable for $FeNO$ and periostin (see Figures E2A–E2C).

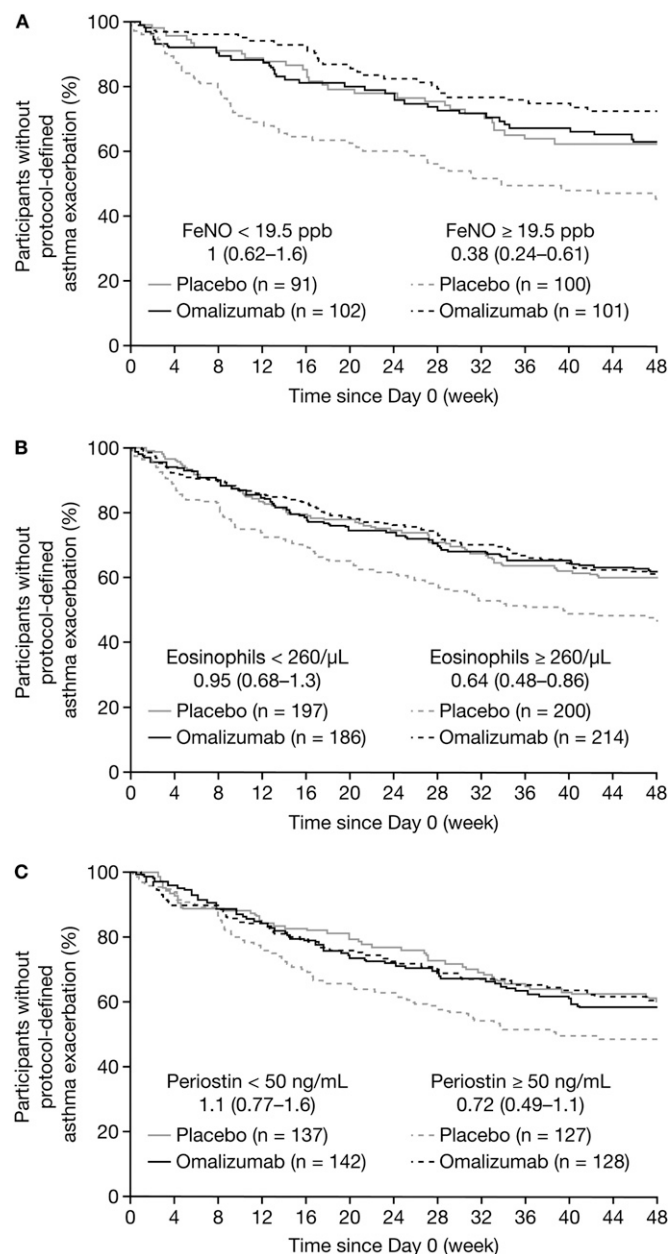


Figure 4. Time to first protocol-defined asthma exacerbation in baseline (A) fractional exhaled nitric oxide ($FeNO$) low (<19.5 ppb) and high (≥19.5 ppb) subgroups, (B) peripheral blood eosinophil low (<260/ μ L) and high (≥260/ μ L) subgroups, and (C) serum periostin low (<50 ng/mL) and high (≥50 ng/mL) subgroups.

A post hoc analysis was conducted using 24 ppb for $FeNO$ because it was also a clinically relevant cut-off based on the ATS guidelines (17). Similar results were noted in the post hoc analysis using an alternative $FeNO$ cut-off; exacerbation rates in the $FeNO$ low (<24 ppb) and high (≥24 ppb) subgroups were 0.81 and 1.01, respectively, in the placebo group and 0.56 and 0.55, respectively, in the omalizumab group. The omalizumab group demonstrated a 46% (95% CI, 14–66; $P = 0.009$) reduction in exacerbations versus the placebo group in the high $FeNO$ subgroup, compared with a 31% (95% CI, –10 to 55; $P = 0.087$) reduction in the low $FeNO$ subgroup after 48-weeks of treatment (Figure 3).

Time to first protocol-defined exacerbation. The Kaplan-Meier curves and the hazard ratios of the exacerbation-free rate in each of the three biomarker-high subgroups favored the omalizumab

group over placebo; in each of the biomarker-low subgroups, the difference between the omalizumab and placebo group was minimal (Figure 4; *see* Figure E3). Analyses of time to first protocol-defined exacerbation were consistent with the greater percentage reduction in protocol-defined exacerbations during the 48-week treatment period observed in patients in each of the high-biomarker subgroups, compared with those patients in the low-biomarker subgroups.

Evaluation of secondary endpoints. There was a trend toward greater treatment benefits from omalizumab in the F_{ENO} high subgroup compared with the F_{ENO} low subgroup for the secondary endpoints of change from baseline at Week 48 in albuterol use, AQLQ score, and % predicted FEV_1 (Table 2; *see* Table E2). However, no consistent predictive trends were observed for either peripheral blood eosinophil count or serum periostin for these secondary endpoints. Changes from baseline in % predicted FEV_1 in the biomarker subgroups are shown in Figures E4 and E5. Although the relative change from baseline was consistently higher in the omalizumab treatment group compared with placebo, none of the biomarkers showed a clear predictive or prognostic effect (Table 2; *see* Figures E4 and E5).

Safety

The incidence of treatment-emergent AEs was similar in the biomarker-low and biomarker-high subgroups, and no new safety signal was detected (Table 3). Similar trends were seen across treatment and biomarker subgroups for the incidence of serious AEs (data not shown). The most common AE in each low- and high-biomarker subgroup, irrespective of study treatment group, was upper respiratory tract infection (range across subgroups, 21–26.1% of patients). Three deaths occurred during the study, all in the placebo group. No clinically relevant abnormalities in laboratory tests (including platelet count) were observed.

