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Omalizumab stabilises lung function and reduces bronchial mucosal inflammation in non-atopic asthma

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ABSTRACT

Background

Control of severe, unstable asthma remains a challenge particularly in non-atopic patients who are currently denied anti-IgE therapy which is perceived as ineffective.

Objective

Utilise a randomised, double-blind, placebo controlled study to establish proof of principle that omalizumab can stabilise lung function while reducing bronchial mucosal inflammation in non-atopic asthmatics.

Methods

16 symptomatic, non-atopic asthmatics were destabilised following randomisation (1:1) to receive omalizumab or identical placebo treatment for 20 weeks. Lung function (FEV_1), asthma-related symptoms (Juniper ACQ, ACD) and quality of life (Juniper mini-AQLQ) were monitored. Inflammatory cells were enumerated in sections of bronchial mucosal biopsies collected before and after 16 weeks of treatment.

Results

Substantial, supervised reduction of regular therapy resulted in a decline in lung function in the placebo treated patients which was reversed in the omalizumab treated patients with highly significant differences in absolute (p=0.02) and %

predicted FEV_1 (p=0.009) with corresponding clinically, if not statistically significant improvements in asthma symptoms (ACQ) and quality of life (mini-AQLQ). Omalizumab, compared with placebo therapy was also associated with highly significant median percentage reductions in the numbers of bronchial mucosal total IgE^+ cells (p<0.001), mast cells (p<0.001) and plasma cells (p=0.005). IgE^+ mast cells were also reduced but not significantly. Mucosal B lymphocytes and eosinophils were not altered.

Conclusion

Omalizumab stabilises and improves lung function in non-atopic asthmatics, possibly at least partly by reducing bronchial mucosal inflammation and IgE expression. A policy of restricting therapy to asthmatics with the "endotype" of atopy as conventionally defined may exclude potential responders.

Clinical Implication

Omalizumab is currently denied to asthmatics categorised as "non-atopic" by conventional criteria. Our data suggest that the "endotype" of non-atopy is inappropriate for excluding potential responders.

Capsule Summary

Treatment of severe, unstable asthma ~~s~~ remains a challenge. In this randomized, double-blinded, placebo controlled proof of concept study, we addressed the effects of omalizumab on lung function and bronchial mucosal inflammation in non-atopic asthmatics.

Key words

Asthma; omalizumab; anti-IgE; non-atopic

Abbreviations

ACD	Asthma Control Diary
ACQ	Asthma Control Questionnaire
AQLQ	Asthma Quality of Life Questionnaire
CTA	Clinical Trial Authorisation
FEV1	Forced Expiratory Volume in the first second
FITC	Fluorescein isothiocyanate
GCP	Good Clinical Practice
NICE	National Institute of Health and Care Excellence
OCT	Optimal Cutting Temperature
PBS	Phosphate Buffered Saline
PFA	Paraformaldehyde
SIQR	Semi-Interquartile Range

INTRODUCTION

Asthma remains a leading cause of suffering affecting about 300 million people worldwide[1] and 25.7 million in the US[2]. In the UK, 10% of the 5.2 million sufferers are estimated to retain daily symptoms and remain vulnerable to acute exacerbations despite the regular and efficient delivery of conventional anti-asthma therapy, including systemic corticosteroid[3].

The humanised, monoclonal IgG_1 anti-IgE antibody omalizumab is the vanguard of what will hopefully form an arsenal of new biologicals able to improve the lives of severe asthma sufferers. That omalizumab therapy stabilises asthma control, reducing disease exacerbations, and consequently unplanned hospital admissions and exposure to systemic corticosteroid therapy in a substantial proportion of severe asthmatics is now acknowledged by professional and regulatory authorities worldwide, including the British Thoracic Society (BTS) and NICE[4] in the UK.

A major challenge when deploying treatment with biologicals for severe asthma is the possibility of mechanistic variation in the disease, requiring pre-identification of potential responders to any specific agent. This has generated intense interest in identifying "phenotypes" or "endotypes" of asthma. In the case of omalizumab, the *prima facie* effect of which is to prevent, and possibly reverse binding of IgE to its high- and low-affinity receptors[5, 6], the tacit assumption has been that it improves asthma stability fundamentally by

reducing or abolishing mast cell and basophil activation by cross-linking of surface-bound allergen-specific IgE by allergen in suitably sensitised, "atopic" patients. Consequently, key clinical trials investigating its efficacy, such the INNOVATE study[7] have been limited to atopic asthmatics while its marketing authorisation restricts its use to patients with "convincing IgE-mediated asthma". This phrase is not universally defined but is in practice usually equated with evidence of IgE sensitisation (by skin prick or *in vitro* testing) to one or more common perennial aeroallergens. Conversely, the therapy has been denied to at least, we estimate, 20,000 otherwise eligible non-atopic severe asthmatics in the UK, and many more worldwide (the prevalence of non-atopic, severe asthma was estimated at 50% of the total in the ENFUMOSA cross sectional study[8] and 17–34% in the SARP study)[9].

