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Effect of omalizumab on peripheral blood eosinophilia in allergic asthma

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Summary

Eosinophilia is an established marker of asthma-related inflammation. We assessed the effect of omalizumab on peripheral blood eosinophil counts using a pooled analysis of data from five randomized, double-blind, placebo-controlled studies in patients with moderate-to-severe persistent allergic asthma receiving moderate-to-high-dose inhaled corticosteroids (omalizumab, $n = 1136$; placebo, $n = 1100$). Relationships between omalizumab, peripheral blood eosinophils, serum free IgE concentrations and clinical outcomes were explored. Baseline mean eosinophil counts were similar in each treatment group. Post-treatment eosinophil counts were significantly reduced from baseline in the omalizumab group ($p < 0.0001$) but were not significantly different in the placebo group. Greater reductions in eosinophil counts were observed in patients who had post-treatment free IgE levels < 50 ng/mL. Three studies included steroid-stable and steroid-reduction phases. At the end of each phase in these studies, a significantly greater reduction in eosinophil counts was achieved in the omalizumab group compared with the placebo group ($p < 0.0001$). A consistent pattern of improved clinical outcomes/decreased eosinophils and worsened clinical outcomes/increased eosinophils was observed for both omalizumab and placebo treatment groups. The findings from our analysis of a large patient population are consistent with earlier reports of the inhibitory effect of omalizumab on eosinophils.

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Introduction

Asthma is a chronic inflammatory disorder of the airways that is often allergic in nature and characterized by the presence of immunoglobulin E (IgE) antibodies against one or more common environmental allergens.^{1–5} The main

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phases of allergic asthma are: sensitization to an allergen, an early asthmatic response (within minutes of allergen exposure), a late asthmatic response (within hours/days of allergen exposure), and airway remodelling.

The important role of eosinophils in inflammation is well documented, and eosinophilia is established as a good marker of inflammation in patients with asthma.^{3,6} Eosinophils release a variety of pro-inflammatory mediators including granule-stored cationic proteins, newly synthesized eicosanoids and cytokines.^{3,6} Furthermore, sputum eosinophil counts correlate closely with asthma severity⁷ and both the risk and severity of exacerbations.^{8,9}

Anti-IgE therapy with omalizumab, added to medium- or high-dose inhaled corticosteroids (ICS), with or without a long-acting β_2 -agonist, has proven effective in the treatment of patients with moderate-to-severe and severe allergic (IgE-mediated) asthma by reducing exacerbations and associated use of rescue bursts of systemic corticosteroids in addition to improving other clinical outcomes.^{10–16} Omalizumab results in a rapid reduction in free IgE levels and correspondingly downregulates Fc ϵ RI expression on basophils and mast cells. These actions reduce IgE-mediated responses to allergens, mast cell degranulation and release of pro-inflammatory mediators.^{4,17–20} Omalizumab has also been shown to reduce Fc ϵ RI expression on antigen-presenting cells (dendritic cells [DCs]) prior to their trafficking into tissues,²¹ suggesting that omalizumab may have the unique capacity to block both sensitization and effector phases of the allergic inflammatory process. There is evidence that blocking IgE with omalizumab leads to an inhibitory effect on eosinophils. Djukanović et al.¹⁷ reported that omalizumab monotherapy significantly reduced the number of sputum and bronchial submucosal eosinophils in patients with allergic asthma not receiving ICSs. This observed decrease in sputum and bronchial submucosal eosinophils was accompanied by a reduction in submucosal IgE+ and Fc ϵ RI+ cells.

The aim of this analysis was to assess the effect of omalizumab on peripheral blood eosinophil counts in patients with moderate-to-severe persistent allergic asthma. In addition, the relationships between peripheral blood eosinophils, serum free IgE concentrations and clinical outcomes were explored.

Methods

Studies and patients

This pooled analysis included data from five randomized, double-blind, placebo-controlled pivotal registration trials that assessed efficacy and safety of omalizumab in the treatment of patients with moderate-to-severe persistent allergic asthma (Table 1).^{10–14} In all studies, patients were ≥ 12 years of age and were receiving ongoing therapy with at least moderate-to-high doses of ICS at baseline. A monthly dose of omalizumab of at least 0.016 mg/kg/IU IgE was administered subcutaneously every 2 or 4 weeks, according to a dosing table nomogram incorporating the patient's bodyweight and baseline IgE levels.