DISCUSSION

Examination of reductions in exacerbations between subgroups of patients with low and high levels of biomarkers showed that omalizumab treatment was associated with greater effects in all

three high-biomarker subgroups (F_{ENO} , peripheral blood eosinophils, and serum periostin). No consistent trends were observed in subgroup analyses of the secondary endpoints of change from baseline at Week 48 in total asthma symptom score, albuterol use, AQLQ score, and % predicted FEV_1 , with the exception of F_{ENO} (albuterol use, AQLQ score, and % predicted FEV_1 only), although it should be noted that the improvements in AQLQ score and % predicted FEV_1 are small and would likely not be considered clinically meaningful (33, 34). Potential prognostic effects were also observed for all three biomarkers, as demonstrated by higher exacerbation rates in patients with high baseline biomarkers in both the omalizumab and placebo groups. Previous investigations have not shown that potential markers, including allergen-specific IgE and total IgE, serum tryptase, and soluble CD23, were able to predict effects of omalizumab (data not shown) (14, 35).

The value of F_{ENO} as a predictor of a patient's likelihood to respond to ICS treatment is high, and has been described previously (17); however, because responses to corticosteroid treatment were not assessed as part of the present study, these findings could not be confirmed. It should be noted that the prespecified cut-off for F_{ENO} used in our analyses is lower than that recommended by the ATS, and that the overall F_{ENO} levels recorded in patients in EXTRA were lower than those usually expected for patients with severe asthma, possibly because of high doses of inhaled and OCS treatment at baseline. We did, however, also perform a *post hoc* analysis using a higher cut-off similar to that recommended by the ATS (17); results were similar to those using the prespecified lower F_{ENO} cut-off but results should be interpreted with caution because of smaller sample size per subgroup and the *post hoc* nature of the analysis.

Exhaled NO seems to originate from the airway epithelium as a consequence of up-regulation of NO synthase, which is associated with inflammation (17). F_{ENO} therefore provides an indirect marker of up-regulated airway inflammation (17). Similarly, eosinophilia and high periostin also indicate a greater degree of airway inflammation (10, 18, 19). Patients with greater levels of airway inflammation, as evidenced by elevated F_{ENO} , blood eosinophils, or serum periostin, may be a population of patients with

TABLE 2. SECONDARY EFFICACY ENDPOINTS OF TOTAL ASTHMA SYMPTOM SCORE, ALBUTEROL USE, AQLQ, AND % PREDICTED FEV_1 ; EVALUATION BY LOW AND HIGH SUBGROUPS OF EACH OF THE THREE BIOMARKERS (BASELINE F_{ENO} , PERIPHERAL BLOOD EOSINOPHIL COUNT, SERUM PERIOSTIN)

	Total Asthma Symptom Score		Albuterol Use		AQLQ Score		Change from Baseline in % Predicted FEV_1	
	Low Subgroup	High Subgroup	Low Subgroup	High Subgroup	Low Subgroup	High Subgroup	Low Subgroup	High Subgroup
F_{ENO}								
Placebo (LSM)	−1.11	−1.51	−1.35	−1.12	0.76	0.89	1.17	3.37
Omalizumab (LSM)	−1.32	−1.80	−1.48	−1.85	1.00	1.28	3.34	6.62
LSM difference	−0.21	−0.29	−0.14	−0.73	0.24	0.39	1.97	3.26
(95% CI)	(−0.68 to 0.25)	(−0.77 to 0.19)	(−0.73 to 0.45)	(−1.30 to −0.16)	(−0.09 to 0.57)	(0.06 to 0.73)	(−1.83 to 5.77)	(−0.33 to 6.84)
P value	0.37	0.23	0.65	0.01	0.16	0.02	0.31	0.08
Eosinophils								
Placebo (LSM)	−1.06	−1.47	−1.18	−1.42	0.84	1.01	2.05	5.61
Omalizumab (LSM)	−1.41	−1.63	−1.30	−1.76	1.13	1.14	3.77	6.92
LSM difference	−0.34	−0.16	−0.13	−0.34	0.26	0.14	1.72	1.3
(95% CI)	(−0.69 to −0.02)	(−0.51 to 0.19)	(−0.61 to 0.30)	(−0.83 to 0.11)	(0.06 to 0.51)	(−0.11 to 0.36)	(−1.06 to 4.51)	(−1.23 to 3.84)
P value	0.05	0.37	0.60	0.14	0.01	0.29	0.22	0.31
Periostin								
Placebo (LSM)	−1.07	−1.50	−1.25	−1.60	0.82	1.07	3.40	5.68
Omalizumab (LSM)	−1.69	−1.46	−1.66	−1.49	1.32	1.17	5.20	6.01
LSM difference	−0.63	0.04	−0.41	0.12	0.50	0.10	1.79	0.42
(95% CI)	(−1.02 to −0.24)	(−0.40 to 0.48)	(−0.91 to 0.10)	(−0.46 to 0.69)	(0.22 to 0.77)	(−0.19 to 0.40)	(−1.15 to 4.73)	(−3.22 to 4.06)
P value	0.0017	0.86	0.11	0.69	0.0005	0.51	0.23	0.82

Definition of abbreviations: AQLQ = Asthma Quality of Life Questionnaire; CI = confidence interval; F_{ENO} = fractional exhaled nitric oxide; LSM = least squares mean. P values represent omalizumab versus placebo in each biomarker subgroup.

TABLE 3. NUMBER (%) OF PATIENTS WITH ADVERSE EVENTS IN EACH BIOMARKER SUBGROUP (SAFETY-EVALUABLE POPULATION)

TEAEs, n (%)	Omalizumab		Placebo	
	Low-Biomarker Subgroup at Baseline	High-Biomarker Subgroup at Baseline	Low-Biomarker Subgroup at Baseline	High-Biomarker Subgroup at Baseline
FE _{NO}	86 (83.5)	81 (80.2)	72 (80.0)	73 (73.0)
Peripheral blood eosinophils	150 (80.6)	172 (80.0)	161 (81.7)	158 (79.4)
Serum periostin	119 (84.0)	105 (82.0)	112 (82.0)	103 (81.0)

Definition of abbreviations: FE_{NO} = fractional exhaled nitric oxide; TEAEs = treatment-emergent adverse events.

asthma at a higher risk of asthma exacerbations and therefore more likely to benefit from omalizumab.

