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Omalizumab-induced decrease of Fc ϵ RI expression in patients with severe allergic asthma

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KEYWORDS

Basophils;
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Summary

Background: It is documented that omalizumab treatment reduces the cell surface expression of immunoglobulin E high-affinity receptor (Fc ϵ RI) on several cell types. This has not been investigated in patients with uncontrolled severe persistent allergic asthma.

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Plasmacytoid dendritic cells;
Uncontrolled severe allergic asthma

Methods: In a double-blind, randomized, placebo-controlled study, patients with severe allergic asthma uncontrolled by high dose inhaled corticosteroids and long-acting β_2 -agonist received either omalizumab ($n = 20$) or placebo ($n = 11$) over 16 weeks at appropriate doses and frequencies. Baseline and end of study (week 16) Fc ϵ RI expression on basophils and plasmacytoid dendritic cells was determined by flow cytometry for the primary endpoint. Secondary efficacy endpoints included asthma control and lung function as part of an initial investigation into the use of Fc ϵ RI expression as a marker of response.

Results: In the omalizumab group, and with respect to placebo, Fc ϵ RI expression was significantly reduced at end of study on basophils (-82.6% , $p < 0.01$) and plasmacytoid dendritic cells (-44.2% , $p = 0.029$). Fc ϵ RI expression reduction was not found to be correlated with clinical response.

Conclusions: Long-term omalizumab treatment induced reduction of Fc ϵ RI expression on circulating basophils and plasmacytoid dendritic cells. These changes were not associated with those of clinical features related to severe asthma, which does not support further investigation into its use as a predictive marker of response.

Trial registration: The study was registered with ClinicalTrials.gov (identifier: NCT00454051) and the European Clinical Trials Database, EudraCT (identifier: 2006-003591-35)

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Introduction

Atopy and asthma have long been associated, although the causative relationship between aeroallergen sensitivity and asthma is not fully understood.^{1,2} In the initial allergic response, and several aspects of chronic inflammation, immunoglobulin E (IgE) plays a key role. Its binding to the IgE high-affinity receptor (Fc ϵ RI) at the surface of mast cells and basophils is pivotal for the release of pro-inflammatory mediators.^{3,4}

Omalizumab (Xolair,[®] Novartis Pharma SAS.), a humanized recombinant murine monoclonal anti-IgE antibody, was designed to treat allergic asthma by reducing the circulating pool of IgE able to bind to Fc ϵ RI.^{5,6} In this regard, omalizumab treatment is successful, resulting in a marked reduction of free IgE.^{7–10} It is known that IgE is able to both up- and down-regulate Fc ϵ RI expression¹¹ but also that serum IgE is positively correlated with Fc ϵ RI density on basophils¹² and dendritic cells.¹³ Consistent with this, several studies have shown substantial reduction in the density of Fc ϵ RI expressed on circulating basophils⁸ and dendritic cells⁹ of atopic patients treated with omalizumab.

Severe-persistent asthma, uncontrolled by inhaled corticosteroids and long-acting β_2 -agonists¹⁴ may be considered different to mild-to-moderate asthma in several regards including the prevalence of atopy.^{15–17} But, although atopy is less often found in severe asthmatics than in those with controlled asthma, positive skin-prick tests have been found in approximately 60% of patients with severe uncontrolled asthma.¹⁵ Omalizumab has demonstrated efficacy in moderate-to-severe allergic asthma^{18–20} and more recently the INNOVATE study²¹ confirmed this efficacy in patients with severe allergic asthma, uncontrolled by GINA step 4 treatment.¹⁴ Subsequent reports of the INNOVATE study found that it was difficult to predict clinical response from baseline factors²² and that free IgE levels varied in function of omalizumab concentrations, independently of response.¹⁰ The INNOVATE study did not however investigate the effect of omalizumab on Fc ϵ RI expression, and previous investigations that have done so

did not consider the same patient group nor attempt to correlate this with disease response.^{8,9}

The present study aimed to further understand the effects of omalizumab therapy in uncontrolled severe persistent allergic asthma by investigating the changes in the expression of Fc ϵ RI on basophils and plasmacytoid dendritic cells (pDC^{13,23}) after 16-week treatment with omalizumab in comparison with placebo. It was also a pilot study to investigate the potential for Fc ϵ RI to be used as a predictive marker of response in such patients, thus correlation between change and clinical response at 16 weeks was also assessed.

Methods

The study was conducted according to the Declaration of Helsinki, approved by the Bordeaux ethics committee (Comité de Protection des Personnes), and all participants gave written informed consent. The study was registered with ClinicalTrials.gov (identifier: NCT00454051) and the European Clinical Trials Database, EudraCT (identifier: 2006-003591-35).

Patients

Were consecutively enrolled; adults (≥ 18 years) with severe persistent allergic asthma with the following characteristics: FEV₁ $< 80\%$ of predicted, frequent daily symptoms (≥ 4 days/week average) or nocturnal awakening (≥ 1 /week average), multiple severe asthma exacerbations (≥ 2), severe asthma exacerbations requiring an unscheduled medical intervention with systemic corticosteroid in the past year, or hospitalization/emergency room for an asthma exacerbation in the past year, high dose inhaled corticosteroid > 1000 μg beclometasone dipropionate or equivalent and an inhaled long-acting β_2 -agonist, allergy to a perennial allergen (positive prick skin test or *in vitro* reactivity to a perennial aeroallergen, RAST), total serum IgE level ≥ 30 to ≤ 700 IU/ml and suitable serum total IgE level and weight according to omalizumab dosing tables.