The treatment period in three studies consisted of two phases: (1) a 16-week 'steroid-stable' phase during which patients received omalizumab or placebo treatment in

Table 1 Studies included in the pooled analysis.

Study	Patient population	Patients (n)	Duration (weeks)
Humbert et al. ¹⁰	Inadequately controlled severe allergic asthma despite GINA 2002 Step 4 therapy	419	28
Vignola et al. ¹¹	Moderate-to-severe allergic asthma and rhinitis	405	28
Busse et al. ¹²	Severe allergic asthma	525	28 ^{a,b}
Solèr et al. ¹³	Moderate-to-severe allergic asthma	546	28 ^{a,b}
Holgate et al. ¹⁴	Severe allergic asthma dependent on high-dose ICS	341	32 ^c

^a 16-week steroid-stable phase followed by a 12-week steroid-reduction phase.

^b Additional 24-week extension phase.

^c 16-week steroid-stable phase followed by a 16-week steroid-reduction phase.

addition to a fixed dose of ICS; and (2) a 12-week^{12,13} or 16-week¹⁴ 'steroid-reduction' phase. During the steroid-reduction phase ICS doses were adjusted to provide the lowest dose consistent with asthma control.^{12–14} Patients who received omalizumab achieved median reductions in ICS dose of 75%,²² 83%²³ and 60%¹⁴ compared with approximately 50%^{12–14} in the placebo group ($p < 0.01$). Two studies^{12,13} were also followed by extension phases that were similar in design.^{22,23} Briefly, during the 24-week double-blind extension phase, patients were maintained on omalizumab or placebo and the lowest sustainable dose of beclomethasone dipropionate (BDP). The use of other asthma medications was permitted and investigators were allowed to switch patients from BDP to other ICS medications. Full details of the five study designs have been reported elsewhere.^{10–14}

Circulating eosinophil counts and serum free IgE concentrations

Blood samples were obtained at baseline and post-treatment for measurement of the complete blood count (CBC) including eosinophils. The CBC was analyzed using the Bayer Advia 120 automated hematology system. Eosinophil counts greater than 15% were confirmed by manual differential. The post-treatment (omalizumab or placebo) time point was taken as the first available eosinophil laboratory evaluation after (and including) Week 16. In the three steroid-reduction studies,^{12–14} eosinophil counts were also calculated at the end of the 'steroid-reduction' phase. In the two studies with extension phases,^{22,23} change in eosinophil counts were calculated at the end of the extension phase, taken as the eosinophil laboratory evaluation at Week 52.

Blood samples were also collected for measurement of serum total and free IgE at baseline and post-treatment. Baseline pretreatment total IgE concentrations in serum

was determined by a microparticle enzyme immunoassay test kit commercially available from Abbott Inc. Free IgE was measured using a solid-phase ELISA, as described previously.²⁴

Eosinophil counts in subgroups of serum free IgE levels < 50 ng/mL and ≥50 ng/mL

For the studies from which IgE data were available,^{10,12–14} changes from baseline to post-treatment in mean eosinophil counts were grouped according to patients who achieved suppression of free IgE levels to <50 ng/mL^a vs patients who had not (i.e. free IgE levels ≥50 ng/mL).

Eosinophil counts in subgroup of patients receiving maintenance oral corticosteroids (OCS) at baseline

Changes from baseline to post-treatment in mean eosinophil counts were determined for those patients receiving maintenance OCS at baseline.

Clinical outcomes

The clinical outcomes assessed were: (1) total number of steroid bursts associated with treatment of asthma exacerbations^{10,11}; (2) investigator's global evaluation of treatment effectiveness (GETE) which was rated as 'excellent/good', 'moderate', or 'poor/worse'; and (3) lung function assessed as change from baseline in forced expiratory volume in 1 s (FEV₁). The effect of omalizumab on steroid bursts and GETE was determined at the end of the 'steroid-reduction' phase^{12–14} or the end of the study.^{10,11} A steroid burst was defined as a dose of oral prednisone (40–60 mg) in single or two divided daily doses for adults or 1–2 mg/kg/day (maximum 60 mg/day) for patients less than 18 years, generally administered for 3–10 days. The last non-missing observation in the 'steroid-stable' phase was considered the end of study FEV₁ measurement for the three studies that had a 'steroid-reduction' phase.^{12–14} The last non-missing observation after the treatment phase was considered the end of study FEV₁ measurement for the remaining two studies.^{10,11} The relationships between changes in eosinophil counts and clinical outcomes were explored.