Despite earlier efforts using samples and datasets from prior omalizumab studies, we were unable to demonstrate that many commonly available serum biomarkers (specific-to-total IgE ratios, serum tryptase, eosinophil cationic protein, or soluble CD23) were able to predict outcomes with omalizumab (data not shown). Before specifically examining FE_{NO}, peripheral blood eosinophils, and serum periostin, limited exploratory analyses were performed using data from the EXTRA study. Neither FE_{NO} nor periostin had been assessed with respect to omalizumab effects previously, because data for these biomarkers were not collected in previous large placebo-controlled studies of omalizumab. To minimize a selection bias associated with testing multiple markers, two of the three biomarkers presented here were prespecified as markers of interest for this analysis. The thresholds used to define the low and high subgroup were also prespecified arbitrarily as a median to provide an approximately equal distribution of data across the subgroups; these were the only thresholds initially considered with the aim of reducing the chance of a false-positive finding associated with multiple testing. Although STEPP analyses were used to explore alternative thresholds, it should be noted that in typical situations, cut-off selection involves a tradeoff between the size of the high-biomarker subgroup and the treatment effect in that subgroup. In addition, caution should be used in interpreting these results, because the CIs are wide and multiplicity issues are of potential concern when looking at multiple cut-offs and a large number of associated subgroup analyses. Additional analyses to explore alternative thresholds and to prospectively verify these findings in future studies with larger datasets are necessary to optimize and validate the predictive potential of these biomarkers.

The potential of periostin as a biomarker was noted in a recent study of an anti-IL-13 therapy currently in clinical development for asthma (20). Patients with high baseline periostin demonstrated a greater improvement in lung function with anti-IL-13 therapy compared with those with low periostin levels (20). A *post hoc* analysis also showed that the efficacy of anti-IL-13 therapy (in terms of improvement in FEV₁) was greater in patients with high baseline FE_{NO} than in those with low FE_{NO}. However, inpatient variability was greater for baseline FE_{NO} (mean coefficient of variation, 19.8%) than for periostin (5%), indicating that periostin might be a more stable biomarker.

Several recent asthma clinical studies have investigated variability in patients' responses to treatment, and have included measurement of biomarkers as part of study designs. Although a recent review by Szeffler and coworkers (36) only identified multiallergen screening to define atopy as a core asthma outcome biomarker that should be included in future studies, the importance of supplemental biomarkers, such as blood eosinophil counts and FE_{NO}, was highlighted.

There are several limitations to this study. Biomarker data were not available in all patients enrolled, and FE_{NO} data were available for less than half of the patients in the overall study;

patient numbers were, however, still adequate across the subgroups. It should be noted that the overall sample size of this study did not provide sufficient statistical power to show a difference between the biomarker-low and biomarker-high subgroups. Simulation studies showed that a much higher sample size is required to test the interaction term with adequate power (Genentech, data on file). In addition, most of the analyses presented are *post hoc* and exploratory, and no statistical type I error control plan was implemented. The *P* values in the analysis should therefore be used to assess the strength of evidence and do not provide a basis for claiming statistical significance. Previous documentation of the high visit-to-visit variability of blood eosinophils as a results of diurnal fluctuations should also be considered when interpreting these results (36).

Overall, omalizumab was well tolerated and a similar incidence of AEs was observed in the omalizumab and placebo groups, and across the low- and high-biomarker subgroups. Safety data were consistent with the established profile of omalizumab (37), and importantly, no new safety signals were observed.

We found that the biomarkers FE_{NO}, peripheral blood eosinophil counts, and serum periostin provided information on patient subgroups that may achieve greater benefit from omalizumab therapy. Given the exploratory and retrospective nature of these analyses, caution is warranted in overly interpreting these findings, because additional studies are required to further explore the characteristics and prognostic effects of these biomarkers. Nonetheless, these observations provide preliminary information that biomarkers may exist to identify patients more likely to benefit from omalizumab in relationship to preventing exacerbations. Confirmation of these observations will facilitate the selection of candidates for this treatment to potentially make this selection process more effective and therefore cost saving.

Author disclosures are available with the text of this article at www.atsjournals.org.

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References

- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161:1720–1745.
- Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2012. Available from: <http://www.ginasthma.org>.
- Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, Pedersen SE. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. *Am J Respir Crit Care Med* 2004;170:836–844.
- Humbert M, Beasley R, Ayres J, Slavin R, Hebert J, Bousquet J, Beeh KM, Ramos S, Canonica GW, Hedgecock S, *et al*. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005;60:309–316.