Exclusion criteria included: Smoking history >20 pack-years, asthma exacerbation within 4 weeks prior to randomization, history of food or drug-related severe anaphylactoid/anaphylactic reaction, elevated serum IgE levels for reasons other than allergy, previous use of omalizumab and uncontrolled chronic diseases including cancer.

Study design

This randomized, placebo-controlled, double-blinded, multi-centre study to assess the expression of FcεRI on blood basophils and pDC2 comprised an initial screening visit to determine eligibility four weeks before the 16-week add-on treatment phase. It was performed from December 2006 to March 2008; six centres participated, all were in France. At screening and subsequent visits, asthma and concomitant treatments were recorded. All concomitant treatments were allowed; maintenance asthma treatment was to remain unchanged throughout the study period. Study drug dose was determined following the European SmPC of omalizumab²⁴ using total IgE measured at screening and body weight at week 0 of treatment phase (baseline). During the treatment phase, patients made study visits at weeks 0, 4, 8, 12 and 16 (additional visits if injection every 2 weeks was required). The primary efficacy variable was change from baseline in FcεRI expression on blood basophils and pDC2 after the 16-week treatment phase. Blood samples were taken at screening (or week 0 before study drug administration in case of technical issue with the original sample) and at week 16 for this assessment. Primary comparison concerned the treatment groups in terms of reduction of FcεRI expression on basophils, the comparison regarding FcεRI expression on pDC2 was secondary to this.

Treatment groups were also compared with regard to secondary efficacy variables. Kinetics of change in FcεRI expression on blood basophils and pDC2 was assessed in patients of a sub-set of centres ($n = 3$). For those receiving treatment every two weeks, additional blood samples were taken at week 2 and for all patients of the centres concerned, at weeks 4, 8 and 12. Asthma symptoms were recorded in a patient diary card issued at screening (along with a peak flow meter) and that was reviewed at each visit. Asthma control and change from baseline was investigated by the number of non-satisfied items of the first 6 defining acceptable control according to the French National Agency for Accreditation and Evaluation in Healthcare (ANAES): daytime symptoms <4 days per week, nighttime symptoms (awakening) <1 night per week, normal physical activity (no impairment in daily activities), mild and infrequent exacerbations (absence of treatment with oral corticosteroids), no absence from work/school, short-acting β_2 -agonist <4 doses/week. Physicians gave overall assessment of treatment effectiveness at week 16. Adverse events, independently of relation to study drug, and asthma exacerbations were recorded at each visit.

Patients were centrally randomized to either omalizumab or placebo at a ratio of 2:1, stratified by centre. Omalizumab was supplied by Novartis Pharma SAS, as a sterile, freeze-dried preparation. Placebo was a physiological salt solution, administered according to the same

scheme to respect the same dosing frequency and injected volume. Study medications were stored in the Pharmacy Department of each centre. Since reconstitution time and viscosity are different for omalizumab and placebo, individuals independent of the investigator reconstituted drugs. The nurse who injected the drug was instructed not to inform the patient or investigator of facts likely to unblind treatment.

Analysis of FcεRI expression

Blood samples (5 ml, EDTA tubes) were to be maintained at ambient temperature (strictly >10 °C) and analyzed within 24 h of collection by the cellular immunology laboratory of the Bordeaux teaching hospitals. Flow cytometry analysis (FC-500, Beckman Coulter) was performed on 100 μ l samples using the mAbs: anti-CD123 PE (Becton Dickinson), anti-BDCA2 FITC (Miltenyi Biotec) and isotype control IgG₂b APC (Clinisciences), or, anti-CD123 PE, anti-BDCA2 FITC and anti-FcεRI APC (Clinisciences). The proportion of FcεRI-positive cells and FcεRI expression (mean fluorescence intensity) were determined either on basophils or pDC2 gated according to phenotype (CD123+/BDCA2-, CD123+/BDCA2+ respectively).

Statistical analysis

Based on previously reported results^{8,9} and anticipating probability of 0.9 that FcεRI reduction would be greater in the omalizumab group compared to placebo, 10 patients would give power of 87% (Wilcoxon test, risk = 0.05). An additional 10 patients were included in the omalizumab

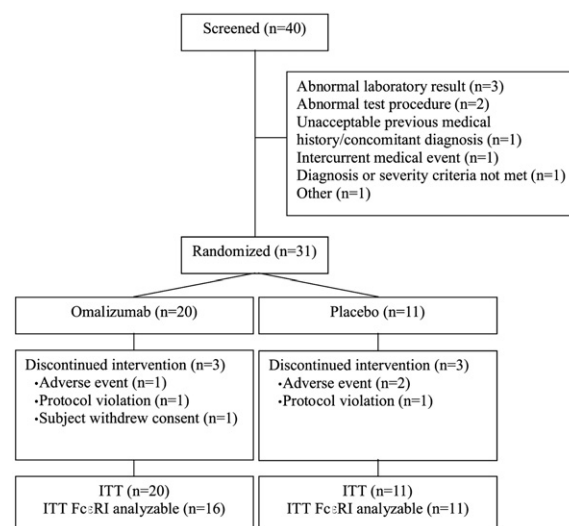


Figure 1 Patient flow. All patients receiving one dose of study medication with at least one post-dose efficacy assessment were included in the Intent-To-Treat (ITT) population. The patients with missing pre- and/or post-treatment assessment(s) of FcεRI expression were excluded from the ITT FcεRI analyzable population for the primary endpoint. Abnormal laboratory result refers to serum IgE outside dosing table for omalizumab and abnormal test procedure refers to a technical issue with serum IgE determination.