Much indirect evidence suggests that IgE may play a role in asthma regardless of conventional atopic status. Epidemiologically, asthma was 5 fold more prevalent in a cohort of non-atopic subjects with elevated total serum IgE[10]. We and others have shown that atopic and non-atopic asthma are virtually identical in terms of their bronchial mucosal cellular and molecular immunopathology[11-18], evidence of local B cell switching to IgE synthesis[19, 20], elevated local FcεRI receptor expression[21] (local IgE up regulates FcεRI on expressing cells) and, very recently, elevated total bronchial mucosal IgE concentrations[22]. Furthermore, there is ample evidence that IgE directed against antigens other than aeroallergens, such as viral antigens[23] and Staphylococcal

enterotoxins[24] which also act as superantigens, may play a role in asthma pathogenesis. IgE may influence the functions of mast cells by antigen-independent mechanisms[25, 26]. Finally, IgE may exacerbate asthmatic bronchial mucosal inflammation by mechanisms other than causing degranulation of mast cells and basophils, such as by enhancing antigen capture by antigen-presenting cells and activating monocyte/macrophages[27]. All of these data lend weight to the view that the presence or absence of atopy as operationally defined might not be an appropriate phenotypic or endotypic criterion for predicting responsiveness to omalizumab therapy.

To address this, we hypothesised that omalizumab therapy provides clinical benefit in chronic, severe asthmatics designated non-atopic by conventional criteria. Rather than embarking on a large, lengthy and costly clinical trial with frequency of exacerbations as a primary outcome measure, we elected in the first instance to provide proof of concept in a double-blind, placebo controlled study that omalizumab therapy can maintain or improve lung function in these patients despite provocation of the disease in the shorter term by supervised reduction of therapy. We also sampled their bronchial mucosa at fibreoptic bronchoscopy before and after therapy to address the hypothesis that omalizumab reduces local expression of IgE as well as the numbers of B cells, plasma cells and mast cells.

STUDY DESIGN, PATIENTS AND LABORATORY METHODS

Study protocol

This was a randomised, placebo-controlled, double-blind, parallel-group, proof of concept trial of 20 weeks' duration. Omalizumab and identical vehicle control manufactured to GCP standards were kindly supplied by the manufacturers (Novartis Pharmaceuticals). The trial was approved and monitored by Guy's Research Ethics Committee (REC Ref: 09/H0804/43) and the Medicines and Healthcare Products Regulatory Agency (CTA No: 14523/0219/001/0001) and registered on clinicaltrials.gov (reference NCT01113437). Eligible patients were moderate/severe, non-atopic asthmatics who provided written, informed consent recruited from the asthma clinics at Guy's and St. Thomas', the Royal Brompton and the Homerton University Hospitals in London, the departmental database or through advertisement.

Asthma was defined as a history of relevant symptoms and documented (i) ≥ 12% reversibility of FEV_1 in response to inhaled bronchodilator and/or (ii) ≥ 8% variability of the peak expiratory flow (PEF) during a 24 hour period or ≥ 20% variability over a period of 1-2 weeks. Moderate/severe asthma was defined as regular (at least 3 days per week) day- and night-time symptoms in the 3 months prior to screening despite regular step 3-5 asthma treatment according to the BTS guidelines[28]. Non-atopic was defined as negative skin prick and/or *in vitro* IgE tests (Phadia ImmunoCAP® Grade 0 or ≤ 0.35 kU/L)

to the following local UK aeroallergens: mixed grass, mixed tree, mixed mould, cat, dog and house dust mite. The non-atopic status of these participants was further confirmed by full ISAC (Phadia) screening of their sera and bronchial biopsy homogenates (data presented elsewhere) [22]. Exclusion criteria are listed in the online repository section.