Statistical methods

Absolute eosinophil counts at baseline and post-baseline time points were summarized descriptively for the omalizumab and placebo group and the mean change from baseline calculated. *T*-tests were used to determine if mean changes were different from zero for each treatment group. The two-tailed null hypothesis stated that the mean change from baseline to the post-baseline time point in eosinophil counts was not different from zero. For each post-baseline time point, change from baseline was analyzed using an analysis of covariance (ANCOVA) model

with baseline eosinophil value, age, gender, treatment group (omalizumab or placebo), dosing schedule (every 2 or 4 weeks) and study centre as explanatory variables. Least squares means (LSM) were computed for each treatment group as well as 95% confidence intervals (CIs) for LSM treatment differences and *p*-values for the treatment difference. The number of asthma exacerbations requiring steroid bursts was analyzed using Poisson regression with treatment, age, gender, use of oral steroids at baseline, baseline FEV₁ (≥80%, 60–79%, <60%), dosing schedule and study as explanatory variables.^{25,26} The relative risk (RR) for omalizumab versus placebo was calculated and presented with corresponding 95% CI. Investigator-determined GETE were analyzed using Cochran–Mantel–Haenszel mean score test adjusting for study.²⁵ Change from baseline in FEV₁ was analyzed using ANCOVA with treatment, age, gender, baseline FEV₁, dosing schedule and study as explanatory variables; LSM of the change from baseline and 95% CI were calculated. All analyses were performed using SAS Version 9.1 (Copyright © 2002–2003 by SAS Institute Inc., Cary, NC, USA).

Table 2 Baseline demographic and clinical characteristics.

	Omalizumab (n = 1136)	Placebo (n = 1100)
<i>Age, years</i>		
Mean (SD)	40.5 (14.3)	40.2 (14.09)
Min, Max	12, 79	12, 74
<i>Sex, n (%)</i>		
Males	476 (41.9)	458 (41.6)
Females	660 (58.1)	642 (58.4)
<i>Baseline FEV₁, n (%)</i>		
≥80%	313 (27.6)	305 (27.7)
60–79%	519 (45.7)	502 (45.6)
<60%	304 (26.8)	293 (26.6)
Serum total IgE (IU/mL), mean (SD)	213 (169.3)	209.5 (163.88)
Total clinical symptoms score, mean (SD)	5.9 (6.34)	5.8 (6.26)
Baseline ICS in BDP equivalent (μg/day), mean (SD)	1409 (1076)	1402 (1008.1)
<i>Baseline oral corticosteroid use, n (%)</i>		
Yes	99 (8.7)	87 (7.9)
No	1037 (91.3)	1013 (92.1)
<i>Baseline LABA use, n (%)</i>		
Yes	376 (33.1)	349 (31.7)
No	760 (66.9)	751 (68.3)

BDP = beclomethasone dipropionate; FEV₁ = forced expiratory volume in 1 s; ICS = inhaled corticosteroids; IgE = immunoglobulin E; LABA = long-acting β₂-agonist; SD = standard deviation.

^a 2.44 ng/mL = 1 IU.

Table 3 Eosinophil counts ($10^9/L$) at baseline and post-treatment (Week 16), change from baseline, and percentage change from baseline in patients treated with omalizumab or placebo.