Table 1 Characteristics of the ITT population.

	Omalizumab N = 20	Placebo N = 11	All patients N = 31
Sex, n (%)			
Male	6 (30)	6 (54.5)	12 (38.7)
Age, years			
Mean (SD)	45.7 (13.30)	50.6 (16.31)	47.4 (14.37)
Median (range)	42.0 (23; 74)	56.0 (25; 74)	49.0 (23; 74)
Weight, kg ^a			
Mean (SD)	74.2 (16.74)	74.5 (15.35)	74.3 (16.00)
Median (range)	70.5 (50; 112)	80.0 (51; 98)	71.0 (50; 112)
Asthma duration, years			
Mean (SD)	31.2 (18.69)	31.8 (19.17)	31.4 (18.55)
Median (range)	30.0 (3; 74)	26.0 (1; 59)	28.0 (1; 74)
FEV ₁ , % of predicted value			
Mean (SD)	61.3 (14.83)	66.6 (11.38)	63.2 (13.75)
Median (range)	64.2 (35; 79)	64.6 (50; 90)	64.6 (35; 90)
Total IgE, IU/mL			
Mean (SD)	202.0 (129.12)	253.3 (189.03)	220.2 (151.96)
Median (range)	246.5 (29.0; 384.0)	216.0 (31.0; 602.0)	234.0 (29.0; 602.0)
Asthma treatments ^b , n (%)			
Inhaled corticosteroid	20 (100)	11 (100)	31 (100)
Long-acting β_2 -agonists	20 (100)	11 (100)	31 (100)
Oral corticosteroids	3 (15) ^c	4 (36.4)	7 (22.6) ^c
Theophylline	1 (5)	1 (9.1)	2 (6.5)
Montelukast	8 (40)	4 (36.4)	12 (38.7)
Anticholinergics	6 (30)	6 (54.5)	12 (38.7)
Inhaled corticosteroid daily dose, μ g BDP eq./day ^d			
Mean (SD)	3712 (1042.7)	3272 (1348.4)	3556 (1157.8)
Median (range)	4000 (2000; 6500)	4000 (2000; 6000)	4000 (2000; 6500)
Asthma exacerbations requiring systemic corticosteroids in previous year, n			
Mean (SD)	4.7 (3.33)	4.0 (2.90)	4.4 (3.15)
Median (range)	3.5 (2; 15)	3.0 (2; 12)	3.0 (2; 15)
Hospitalizations in previous year, n			
Mean (SD)	0.9 (2.03)	0.3 (0.65)	0.6 (1.68)
Median (range)	0.0 (0; 8)	0.0 (0; 2)	0.0 (0; 8)
Total nights in hospital in previous year, n			
Mean (SD)	15.7 (31.54)	14.0 (9.90)	15.3 (26.93)
Median (range)	3.0 (1; 80)	14.0 (7; 21)	4.0 (1; 80)
Emergency room visits in previous year, n			
Mean (SD)	0.9 (1.42)	0.3 (0.65)	0.6 (1.23)
Median (range)	0.0 (0; 5)	0.0 (0; 2)	0.0 (0; 5)
Physician visits for urgent treatment in previous year, n			
Mean (SD)	3.9 (2.94)	2.7 (1.42)	3.5 (2.57)
Median (range)	3.0 (0; 10)	2.5 (0; 5)	3.0 (0; 10)
Absenteeism from school or work in previous year (days)			
Mean (SD)	49.4 (119.40)	0.6 (1.77)	33.8 (100.22)
Median (range)	3.0 (0; 365)	0.0 (0; 5)	0.0 (0; 365)
Hospitalizations in intensive care unit in previous year — yes, n (%)	2 (10)	None	2 (6.5)
Intubations/assisted ventilations in previous year — yes, n	None	None	None

^a Data obtained at visit 2 (baseline), all other data obtained at visit 1 (screening).^b That were to be maintained over study period.^c Does not included one subject who received hydrocortisone to prevent adrenal insufficiency that was not considered to be for asthma treatment.^d Beclometasone dipropionate (BDP) equivalent: 1 μ g BDP = 1 μ g budesonide propionate = 0.5 μ g fluticasone propionate.