5. Szeffler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, Craig TJ, Dolovich M, Drazen JM, Fagan JK, *et al.* Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002;109:410–418.
6. Szeffler SJ, Martin RJ. Lessons learned from variation in response to therapy in clinical trials. *J Allergy Clin Immunol* 2010;125:285–292.
7. Chung KF. Anti-IgE monoclonal antibody, omalizumab: a new treatment for allergic asthma. *Expert Opin Pharmacother* 2004;5:439–446.
8. Shields RL, Whether WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, Thomas D, Saban R, Jardieu P. Inhibition of allergic reactions with antibodies to IgE. *Int Arch Allergy Immunol* 1995;107:308–312.
9. Levine SJ, Wenzel SE. Narrative review: the role of Th2 immune pathway modulation in the treatment of severe asthma and its phenotypes. *Ann Intern Med* 2010;152:232–237.
10. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, Koth LL, Arron JR, Fahy JV. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388–395.
11. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008;118:3546–3556.
12. Cameron L, Hamid Q, Wright E, Nakamura Y, Christodoulou P, Muro S, Frenkiel S, Lavigne F, Durham S, Gould H. Local synthesis of epsilon germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. *J Allergy Clin Immunol* 2000;106:46–52.
13. Bousquet J, Wenzel S, Holgate S, Lumry W, Freeman P, Fox H. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. *Chest* 2004;125:1378–1386.
14. Bousquet J, Rabe K, Humbert M, Chung KF, Berger W, Fox H, Ayre G, Chen H, Thomas K, Blogg M, *et al.* Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med* 2007;101:1483–1492.
15. Wahn U, Martin C, Freeman P, Blogg M, Jimenez P. Relationship between pretreatment specific IgE and the response to omalizumab therapy. *Allergy* 2009;64:1780–1787.
16. Chibana K, Trudeau JB, Mustovich AT, Hu H, Zhao J, Balzar S, Chu HW, Wenzel SE. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. *Clin Exp Allergy* 2008;38:936–946.
17. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–615.
18. Massanari M, Holgate ST, Busse WW, Jimenez P, Kianifard F, Zeldin R. Effect of omalizumab on peripheral blood eosinophilia in allergic asthma. *Respir Med* 2010;104:188–196.
19. Jia GA, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, Shikotra A, Carter R, Audusseau S, Hamid Q, *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthma. *J Allergy Clin Immunol* 2012;130:647–654.
20. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, Harris JM, Scheerens H, Wu LC, Su Z, *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–1098.
21. Novartis US and Genentech. Omalizumab (Xolair) label [updated 2010 Jul; accessed 2013 Apr 3]. Available from: www.gene.com
22. Bousquet J, Cabrera P, Berkman N, Buhl R, Holgate S, Wenzel S, Fox H, Hedgecock S, Blogg M, Cioppa GD. The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe persistent asthma. *Allergy* 2005;60:302–308.
23. Bousquet J, Siergiejko Z, Swiebocka E, Humbert M, Rabe KF, Smith N, Leo J, Peckitt C, Maykut R, Peachey G. Persistency of response to omalizumab therapy in severe allergic (IgE-mediated) asthma. *Allergy* 2011;66:671–678.
24. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, van AA, Gupta N. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184–190.
25. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, Gruchalla RS, Kattan M, Teach SJ, Pongracic JA, *et al.* Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011;364:1005–1015.
26. Hanania NA, Alpan O, Hamilos DL, Condemi JJ, Reyes-Rivera I, Zhu J, Rosen KE, Eisner MD, Wong DA, Busse W. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: a randomized trial. *Ann Intern Med* 2011;154:573–582.
27. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson GB, Chung KF, Bousquet J, Kerstjens HA, Fox H, Thirlwell J, *et al.* Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;34:632–638.
28. Kulus M, Hebert J, Garcia E, Fowler TA, Fernandez VC, Blogg M. Omalizumab in children with inadequately controlled severe allergic (IgE-mediated) asthma. *Curr Med Res Opin* 2010;26:1285–1293.
29. Lanier B, Bridges T, Kulus M, Taylor AF, Berhane I, Vidaurre CF. Omalizumab for the treatment of exacerbations in children with inadequately controlled allergic (IgE-mediated) asthma. *J Allergy Clin Immunol* 2009;124:1210–1216.
30. Soler M, Matz J, Townley R, Buhl R, O'Brien J, Fox H, Thirlwell J, Gupta N, Della CG. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 2001;18:254–261.
31. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–1136.
32. Lazar AA, Cole BF, Bonetti M, Gelber RD. Evaluation of treatment-effect heterogeneity using biomarkers measured on a continuous scale: subpopulation treatment effect pattern plot. *J Clin Oncol* 2010;28:4539–4544.
33. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific quality of life questionnaire. *J Clin Epidemiol* 1994;47:81–87.
34. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chaney P, Enright PL, Gibson PG, *et al.* An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180:59–99.
35. Harris JM, Wong DA, Kapp AV. Development of the Asthma Control Composite outcome measure to predict omalizumab response. *Ann Allergy Asthma Immunol* 2011;107:273–280.
36. Szeffler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, Hunt JF, Kita H, Liu AH, Panettieri RA Jr, *et al.* Asthma outcomes: biomarkers. *J Allergy Clin Immunol* 2012;129:S9–S23.
37. Corren J, Casale TB, Lanier B, Buhl R, Holgate S, Jimenez P. Safety and tolerability of omalizumab. *Clin Exp Allergy* 2009;39:788–797.

**Exploring the effects of omalizumab in allergic asthma: an analysis of
biomarkers in the EXTRA study**

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Online data supplement

METHODS

Patients

Asthma was considered inadequately controlled if, during the 4 weeks before screening and for 2 consecutive weeks out of up to 4 weeks before randomization, patients had an average of ≥ 1 nighttime awakenings/week and daytime asthma symptoms which required rescue medication use for ≥ 2 days/week. Patients were also required to have had at least one asthma exacerbation requiring treatment with systemic corticosteroids in the 12 months prior to screening. Patients were excluded if they had an asthma exacerbation requiring intubation in the 12 months prior to screening, or an exacerbation requiring systemic corticosteroid treatment in the 30 days before screening. Other exclusion criteria included active lung disease other than asthma, omalizumab treatment in the 12 months prior to screening, non-allergy related elevated serum IgE levels, or pregnancy.

RESULTS

Efficacy

Exacerbation rates by M1, M2 and M3 stratum

In the overall EXTRA study population, it was found that the treatment effect of omalizumab was smallest in the M3 strata. Thus, we conducted an additional analysis in the subset of patients in the M1 and M2 strata. As demonstrated in Figure E1, we did not find that the biomarkers had stronger predictive values in the subset of the M1+M2 population.

Evaluation of patterns of treatment effect for varying levels of each biomarker

STEPP analyses are shown in Figure E2a–c.

Time to first protocol-defined exacerbation

Kaplan-Meier curve and the hazard ratios of the exacerbation-free rate in the FeNO low (<24 ppb) and high (≥ 24 ppb) subgroups are shown in Figure E3.

Evaluation of secondary endpoints

Changes from baseline in % predicted FEV₁ are shown in Figures E4 and E5.

Figure E1. Reduction in protocol-defined asthma exacerbation rate in the M1+M2 strata

Figure E2. Reduction in exacerbation rates in each subpopulation by (a) FeNO, (b) peripheral blood eosinophils, (c) periostin

Note: Each plot provides percent reduction in exacerbations (versus placebo) and corresponding 95% confidence intervals for subgroups of patients with biomarker values within a range of seven possible cutoffs corresponding to the 20th, 30th, 40th, 50th, 60th, 70th and 80th percentiles of biomarker values. Each subgroup contains 40% of patients with biomarker values around the selected cutoff (i.e., 20% with biomarker values below the selected cutoff, and 20% with values equal to or above the selected cutoff). The horizontal grey line denotes the reduction in all patients.