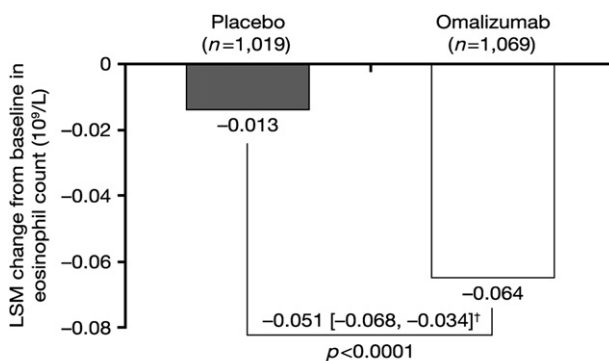
	Omalizumab	Placebo
Baseline, mean (SD)	0.321 (0.2399) [n = 1116]	0.332 (0.2504) [n = 1082]
Post-treatment, mean (SD)	0.262 (0.2020) [n = 1084]	0.320 (0.2596) [n = 1033]
Change from baseline, mean (SD)	-0.057 (0.2178)	-0.012 (0.2497)
p-value*	$p < 0.0001$ [n = 1069]	$p = 0.1199$ [n = 1019]
Percentage change from baseline, mean (SD)	-3.961 (82.0639)	23.089 (179.4656)
Median (min, max)	-18.75 (-100.00, 1218.68) [n = 1060]	-2.097 (-100.00, 4397.92) [n = 1016]

*Two-tailed *t*-test with the following null hypothesis: mean change in eosinophil count from baseline to post-baseline time point is not different from zero.

Results

Baseline demographic and clinical characteristics

Data for 1136 omalizumab-treated patients and 1100 placebo recipients were analyzed. In the three studies that included a steroid-reduction phase, data were available for 1412 patients (omalizumab: $n = 718$; placebo: $n = 694$). In the two studies with an extension phase, data were available for 1071 patients (omalizumab: $n = 542$; placebo: $n = 529$). The treatment groups were well matched in terms of baseline demographic and clinical characteristics (Table 2). Overall, approximately 96% of patients met GINA 2002 criteria for severe persistent asthma based on both symptoms and asthma therapy.¹



*LSM treatment difference (95% confidence interval)

Figure 1 Least squares mean (LSM) change from baseline to post-baseline time point in eosinophil counts (all five studies).

Eosinophil counts and free IgE levels

Mean eosinophil counts at baseline were similar in the two treatment groups (Table 3). Post-treatment eosinophil counts were significantly reduced from baseline in the omalizumab group ($p < 0.0001$) but were not significantly different in the placebo group. The LSM treatment difference for the post-treatment change from baseline in eosinophil counts was $-0.051 \times 10^9/L$ in favour of omalizumab ($p < 0.0001$) (Fig. 1).

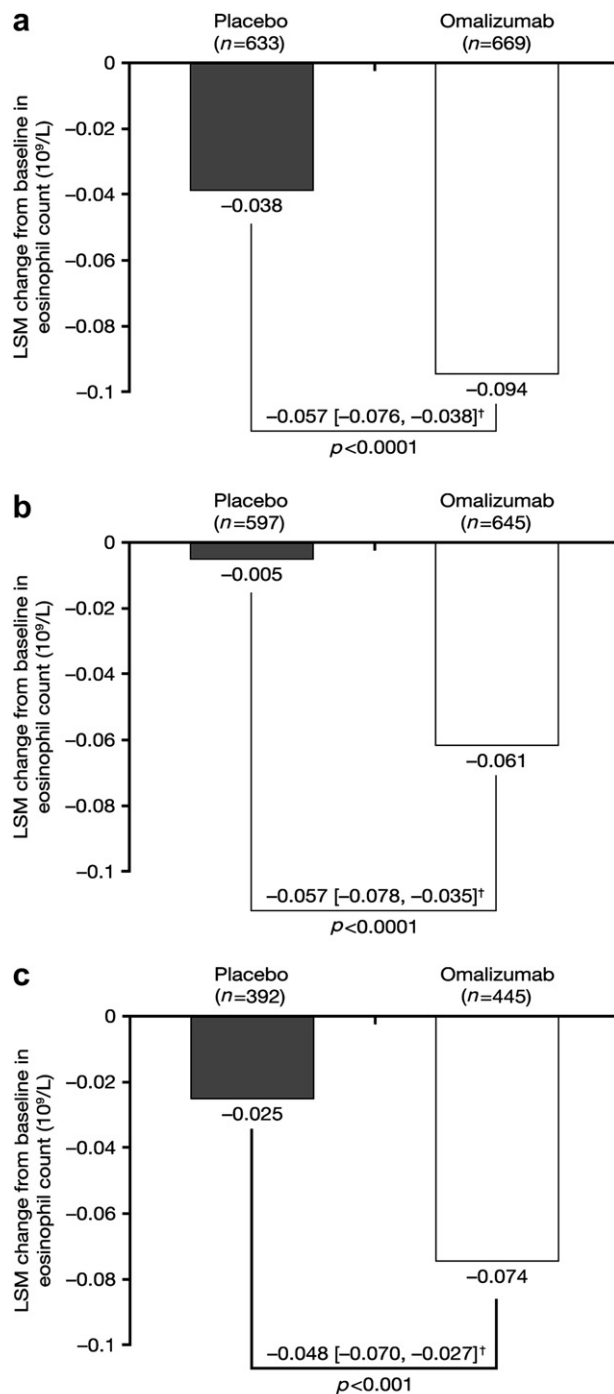
In the three studies that comprised 'steroid-stable' and 'steroid-reduction' phases,^{12–14} eosinophil counts were significantly reduced from baseline in the omalizumab group at the end of each phase (Table 4). There was no significant change in the placebo group at either time point (Table 4).