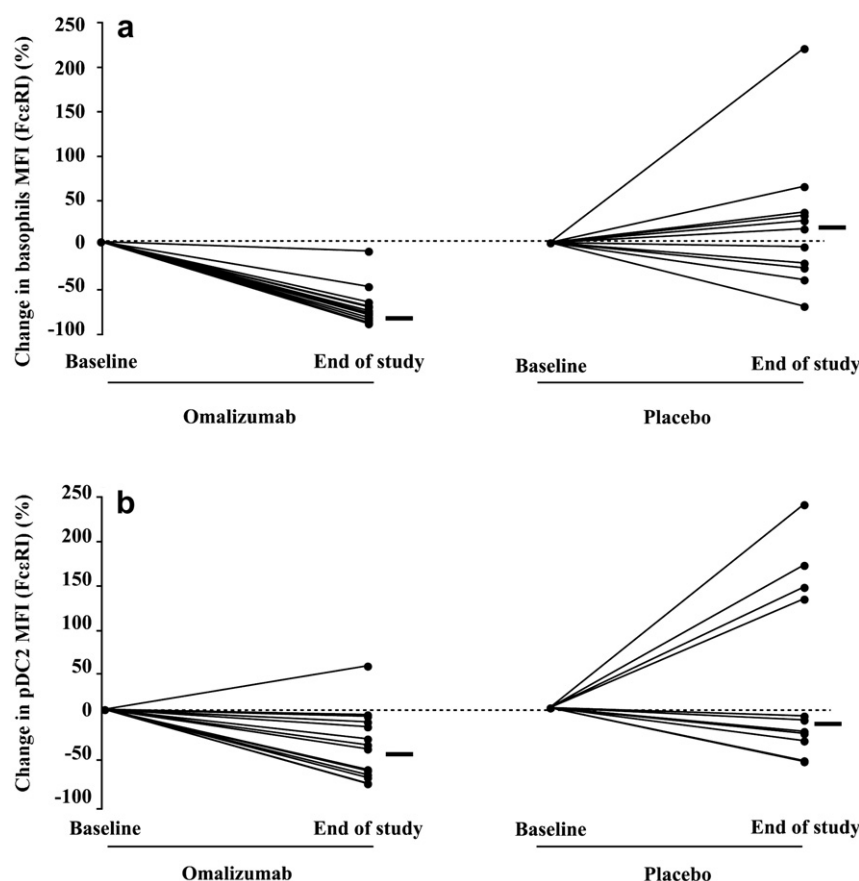


Figure 2 Change in FcεRI expression on basophils and plasmacytoid dendritic cells (pDC2) at the end of study relative to baseline. The change in FcεRI expression (mean fluorescence intensity, MFI) on (a) basophils and (b) pDC2 for individual patients in the omalizumab and placebo groups are shown. Median values are indicated by a solid bar to the right of each plot. Difference between median change found in omalizumab and placebo groups was statistically significant for (a) basophils $p < 0.01$ and (b) pDC2 $p = 0.029$.

group to allow better estimation of efficacy and safety. All patients receiving one dose of study medication with at least one post-dose efficacy assessment were included in the intent-to-treat (ITT) population. All patients with no major protocol deviation were included in the per-protocol population. The reference population for efficacy analysis was the ITT population. The patients with missing pre- and/or post-treatment assessment(s) of basophil FcεRI expression were excluded from the ITT and per-protocol populations analyzable for the primary endpoint. Adverse events were recorded for all patients receiving at least one dose of study medication with at least one post-dose safety assessment.

For the primary efficacy variable, change from baseline (week 0 of treatment phase) was described by the difference between baseline value, defined for each patient as the value observed at screening or at week 0 before the first administration of study drug, and the value observed at week 16, expressed as the % of baseline. Owing to the method of measurement, statistical analyses were not performed on absolute values. The treatment groups were compared using the Wilcoxon non-parametric test.

Secondary efficacy variables including the comparison between treatment groups of the change from baseline

(month between screening visit and treatment phase) to the last month of treatment in asthma symptoms, asthma control and FEV₁ were analyzed by the Wilcoxon test. Correlation between asthma symptoms and FcεRI expression (change from baseline) was analyzed using Spearman rank correlation coefficients. However, the study was not powered to detect a difference in secondary efficacy variables between omalizumab and placebo groups. Analyses used two-sided tests with a 0.05 significance level.

Results

Demographic and baseline characteristics

Forty patients were screened, 31 were randomized (omalizumab $n = 20$, placebo $n = 11$), and 25 (80.6%) completed the study. Three patients discontinued in each treatment group (15.0% in the omalizumab and 27.3% in the placebo group). Analysis populations were intent-to-treat (ITT, $n = 31$) and those with initial and week 16 FcεRI data were included in the ITT FcεRI analyzable ($n = 27$, Fig. 1).

All patients were treated with high dose ICS in combination with a LABA, 22.6% ($n = 7$) were also receiving oral

Table 2 Change from baseline FcεRI expression (mean fluorescence intensity, MFI) and proportion (%) of basophils and dendritic cells expressing FcεRI after 4, 8, 12 and 16 weeks of treatment (patients with repeated measurements of the ITT FcεRI analyzable population).