Figure E3. Time to first protocol-defined asthma exacerbation in baseline FeNO low (< 24 ppb) and high ($\geq$ 24 ppb) subgroups (post-hoc analysis),

Figure E4. Relative change in FEV₁ from baseline in (A) the FeNO low (< 19.5 ppb) and high ($\geq$ 19.5 ppb) subgroups, (B) the peripheral blood eosinophil (< 260/ μ L) and high ($\geq$ 260/ μ L) subgroups, and (C) the periostin low (< 50 ng/mL) and high ($\geq$ 50 ng/mL) subgroups

Figure E5. Relative change in FEV₁ from baseline in the FeNO low (< 24 ppb) and high ($\geq$ 24 ppb) subgroups (post-hoc analysis)

Table E1. PATIENT DEMOGRAPHICS AND BASELINE CHARACTERISTICS FOR THE OVERALL OMALIZUMAB AND PLACEBO PATIENT POPULATIONS, AND THE SUBGROUPS OF PATIENTS WITH BASELINE PERIPHERAL BLOOD EOSINOPHIL COUNTS, FENO AND SERUM PERIOSTIN AVAILABLE

	Overall		Biomarker subgroups					
	Placebo (n = 421)	Omalizumab (n = 427)	FeNO placebo (n = 191)	FeNO omalizumab (n = 203)	Eosinophils placebo (n = 397)	Eosinophils omalizumab (n = 400)	Periostin placebo (n = 264)	Periostin omalizumab (n = 270)
Mean (SD) age, years	45 (14)	44 (14)	45 (14)	43 (14)	46 (14)	44 (14)	45 (14)	44 (14)
Mean body weight (SD), kg	86 (21)	88 (21)	86 (21)	88 (22)	86 (21)	88 (21)	87 (21)	88 (21)
Sex, n (%)								
Female	295 (70)	262 (61)	137 (72)	121 (60)	276 (70)	245 (61)	180 (68)	169 (63)
Race, n (%)								
White	318 (76)	313 (73)	137 (72)	156 (77)	305 (77)	297 (74)	199 (75)	198 (73)
Mean total serum IgE (SD), IU/mL	175 (134)	179 (135)	179 (133)	188 (147)	174 (133)	179 (134)	171 (133)	179 (135)

Mean pre-bronchodilator FEV ₁ (SD), % predicted	64 (14)	65 (15)	64 (13)	66 (15)	65 (14)	65 (15)	64 (14)	65 (15)
Hospitalized for asthma, <i>n</i> (%) [*]	41 (10) [†]	53 (12)	20 (10)	26 (13)	39 (10) [‡]	47 (12)	29 (11)	37 (14)
Mean (SD) number of exacerbations [*]	2 (2)	2 (2)	2 (2)	2 (3)	2 (1)	2 (1)	2 (2)	2 (3)
Mean (SD) total asthma symptom scores	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Mean (SD) number of albuterol per day, puff	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)	4 (3)
Mean (SD) AQLQ	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)
Mean (SD) FeNO, ppb	29 (30)	29 (27)	29 (30)	29 (27)	29 (28)	28 (27)	28 (28)	30 (28)
Mean eosinophil count, per µl	358 (332)	344 (289)	373 (345)	341 (291)	358 (332)	344 (289)	353 (340)	338 (276)
Mean (SD) periostin, ng/mL	53 (19)	53 (19)	55 (22)	54 (20)	53 (19)	53 (18)	53 (19)	53 (19)

Concomitant asthma medications, <i>n</i> (%)									
M1 subgroup	159 (38)	151 (35)	68 (36)	79 (39)	150 (38)	144 (36)	91 (34)	88 (33)	
M2 subgroup	191 (45)	203 (48)	90 (47)	97 (48)	182 (46)	189 (47)	123 (47)	133 (49)	
M3 subgroup	71 (17)	73 (17)	33 (17)	27 (13)	65 (16)	67 (17)	50 (19)	49 (18)	

*during the past 12 months; †*n*=420; ‡*n*=396

FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; IgE, immunoglobulin E; LABA, long-acting β₂-agonist; OCS, oral corticosteroids; SD, standard deviation

M1 = ICS plus LABAs alone; M2 = ICS plus LABAs plus one or more additional asthma controller medications (excluding OCS); M3 = ICS plus LABAs plus OCS

Table E2. SECONDARY EFFICACY ENDPOINT OF % PREDICTED FEV₁; EVALUATION BY LOW (< 24 PPB)/HIGH (≥ 24 PPB) BASELINE FeNO (post-hoc analysis)

Total asthma symptom score	Albuterol use	AQLQ score	Change from baseline in % predicted FEV ₁
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		Low	High	Low	High	Low	High	Low	High
		subgroup	subgroup	subgroup	subgroup	subgroup	subgroup	subgroup	subgroup
FeNO	Placebo (LSM)	−1.23	−1.46	−1.28	−1.16	0.77	0.91	0.77	4.10
	Omalizumab	−1.44	−1.71	−1.52	−1.90	1.05	1.28	2.76	7.3
	(LSM)								
	LSM	−0.21	−0.25	−0.24	−0.73	0.28	0.37	1.99	3.20
	difference	(−0.64,	(−0.77,	(−0.77,	(−1.36, -	(−0.02,	(0.01,	(−1.44,	(−0.74,
	(95% CI)	0.22)	0.26)	0.29)	0.10)	0.59)	0.73)	5.42)	7.14)
	<i>P</i> value	0.35	0.33	0.37	0.02	0.07	0.04	0.26	0.11

Definition of abbreviations: AQLQ = the Asthma Quality of Life Questionnaire; CI = confidence interval; FeNO = fractional exhaled nitric oxide; FEV₁ = forced expiratory volume in 1 second; LSM = least squares mean.