Table 4 Eosinophil counts ($10^9/L$) at baseline, Week 16 and Week 28 or 32, change from baseline, and percentage change from baseline in patients treated with omalizumab or placebo in three studies that included a steroid-reduction phase.^{12–14}

	Omalizumab	Placebo
Baseline, mean (SD)	0.316 (0.2502) [n = 701]	0.317 (0.2288) [n = 680]
End of the steroid-stable phase, mean (SD)	0.242 (0.1766) [n = 683]	0.301 (0.2216) [n = 645]
Change from baseline, mean (SD)	-0.069 (0.2244)	-0.016 (0.2288)
p-value*	$p < 0.0001$ [n = 669]	$p = 0.0838$ [n = 633]
Percentage change from baseline, mean (SD), median (min, max)	-8.002 (66.4733), -21.238 (-100.00, 660.36) [n = 665]	18.579 (193.3906), -3.667 (-100.00, 4397.92) [n = 631]
End of the steroid-reduction phase, mean (SD)	0.266 (0.2090) [n = 657]	0.326 (0.2238) [n = 608]
Change from baseline, mean (SD)	-0.043 (0.2518)	0.010 (0.2262)
p-value*	$p < 0.0001$ [n = 645]	$p = 0.2718$ [n = 597]
Percentage change from baseline, mean (SD), median (min, max)	3.410 (89.0318), -14.583 (-100.00, 860.87) [n = 641]	26.603 (111.3232), 3.589 (-100.00, 1584.38) [n = 595]

*Two-tailed *t*-test with the following null hypothesis: mean change in eosinophil count from baseline to post-baseline time point is not different from zero.

The LSM treatment difference for the change from baseline in eosinophil counts was $-0.057 \times 10^9/\text{L}$ in favour of omalizumab ($p < 0.0001$) at the end of the 'steroid-stable' phase (Fig. 2a) and $-0.057 \times 10^9/\text{L}$ in favour of



†LSM treatment difference (95% confidence interval)

Figure 2 Least squares mean (LSM) change from baseline in eosinophil counts at the end of the steroid-stable phase (Week 16) [a], and at the end of the steroid reduction phase (Week 28 or 32) in the three studies of this design [b]; and at the end of the extension phase in the two studies with extension phases [c].

Table 5 Eosinophil counts ($10^9/\text{L}$) at baseline, end of steroid-reduction phase and end of study extension phase, change from baseline in patients, and percentage change from baseline treated with omalizumab or placebo in the two studies with extension phase.^{12,13,22,23}

	Omalizumab	Placebo
Baseline, mean (SD)	0.304 (0.1960) [n = 530]	0.325 (0.2179) [n = 520]
End of the steroid-reduction phase, mean (SD)	0.265 (0.2065) [n = 494]	0.329 (0.2056) [n = 450]
Change from baseline, mean (SD)	-0.033 (0.2118)	0.006 (0.1973)
p-value*	$p < 0.001$ [n = 485]	$p = 0.512$ [n = 442]
Percentage change from baseline, mean (SD), median (min, max)	3.480 (87.4590), -14.039, (-100.00, 860.87)	20.756 (102.7313), 2.349 (-100.00, 1584.38)
End of the extension phase, mean (SD)	0.252 (0.1669) [n = 455]	0.310 (0.2062) [n = 396]
Change from baseline, mean (SD)	-0.049 (0.1775)	-0.010 (0.2017)
p-value*	$p < 0.001$ [n = 445]	$p = 0.318$ [n = 392]
Percentage change from baseline, mean (SD), median (min, max)	-1.440 (66.4215), -15.749, (-100.00, 520.23) [n = 444]	16.562 (91.4919), -0.142 (-100.00, 1047.92) [n = 392]