	Omalizumab N = 8	Placebo N = 4	p-value ^a
FcεRI expression (MFI), % change from baseline, Median (range)			
<i>Basophils</i>			
Week 4	−87.0 (−92; −73)	−37.7 (−96; −12)	0.219
Week 8	−81.6 (−94; −67)	−10.2 (−50; 25)	0.008
Week 12	−87.9 (−95; −74)	−31.0 (−89; 54)	0.107
Week 16	−90.0 (−94; −82)	10.2 (−23; 219)	0.011
<i>Dendritic cells</i>			
Week 4	−61.0 (−81; 37)	−37.8 (−61; −9)	0.637
Week 8	−35.9 (−72; 36)	−4.9 (−64; 25)	0.799
Week 12	−52.2 (−81; 103)	−21.5 (−70; 57)	0.445
Week 16	−53.9 (−83; −6)	45.9 (−37; 133)	0.108
% of cells expressing FcεRI, change from baseline, Median (range)			
<i>Basophils</i>			
Week 4	−1.8 (−4; 19)	−1.2 (−18; −1)	0.777
Week 8	−1.0 (−4; 23)	1.3 (−1; 2)	0.669
Week 12	−2.6 (−24; 50)	−1.3 (−24; 1)	0.799
Week 16	1.0 (−3; 21)	−0.8 (−6; 2)	0.395
<i>Dendritic cells</i>			
Week 4	−3.1 (78; 25)	−3.2 (−27; 15)	0.637
Week 8	−0.8 (−22; 25)	5.8 (−2; 15)	0.203
Week 12	−7.0 (−49; 11)	1.6 (−41; 26)	0.671
Week 16	−11.2 (−24; 9)	17.6 (0; 57)	0.018

^a Wilcoxon test on change from baseline (post-hoc).

corticosteroids. In the year preceding the study, the mean (SD) number of asthma exacerbations requiring systemic corticosteroids and visits for urgent treatment were 4.4 (3.15) and 3.5 (2.57) respectively (Table 1).

Primary efficacy assessments

In the ITT-analyzable population and at end of study (week 16) with regard to baseline (omalizumab $n = 16$, placebo $n = 11$), the median change in FcεRI expression (mean fluorescence intensity, MFI) on basophils significantly decreased in the omalizumab group (−82.6%, range −94; −11%) compared to the placebo group (15.6%, range −71; 219%; $p < 0.01$) and decrease was found for all patients. All-but-one (93.8%) of the patients treated with omalizumab exhibited a reduction of FcεRI expression $\geq 50\%$, whereas all-but-one (90.9%) of the patients treated with placebo exhibited a reduction of $< 50\%$, no change or increase (Fig. 2a). The median proportion of basophils expressing FcεRI also decreased in the patients of the omalizumab group (−0.9%, range −13; 21%) compared to those of the placebo group (1.0%, range −6; 15%), but this was not found to be statistically significant ($p = 0.199$).

In the ITT-analyzable population with data regarding pDC2 (omalizumab $n = 15$, placebo $n = 11$), the median change in the level of FcεRI expression (MFI) was also significantly decreased in the omalizumab group (−44.2%, range −83; 49) compared to the placebo group (−13.6%, range −60; 225%; $p = 0.029$). The proportion of patients

exhibiting a reduction of FcεRI $\geq 50\%$ was 43.8% ($n = 7$) in the omalizumab group and 18.2% ($n = 2$) in the placebo group (Fig. 2b). The median proportion of pDC2 expressing FcεRI significantly decreased in the omalizumab group (−11.2, range −50; 68) compared to the placebo group (6.4%, range −11; 57%; $p = 0.02$).

In the omalizumab group, 7 patients (43.8%) exhibited a reduction $\geq 50\%$ in FcεRI expression on both cell types (basophils and pDC2), 8 (50%) on basophils only and none on pDC2 only. Thus, all patients exhibiting such a reduction on dendritic cells also exhibited this on basophils. Reduction in the proportion of cells expressing FcεRI was found on both cell types in 6 patients (37.5%), on basophils only in 2 patients (12.5%) and on pDC2 only in 3 patients (18.8%). In the placebo group, reduction $\geq 50\%$ in FcεRI expression on both cell types was found in one patient (9.1%) and on pDC2 only in one patient (9.1%). Reduction in the proportion of cells expressing FcεRI was found on both cell types in one patient (9.1%), basophils only in 2 patients (18.2%) and pDC2 only in 2 patients (18.2%).

Secondary efficacy assessments

In the sub-group of the ITT-analyzable population with repeated FcεRI measurements (at weeks 4, 8, 12 and 16), reduction of FcεRI expression on basophils in the omalizumab group was present as early as week 4 and statistically significant compared to the placebo group at weeks 8 and 16 (post-hoc analysis). There was no significant effect on

Table 3 Baseline values and changes from baseline at end of study in data obtained from patient diary card (ITT population).