Note: *P* values represent omalizumab versus placebo in each biomarker subgroup

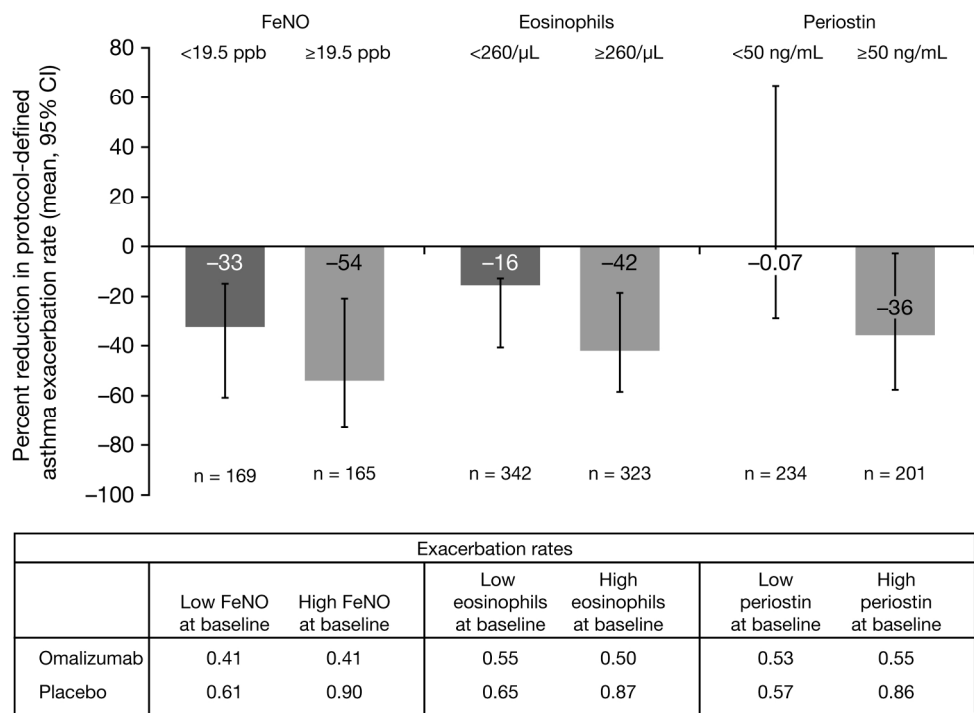


Figure E1. Reduction in protocol-defined asthma exacerbation rate in the M1+M2 strata
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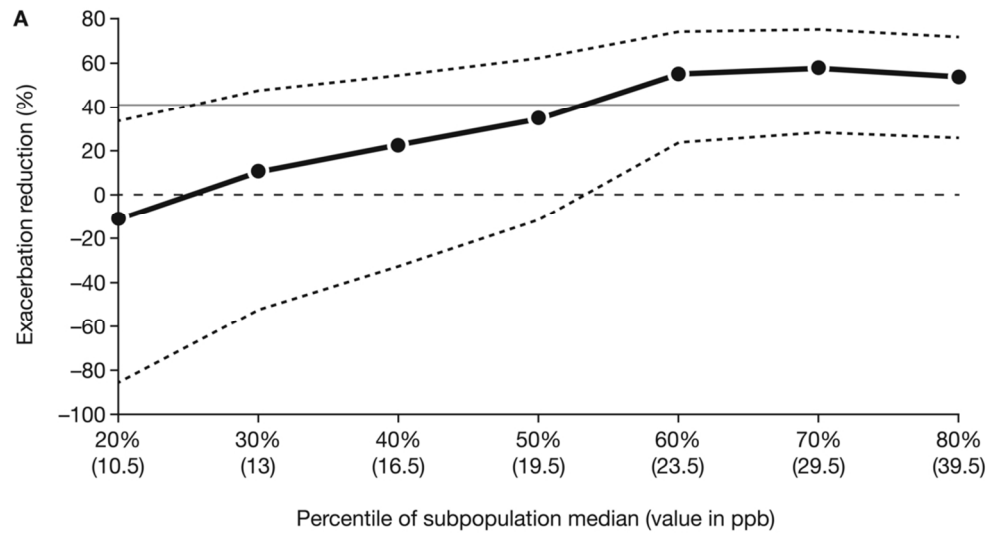


Figure E2. Reduction in exacerbation rates in each subpopulation by (a) FeNO, (b) peripheral blood eosinophils, (c) periostin

Note: Each plot provides percent reduction in exacerbations (versus placebo) and corresponding 95% confidence intervals for subgroups of patients with biomarker values within a range of seven possible cutoffs corresponding to the 20th, 30th, 40th, 50th, 60th, 70th and 80th percentiles of biomarker values. Each subgroup contains 40% of patients with biomarker values around the selected cutoff (i.e., 20% with biomarker values below the selected cutoff, and 20% with values equal to or above the selected cutoff). The horizontal grey line denotes the reduction in all patients.

104x55mm (300 x 300 DPI)

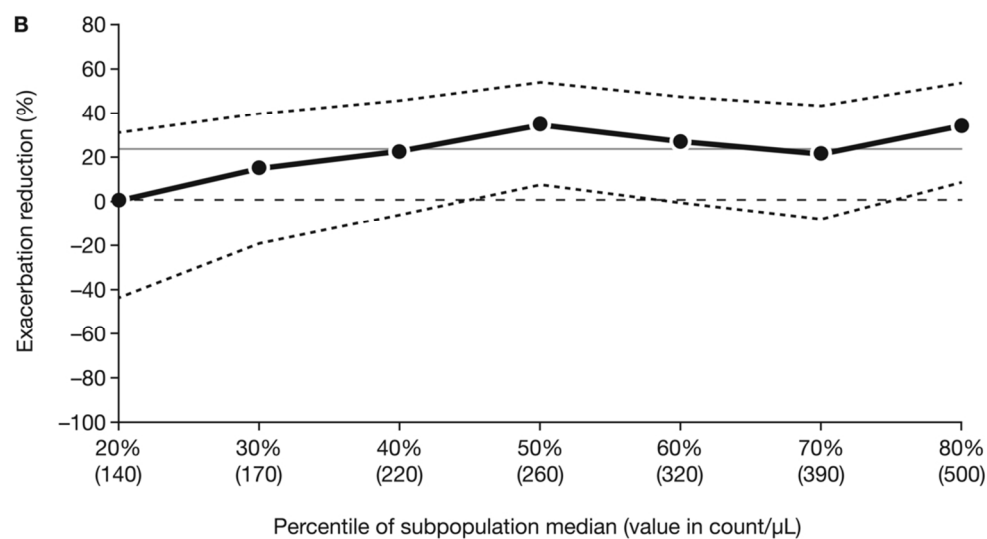


Figure E2. Reduction in exacerbation rates in each subpopulation by (a) FeNO, (b) peripheral blood eosinophils, (c) periostin

Note: Each plot provides percent reduction in exacerbations (versus placebo) and corresponding 95% confidence intervals for subgroups of patients with biomarker values within a range of seven possible cutoffs corresponding to the 20th, 30th, 40th, 50th, 60th, 70th and 80th percentiles of biomarker values. Each subgroup contains 40% of patients with biomarker values around the selected cutoff (i.e., 20% with biomarker values below the selected cutoff, and 20% with values equal to or above the selected cutoff). The horizontal grey line denotes the reduction in all patients.