*Two-tailed t-test with the following null hypothesis: mean change in eosinophil count from baseline to post-baseline time point is not different from zero.

omalizumab ($p < 0.0001$) at the end of the 'steroid-reduction' phase (Fig. 2b).

In the two studies with extension phases,^{22,23} eosinophil counts were significantly reduced from baseline in the omalizumab group at the end of the extension phase (Table 5). Changes from baseline in the placebo group were not significant (Table 5). The LSM treatment difference for the change from baseline in eosinophil counts at the end of the extension phase was $-0.048 \times 10^9/\text{L}$ in favour of omalizumab ($p < 0.001$; Fig. 2c).

Eosinophil counts in subgroups of serum free IgE levels $< 50 \text{ ng/mL}$ and $\geq 50 \text{ ng/mL}$

Patients with post-treatment free IgE levels $< 50 \text{ ng/mL}$ ($n = 786$) achieved a mean (SD) change in eosinophil counts

($10^9/L$) from baseline to post-baseline time point of -0.059 (0.2218) $\times 10^9/L$ (omalizumab: $n = 753$, -0.062 [0.2254]; placebo: $n = 33$, -0.000 [0.0955] $\times 10^9/L$). In contrast, patients with post-treatment free IgE levels ≥ 50 ng/mL ($n = 718$) had a lesser change in eosinophil counts ($10^9/L$) from baseline to post-baseline time point of -0.008 (0.2560) $\times 10^9/L$ (omalizumab: $n = 25$, -0.065 [0.1328]; placebo: $n = 693$, -0.006 [0.2592] $\times 10^9/L$).

Eosinophil counts in patients receiving maintenance OCS at baseline

Omalizumab patients who were also receiving maintenance OCS at baseline ($n = 88$) achieved a mean (SD) decrease in eosinophil count ($10^9/L$) from baseline to post-baseline time point of -0.010 (0.2470) $\times 10^9/L$, compared with patients receiving placebo ($n = 84$) who experienced a mean (SD) increase of 0.034 (0.2654) $\times 10^9/L$.

Clinical outcomes

The mean number of asthma exacerbations requiring treatment with steroid bursts was lower in the omalizumab group compared with placebo (Table 6). The mean (SD) number of steroid bursts was 0.4 (0.87) and 0.6 (1.23) for omalizumab and placebo, respectively. The relative risk for omalizumab to placebo was 0.66 (95% CI: 0.56, 0.77); thus, it would be expected that an omalizumab-treated patient would require 34% fewer steroid bursts than those receiving placebo ($p < 0.001$).

LSM change from baseline in FEV₁ was improved in the omalizumab group compared with placebo (LSM treatment difference [95% CI] 75.12 [44.41, 105.84], $p < 0.0001$;

Table 6 Clinical outcomes in patients treated with omalizumab or placebo.

	Omalizumab <i>n</i> = 1136	Placebo <i>n</i> = 1100
Mean number of steroid bursts	0.4	0.6
RR versus placebo (95% CI)	0.66 (0.56, 0.77)	
<i>p</i> value for RR	$p < 0.001$	
Mean (%) change from baseline in FEV ₁ , mL	111.5 (6.1%)	30.7 (2.5%)
LSM	116.19	41.07
LSM treatment difference (95% CI)	75.12 (44.41, 105.84)	
<i>p</i> value	$p < 0.0001$	
Investigator GETE, <i>n</i> (%)		
Excellent/good	665 (58.5)	406 (36.9)
Moderate	283 (24.9)	312 (28.4)
Poor/worse	137 (12.1)	332 (30.2)
<i>p</i> value	$p < 0.001$	

RR: relative risk; LSM: least mean squares GETE: global evaluation of treatment effectiveness.