	Omalizumab <i>N</i> = 20	Placebo <i>N</i> = 11	P-value ^a
Number of days with asthma symptoms per week			
Baseline value ^b , median (range)	7 (2; 7)	4.5 (1; 7)	0.140
Change from baseline at end of study ^c , median (range)	−1.4 (−7; 3)	0.0 (−4; 2)	
Number of puffs of rescue SABA per week			
Baseline value ^b , median (range)	6.5 (0; 87)	3.8 (0; 45)	0.477
Change from baseline at end of study ^c , median (range)	1.0 (−45; 17)	0.0 (−22; 4)	
Number of nights with awakening per week			
Baseline value ^b , median (range)	2.1 (0; 7)	1.0 (0; 7)	0.405
Change from baseline at end of study ^c , median (range)	−0.6 (−6; 1)	−0.3 (−4; 2)	
Number of days with impairment in daily activities per week			
Baseline value ^b , median (range)	5.7 (0; 7)	4.0 (1; 7)	0.740
Change from baseline at end of study ^c , median (range)	−0.4 (−7; 2)	−0.3 (−7; 2)	
Number of days with absence from work or school per period			
Baseline value ^b , median (range)	0.0 (0; 28)	0.0 (0; 28)	0.083
Change from baseline at end of study ^c , median (range)	0.0 (−10; 0)	0.0 (0; 1)	
Number of days with hospitalization per period			
Baseline ^b	None	None	NA
End of study ^c	None	None	
Number of unscheduled visits per period			
Baseline value ^b , median (range)	0.0 (0; 2)	0.0 (0; 1)	0.402
Change from baseline at end of study ^c , median (range)	0.0 (−2; 1)	0.0 (−1; 1)	
Morning daily PEF			
Baseline value ^b , median (range)	294.7 (125; 579)	311.0 (219; 482)	0.654
Change from baseline at end of study ^c , median (range)	−0.3 (−79; 166)	14.2 (−51; 55)	

^a Wilcoxon rank-sum test on change from baseline.^b Baseline: month between screening visit and treatment phase.^c End of study: month before last non-missing post-dose assessment.

the proportion of basophils expressing FcεRI, no early effect on the expression of FcεRI on pDC2, nor on the proportion of such cells expressing FcεRI (Table 2).

In the ITT population, the change in asthma symptoms from baseline to end of study (last non-missing post-dose assessment) found better improvement in the omalizumab group compared to the placebo group for all symptoms explored except use of rescue SABA and morning PEF, but

no difference was found to be statistically significant (Table 3). No correlation was found between asthma symptoms and change from baseline at end of study of FcεRI expression on basophils and pDC2: Spearman correlation coefficients ranged from 0.021 to 0.28, and *p*-values from 0.91 to 0.16.

At the end of study, 5 patients in the omalizumab group (25%) and one in the placebo group (9.1%) had an

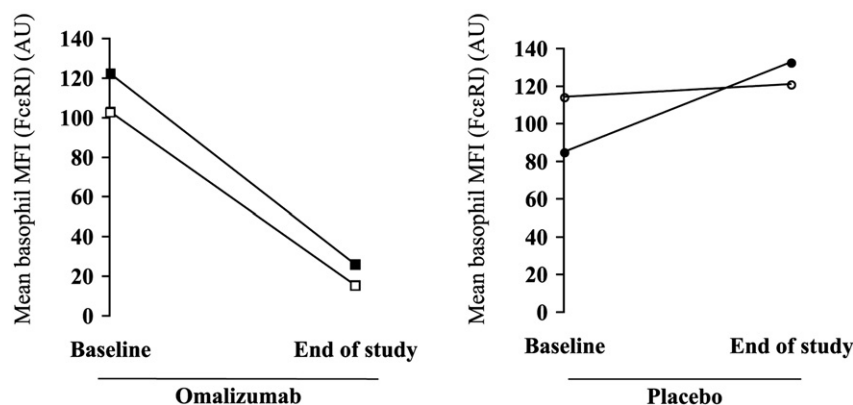


Figure 3 FcεRI expression (MFI) on basophils in response to treatment in physician evaluated responders and non-responders (ITT FcεRI analyzable population). Filled squares: omalizumab responders (*n* = 6), empty squares: omalizumab non-responders (*n* = 9), filled circles: placebo responders (*n* = 3), empty circles: non-responders (*n* = 6).

Table 4 Adverse events (ITT population).

	Omalizumab <i>N</i> = 20 <i>n</i> (%)	Placebo <i>N</i> = 11 <i>n</i> (%)
Total number of patients with adverse events, <i>n</i> (%)	11 (55)	7 (63.6)
Number of serious adverse events	0	1 (9.1)
Primary system organ class affected, <i>n</i> (%)		
Infections and infestations	8 (40)	4 (36.4)
Gastrointestinal disorders	5 (25)	1 (9.1)
Nervous system disorders	3 (15)	1 (9.1)
General disorders and administration site conditions	3 (15)	0
Musculoskeletal and connective tissue disorders	2 (10)	1 (9.1)
Eye disorders	1 (5)	1 (9.1)
Skin and subcutaneous tissue disorders	0	1 (9.1)
Preferred term		
Headache	3 (15)	1 (9.1)
Rhinitis	3 (15)	1 (9.1)
Gastroesophageal reflux disease	2 (10)	1 (9.1)
Back pain	2 (10)	0
Chills	2 (10)	0
Fatigue	2 (10)	0
Nausea	2 (10)	0
Viral infection	2 (10)	0
Bronchitis	1 (5)	1 (9.1)
Conjunctivitis	1 (5)	1 (9.1)
Abdominal pain upper	1 (5)	0
Acute sinusitis	1 (5)	0
Influenza	1 (5)	0
Myalgia	1 (5)	0
Sinusitis	1 (5)	0
Tonsillitis	1 (5)	0
Vomiting	1 (5)	0
Arthralgia	0	1 (9.1)
Dizziness	0	1 (9.1)
Nasopharyngitis	0	1 (9.1)
Oral fungal infection	0	1 (9.1)
Urticaria	0	1 (9.1)
Other clinically significant adverse events, <i>n</i> (%)		
Exacerbation-related serious adverse events	0	1 (9.1)
Discontinued due to asthma exacerbation	1 (5)	2 (18.2) ^a

Preferred terms are ordered by descending frequency in omalizumab column. A subject with multiple occurrences of an AE under one treatment was counted only once in the AE category for that treatment.