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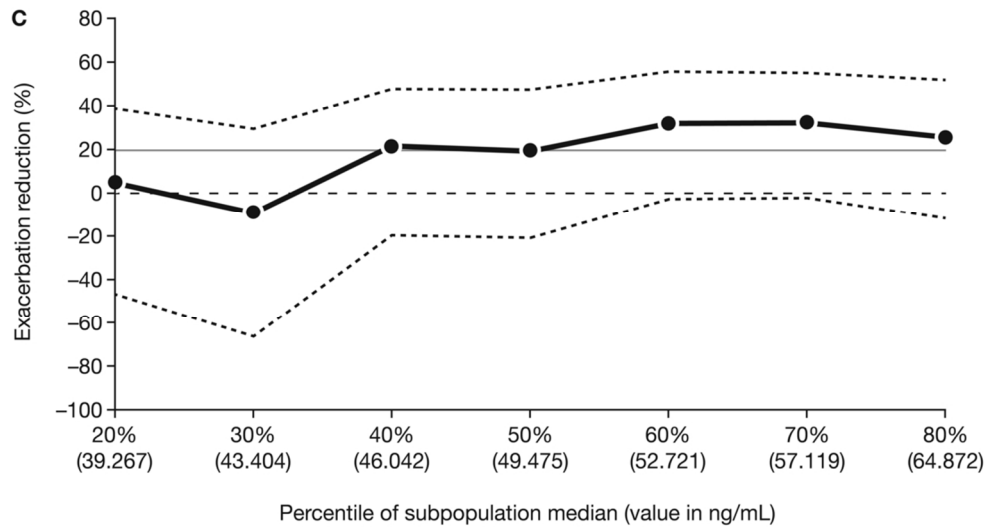


Figure E2. Reduction in exacerbation rates in each subpopulation by (a) FeNO, (b) peripheral blood eosinophils, (c) periostin

Note: Each plot provides percent reduction in exacerbations (versus placebo) and corresponding 95% confidence intervals for subgroups of patients with biomarker values within a range of seven possible cutoffs corresponding to the 20th, 30th, 40th, 50th, 60th, 70th and 80th percentiles of biomarker values. Each subgroup contains 40% of patients with biomarker values around the selected cutoff (i.e., 20% with biomarker values below the selected cutoff, and 20% with values equal to or above the selected cutoff). The horizontal grey line denotes the reduction in all patients.

104x55mm (300 x 300 DPI)

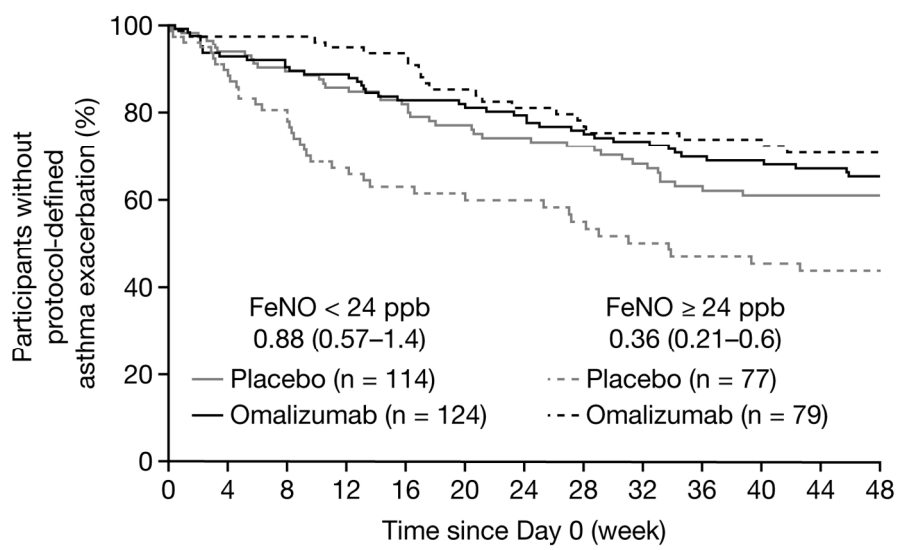


Figure E3. Time to first protocol-defined asthma exacerbation in baseline FeNO low (< 24 ppb) and high (≥ 24 ppb) subgroups (post-hoc analysis).
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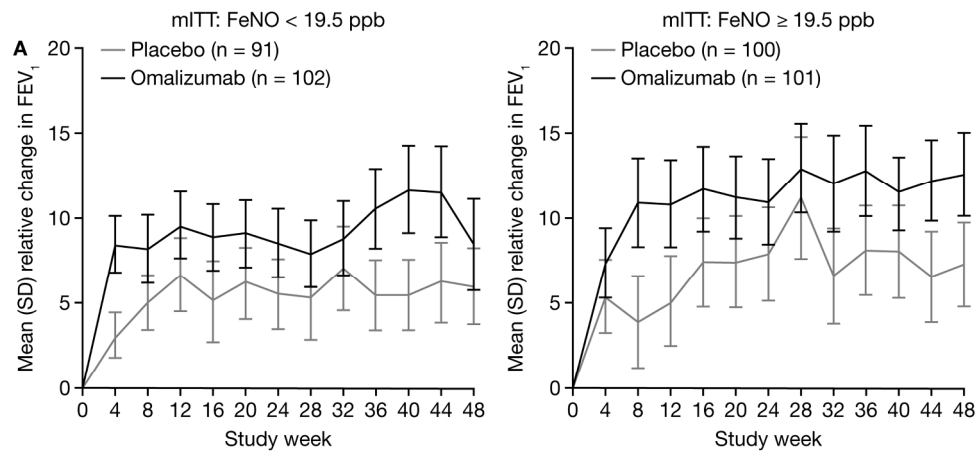