Table 7 Clinical outcomes in patients treated with omalizumab or placebo. Data are presented as percent of patients.

Outcome	Omalizumab <i>n</i> = 1069	Placebo <i>n</i> = 1019
Exacerbations		
No steroid burst and decrease in PBE	48.5	34.2
Steroid burst use and increase in PBE	9.0	17.6
Investigator GETE		
Positive GETE and decrease in PBE	40.5	19.1
Negative GETE and increase in PBE	4.8	14.6
Pulmonary function		
FEV ₁ increase and decrease in PBE	43.6	30.0
FEV ₁ decrease and increase in PBE	15.4	26.4

GETE: global evaluation of treatment effectiveness; PBE: peripheral blood eosinophil.

Table 6). Investigator-determined GETE showed significant improvements in favour of omalizumab. Physicians rated treatment effectiveness as 'excellent' or 'good' in 59% of omalizumab-treated patients compared with 37% in the placebo group ($p < 0.001$; Table 6).

In general, a greater percentage of patients receiving omalizumab experienced an improvement in clinical outcomes and a decrease in eosinophil counts (Table 7).

Discussion

Findings from this pooled analysis demonstrate that reduction of serum free IgE by omalizumab is associated with a significant reduction in peripheral eosinophil counts in patients with moderate-to-severe persistent allergic asthma receiving moderate-to-high doses of ICS. In the pooled analysis of 5 studies, circulating eosinophils were reduced by a median of 18.8% and 2.1% in the omalizumab and placebo groups, respectively (Table 3). In the three studies that included a 'steroid-reduction' phase, reductions in eosinophil counts were maintained despite a significant reduction in ICS dose to levels that are less likely to be associated with systemic activity – circulating eosinophils were reduced in the omalizumab group by a median of 21.2% at the end of the 'steroid-stable' phase and a median of 14.6% at the end of the 'steroid-reduction' phase (at the same time points, eosinophil counts in the placebo group were decreased by a median of 3.7% and increased by 3.6%, respectively; Tables 4 and 5).

In two studies, patients entered a long-term extension following the 'steroid-reduction' phase. During the extension phase, omalizumab sustained the decrease in eosinophil counts observed following steroid reduction. These reductions in eosinophil counts following treatment with

omalizumab were accompanied by improvements in a range of clinical outcome measures.

The significant reduction in eosinophil counts observed in our pooled analysis adds evidence from a large patient population to that obtained from smaller studies, which reported an inhibitory effect of omalizumab on eosinophils. In one study of 35 patients with moderate-to-severe allergic asthma, omalizumab significantly decreased circulating eosinophils at both 16 and 52 weeks ($p < 0.01$ vs placebo).²⁷ Evidence of attenuation of eosinophil infiltration into the lungs was provided by Djukanović and colleagues in a study of 45 patients with corticosteroid-naïve, mild-to-moderate persistent allergic asthma who underwent bronchial biopsy and induced-sputum sampling.¹⁷ In the omalizumab group, the percentage of eosinophils present in induced sputum decreased from 4.8% to 0.6% ($p = 0.05$ vs placebo) and tissue eosinophils in the bronchial submucosa were significantly reduced from 8.0 to 1.5 cells/mm² ($p = 0.03$ vs placebo).

In a study of 19 patients with coexisting allergic asthma and rhinitis, omalizumab therapy induced eosinophil apoptosis and reduced the number of lymphocytes producing GM-CSF, an important factor for eosinophil growth and survival.²⁸ Furthermore, a study of 30 adults with seasonal allergic rhinitis reported that blood eosinophils increased significantly in the placebo group, remained unchanged in the omalizumab group, and that differences between treatment groups were statistically significant.²⁹ The authors also reported that seasonal increases and inhibitory effects of treatment were mirrored in the changes in eosinophils (staining positively for eosinophil peroxidase) in nasal biopsy specimens. Moreover, the number of nasal tissue eosinophils correlated significantly with serum free IgE levels.