^a One was qualified as serious adverse event.

acceptable/optimal control of asthma (defined by an ANAES score of 0), none had such control at baseline. The median (range) change from baseline values of FEV₁ at the end of study (last non-missing post-dose assessment) in the ITT population was 2.6% (−10; 60) in the omalizumab group and 1.7% (−19; 7) in the placebo group, but this difference was not statistically significant ($p = 0.312$). The proportion of patients with no exacerbation was

respectively 45% and 63.6% in the omalizumab and placebo groups.

In the ITT population with available data for physician's overall assessment of treatment effectiveness at week 16 (omalizumab group $n = 17$, placebo $n = 9$), the proportion of responders (response: excellent/good) was 47% ($n = 8$) in the omalizumab group and 33.3% ($n = 3$) in the placebo. Post-hoc analysis in the population with both data sets

($n = 24$) found that omalizumab reduced FcεRI expression in both responders and non-responders (Fig. 3).

Adverse events

Adverse events concerned 11 (55%) of the omalizumab group and 7 (63.6%) of the placebo group, 1 subject of the omalizumab group and 2 of the placebo group discontinued due to asthma exacerbation. One subject of the placebo group experienced an exacerbation-related serious adverse event (Table 4).

Discussion

This double-blind, placebo-controlled study showed that at 16 weeks from initiation of omalizumab treatment FcεRI expression on basophils was significantly reduced in patients with severe uncontrolled asthma. To a lesser extent, but still significantly with respect to placebo, this was also found on plasmacytoid dendritic cells (pDC2²³). Reduced FcεRI expression was found on basophils in all patients and on pDC2 in all-but-one patient, yet there was no correlation between changes in FcεRI expression and clinical response.

MacGlashan et al. report substantial reduction of FcεRI expression on basophils ($\approx 97\%$), but comparison is difficult owing to the higher omalizumab dosing regimen than that used in the current study.⁸ More recent studies did however use the currently recommended dosing regimen (0.016 mg/kg/IU[IgE]/mL per month). In studies of 6 weeks in duration, Lin et al. report a 73% median reduction of FcεRI expression on basophils⁷ and Prussin et al., a 75% median reduction on pDC2.⁹ Both studies found a correlation of FcεRI expression with levels of free serum IgE. These results seem to suggest that the effect of omalizumab was similar on both cell types, however, Prussin et al. also investigated basophils in parallel experiments and found that the magnitude of FcεRI reduction was greater in these than in the dendritic cells tested. Further analysis of data presented by the authors finds that, as in the current study, all patients exhibiting a $\geq 50\%$ reduction of FcεRI expression on pDC2 (and pDC1) also exhibited such a reduction on basophils but not the converse.⁹ Reduction of FcεRI expression on basophils and dendritic cells is reported to be rapid and to reach maximal value within one month of treatment^{7–9} (but this may not be the case for mast cells²⁵). The kinetics of FcεRI expression reduction reported herein for a sub-group of patients are concordant with these results; remaining relatively stable for both basophils and pDC2 cells over the course of the study following an initial reduction within the first 4 weeks of treatment. It is yet to be determined whether increased treatment duration (>16 weeks) would further reduce FcεRI expression or alter the differential effect found between cell types.

The overall incidence of adverse events was slightly higher in the placebo group. All adverse events were as expected for this patient population and pharmacological class. No serious adverse events were reported in the omalizumab group. With respect to the placebo group, in the omalizumab group control was attained by more patients and clinical markers were found to improve. The

latter was not found to be statistically significant at the end of the study, which was not powered to detect clinical difference. Change in asthma symptoms were not correlated with reduction of FcεRI expression, despite this being significant and widespread on both basophils and pDC2 cells. Interestingly, the INNOVATE study found a similarly widespread effect on free IgE levels in the omalizumab group and furthermore this concerned both drug responders (99%) and non-responders (98%).¹⁰ Using the same measure of response (physician's overall assessment, validated using INNOVATE data²²) post-hoc analysis found a similar situation for FcεRI expression on basophils. This suggests the presence of a sub-population of patients clinically resistant to the biological effects of omalizumab,^{21,22} although the sample size limits somewhat the strength of such a conclusion. One caveat is that although omalizumab has been reported to modify lung tissue with respect to the mediators of allergic response in mild-to-moderate asthmatics²⁶ and mild-intermittent asthmatics,²⁷ only circulating cells were investigated here. Independently of such limitations, the widespread effect on FcεRI expression reported here does not support pursuit of investigation into its use as a marker of response.