Figure E4. Relative change in FEV₁ from baseline in (A) the FeNO low (< 19.5 ppb) and high (≥ 19.5 ppb) subgroups, (B) the peripheral blood eosinophil (< 260/μL) and high (≥ 260/μL) subgroups, and (C) the periostin low (< 50 ng/mL) and high (≥ 50 ng/mL) subgroups*
189x90mm (300 x 300 DPI)

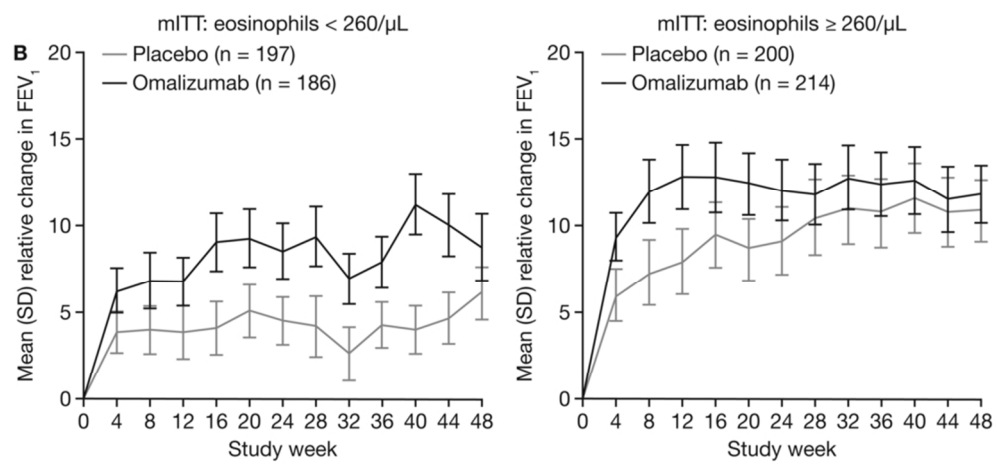


Figure E4. Relative change in FEV1 from baseline in (A) the FeNO low (< 19.5 ppb) and high ($\geq$ 19.5 ppb) subgroups, (B) the peripheral blood eosinophil (< 260/ μ L) and high ($\geq$ 260/ μ L) subgroups, and (C) the periostin low (< 50 ng/mL) and high ($\geq$ 50 ng/mL) subgroups*
85x39mm (300 x 300 DPI)

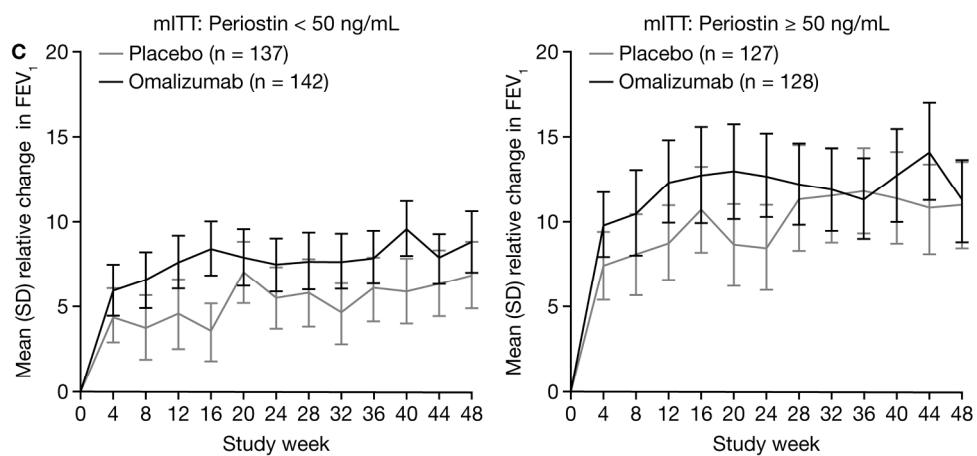


Figure E4. Relative change in FEV₁ from baseline in (A) the FeNO low (< 19.5 ppb) and high (≥ 19.5 ppb) subgroups, (B) the peripheral blood eosinophil (< 260/μL) and high (≥ 260/μL) subgroups, and (C) the periostin low (< 50 ng/mL) and high (≥ 50 ng/mL) subgroups*
189x90mm (300 x 300 DPI)

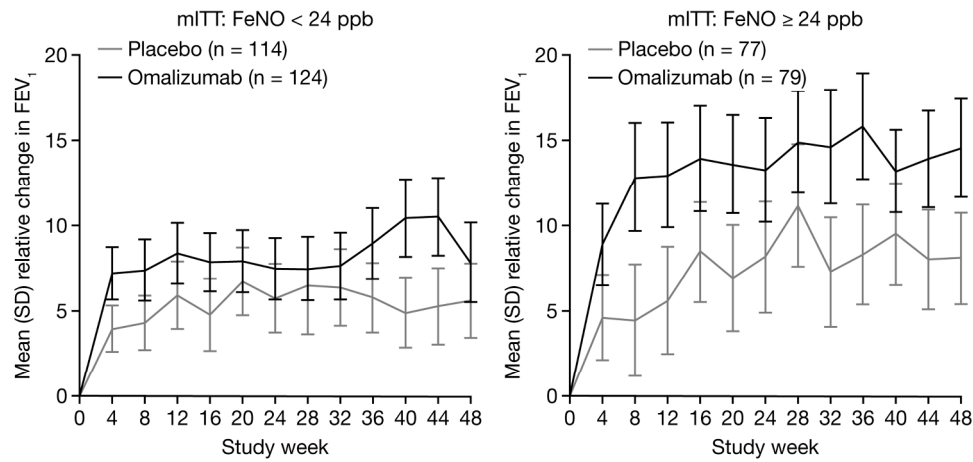


Figure E5. Relative change in FEV1 from baseline in the FeNO low (< 24 ppb) and high (≥ 24 ppb) subgroups (post-hoc analysis)
189x88mm (300 x 300 DPI)