Despite having established a considerable evidence base supporting the effects of omalizumab on circulating and bronchial eosinophils, the mechanism whereby reductions in circulating IgE can ultimately lead to a reduction in eosinophils remains unclear. It is possible that omalizumab may lead to inhibition of the release of pro-inflammatory mediators, cytokines and chemokines from mast cells/basophils, or inhibition of the allergen-induced differentiation of T-cells to Th2 cells by markedly reducing the expression of FcεRI on these cells. The mediators of greatest interest are the Th2 cytokines IL-4, IL-5 and IL-13 as these are responsible for promoting recruitment and activation of mast cells and eosinophils and are the primary effector cells of the allergic response.³⁰ It is known that IL-4, IL-5 and IL-13 levels are increased in the sera of patients with asthma.³¹ Moreover, IL-4 and IL-13 play a central role in promoting eosinophil trafficking, as well as driving Th2 cell differentiation, whereas IL-5 is the major cytokine promoting eosinophil differentiation, proliferation and activation.³

Omalizumab has been shown to significantly reduce IL-4 positive cells in the bronchial submucosa and reduce IL-5 positive cells, although this latter reduction was not statistically significant.¹⁷ A significant reduction in circulating IL-13 and a non-significant reduction in circulating IL-5 have also been reported with omalizumab in patients with moderate-to-severe allergic asthma.²⁷ Eosinophil apoptosis and reduced numbers of lymphocytes producing

GM-CSF have been observed in omalizumab-treated patients with coexisting allergic asthma and rhinitis, which may also contribute to the inhibitory action of omalizumab on eosinophils.²⁸

A direct effect of omalizumab on eosinophils may be possible via the FcεRI receptors that have been detected on eosinophils; however, their functional significance has yet to be established.^{32,33} Studies examining blood from atopic subjects have detected only negligible surface expression of functional FcεRI on eosinophils; however, appreciable intracellular staining for FcεRI was observed.^{33,34} Of note, cross-linking of the surface-expressed receptors did not lead to any detectable degranulation response.^{33,34} Furthermore, a study that sampled eosinophils from transgenic mice found that IL-10 was secreted after FcεRI activation, suggesting some contribution to the regulation of the immune response.³⁵

The anti-eosinophilic effects of ICS are well documented^{36–38} but it is noteworthy that the significant reductions seen with add-on omalizumab in this analysis were achieved in study populations that were already receiving ICS. Leukotriene modifiers have also demonstrated anti-eosinophilic effects but these were of a smaller magnitude than that achieved using ICS.³⁹ Of note, it has been reported that the leukotriene receptor antagonist montelukast failed to reduce sputum eosinophilia in patients with high-dose corticosteroid-dependent asthma.⁴⁰

In our analysis, a greater proportion of patients receiving omalizumab experienced improvement in clinical outcomes (reduced requirement for treatment of exacerbations with oral steroid bursts, increased FEV₁, and positive investigator GETE) and a decrease in peripheral blood eosinophils compared to those receiving placebo. A consistent pattern of improved clinical outcomes/decreased eosinophils and worsened clinical outcomes/increased eosinophils was observed for both omalizumab and placebo treatment groups. However, the clinical significance of the decrease in peripheral blood eosinophils, and in particular how this is related to improvements in FEV₁ and GETE, is uncertain. Weak associations, as measured by Pearson and Spearman correlation coefficients, were observed between change (decrease) from baseline in eosinophil counts and various clinical variables (data not shown).

In summary, our findings from this pooled analysis of five large randomized controlled studies in patients with moderate-to-severe or severe persistent allergic asthma expand the understanding of the mechanism of action of omalizumab beyond binding of serum IgE to include effects on the marker of inflammation, peripheral blood eosinophils.

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Conflict of interest statement

S Holgate undertakes occasional consultant work for Novartis and participates as Chair or speaker in international Symposia at international conferences for which he receives an honorarium.

W Busse has acted as consultant for Genentech/Novartis, Isis, GlaxoSmithKline, Altana, Wyeth, Pfizer, Dynavax and Centocor and has received grants/research support from Novartis, Dynavax, Wyeth, Centocor and GlaxoSmithKline. W Busse has also been on speakers' bureau for GlaxoSmithKline, Novartis, Merck and AstraZeneca.

M Massanari, P Jimenez, F Kianifard and R Zeldin are all employees of Novartis Pharmaceuticals.

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