Taken together, the results presented here along side those of the INNOVATE study, demonstrate that omalizumab treatment in this population with difficult-to-treat asthma is effective in reducing free IgE and induces a reduction of FcεRI expression on circulating basophils and pDC2 as previously proposed.²⁸ The finding that, as for free IgE levels, reduction of FcεRI expression was widespread in treated patients but that not all of these responded to treatment does not support further investigation of FcεRI as a predictive marker of response.

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Conflicts of interest

PC has received pharmaceutical company grant monies, has been a consultant to pharmaceutical companies, and has participated in speaking and advisory committee activities related to industry.

MH has been a consultant for pharmaceutical companies (Actelion, Novartis), and has participated in speaking and advisory committee activities related to industry (Actelion, Astrazeneca, Novartis, GSK, and Pfizer).

FDB has received university grant monies, has been a consultant for pharmaceutical companies (Stallergenes and ALK laboratories), and has participated in speaking and advisory committee activities related to industry (MSD, Novartis, Stallergenes and ALK laboratories).

GG has participated in speaking and advisory committee activities related to industry (GSK, MSD, Novartis and CHIESI).

AD has participated in speaking and advisory committee activities related to industry (MSD, Schering).

MM has been a consultant for a pharmaceutical company (Novartis), and has participated in speaking and advisory committee activities related to industry (Novartis).

IB, PT and VLG are employees of Novartis Pharma SAS.

CCB, CV, MTL, PB, JFM, PR: have no conflicts of interest to disclose.

References

1. Passalacqua G, Ciprandi G. Allergy and the lung. *Clin Exp Immunol* 2008;153(Suppl. 1):12–6.
2. Gaga M, Papageorgiou N, Yiougioti G, Karydi P, Liapikou A, Bitsakou H, et al. Risk factors and characteristics associated with severe and difficult to treat asthma phenotype: an analysis of the ENFUMOSA group of patients based on the ECRHS questionnaire. *Clin Exp Allergy* 2005;35:954–9.
3. Busse WW, Lemanske Jr RF. Asthma. *N Engl J Med* 2001;344:350–62.
4. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med* 2001;164:S1–5.
5. Chang TW. The pharmacological basis of anti-IgE therapy. *Nat Biotechnol* 2000;18:157–62.
6. Infuhr D, Cramer R, Lamers R, Achatz G. Molecular and cellular targets of anti-IgE antibodies. *Allergy* 2005;60:977–85.
7. Lin H, Boesel KM, Griffith DT, Prussin C, Foster B, Romero FA, et al. Omalizumab rapidly decreases nasal allergic response and FcεRI on basophils. *J Allergy Clin Immunol* 2004;113:297–302.
8. MacGlashan Jr DW, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc (ε)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438–45.
9. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcεRI expression. *J Allergy Clin Immunol* 2003;112:1147–54.
10. Slavin RG, Ferioli C, Tannenbaum SJ, Martin C, Blogg M, Lowe PJ. Asthma symptom re-emergence after omalizumab withdrawal correlates well with increasing IgE and decreasing pharmacokinetic concentrations. *J Allergy Clin Immunol* 2009;123:107–13. e3.
11. MacGlashan Jr D. IgE and FcεRI regulation. *Clin Rev Allergy Immunol* 2005;29:49–60.
12. Malveaux FJ, Conroy MC, Adkinson Jr NF, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest* 1978;62:176–81.
13. Foster B, Metcalfe DD, Prussin C. Human dendritic cell 1 and dendritic cell 2 subsets express FcεRI: correlation with serum IgE and allergic asthma. *J Allergy Clin Immunol* 2003;112:1132–8.
14. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention, <http://www.ginasthma.com>. Available from:.
15. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma European network for understanding mechanisms of severe asthma. *Eur Respir J* 2003;22:470–7.
16. Chanez P, Wenzel SE, Anderson GP, Anto JM, Bel EH, Boulet LP, et al. Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol* 2007;119:1337–48.
17. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, lung, and blood Institute's severe asthma Research Program. *J Allergy Clin Immunol* 2007;119:405–13.
18. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184–90.
19. Milgrom H, Fick Jr RB, Su JQ, Reimann JD, Bush RK, Watrous ML, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMab-E25 Study Group. *N Engl J Med* 1999;341:1966–73.
20. Soler M, Matz J, Townley R, Buhl R, O'Brien J, Fox H, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 2001;18:254–61.
21. Humbert M, Beasley R, Ayres J, Slavin R, Hebert J, Bousquet J, 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;60:309–16.
22. Bousquet J, Rabe K, Humbert M, Chung KF, Berger W, Fox H, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med* 2007;101:1483–92.
23. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2002;2:151–61.
24. EMEA Xolair® SmPC Available from, <http://www.emea.europa.eu/humandocs/PDFs/EPAR/Xolair/H-606-PI-en.pdf>.
25. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J Allergy Clin Immunol* 2004;114:527–30.
26. Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004;170:583–93.
27. van Rensen EL, Evertse CE, van Schadewijk WA, van Wijngaarden S, Ayre G, Mauad T, et al. Eosinophils in bronchial mucosa of asthmatics after allergen challenge: effect of anti-IgE treatment. *Allergy* 2009;64:72–80.
28. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005;115:459–65.