The phases of the study protocol are summarised in Figure I:

Screening/baseline: After screening, during a baseline period of up to 4 weeks patients were given and instructed to use, if necessary, a portable peak flow meter (Mini-Wright Standard EU Scale, SKU: 3103387, Clement Clarke International Limited) and blank diary forms (Juniper Asthma Control Diary[29]) in which they documented daily morning and evening peak expiratory flow (PEF) and day and night symptoms (on a 0-6 scale) until the end of the study. Existing anti-asthma medication was not changed at this stage but compliance encouraged.

First bronchoscopy and commencement of therapy: At a second visit patients completed a Juniper Asthma Control Questionnaire[29] (ACQ) and mini-Asthma Quality of Life Questionnaire[30] (mini-AQLQ), then underwent pre-bronchodilator spirometry (Minispir® PC based Spirometer, Winspiro Pro version 4.1.5 software) prior to the obtaining of 10 technically suitable bronchial mucosal biopsies from the right or left second or third generation bronchi at fibreoptic bronchoscopy using an Olympus bronchoscope model BF

XT40 OES. Patients then received their first subcutaneous injection of the trial medication (omalizumab or identical placebo, allocated by the hospital pharmacy using randomisation tables with the patient and attending physician blinded), the dosage and frequency of which (either 2 or 4 weekly) were determined as in standard clinical practice based on their initial body weight and serum total IgE concentration as described in the Omalizumab SmPC. Where serum total IgE was below the lowest concentration in the SmPC dosing table we administered the lowest dosage in the table (75 mg every four weeks). Patients were observed for 2 hours afterwards. At each subsequent dosing visit, patients were examined clinically, encouraged to comply with their usual medication and their diary cards collected and renewed.

Second bronchoscopy: Within a 2 week window between 12 and 14 weeks after commencement of omalizumab/placebo therapy (Time a, Figure I), lung function was re-measured and repeat bronchial biopsies obtained as before.

Therapy reduction phase: Patients were instructed carefully how to use a Turbohaler® device and asked, commencing the day following the second bronchoscopy, to discontinue all existing inhaled and oral anti-leukotriene or theophylline based anti-asthma medications and substitute them with regular budesonide/formoterol combination therapy (Symbicort® 100/6 Turbohaler 2 puffs twice daily initially for 4 weeks and further reduced to 1 puff twice daily until the end of the trial) with additional terbutaline (Bricanyl® Turbohaler 500 μg/puff) as required for immediate relief of symptoms. For patients taking

regular additional oral prednisolone, an attempt was also made progressively to reduce the dosage according to a predetermined regimen depending on the dosage at entry to the study (see Table E I in the online repository). Omalizumab/placebo therapy was continued for a total of 20 weeks while this new therapeutic regimen was pursued.

End of the study (Time B, Figure I): At their penultimate visit, 20 weeks from commencement of omalizumab/placebo therapy, patients completed final ACQ and mini-AQLQ questionnaires then underwent repeat spirometry before being asked to resume their original anti-asthma therapy. A final visit was arranged 2 weeks later to check the patients' wellbeing and enquire about any adverse reactions.

At any time during the study, in the event of an asthma exacerbation, defined as a need for rescue oral corticosteroid medication for deterioration of symptoms and/or lung function, as agreed between the patient and the study physician, patients were treated with a 10 day course of prednisolone 30 mg/day instituted by the study physician. Such patients left the study, resumed their regular anti-asthma medication and were followed up as necessary.

Immunofluorescence

Bronchial biopsies were processed and analysed using double immunofluorescence, single immunohistochemistry and confocal microscopy

where appropriate according to an established protocol described in the online repository section of this manuscript.

Outcome variables

The primary outcome variables were changes in absolute and percentage predicted pre-bronchodilator FEV_1 measured at baseline and the end of the 20 week treatment period. Exploratory clinical outcome variables were changes in morning peak expiratory flow, symptom scores from asthma control diaries (ACD) and asthma quality of life scores (Juniper mini-AQLQ). Laboratory outcome variables were percentage changes in tryptase$^+$ mast cells, CD138$^+$ plasma cells, CD20$^+$ B lymphocytes, cells of these phenotypes co-expressing IgE and total IgE$^+$ cells per unit area of the bronchial mucosal sections.

Statistical analysis

Baseline characteristics and demographic data were summarised using descriptive statistics. Changes in numerical variables at the beginning and end of the study as well as differences in changes between the omalizumab and placebo treated groups were analysed by non-parametric statistics (Mann-Whitney U test). All tests were two sided and $p<0.05$ was accepted as significant. The statistical software package used was GraphPad Prism version 5.

RESULTS

Patients

Of 30 patients screened, 18 were randomised (1:1) and 16 completed the study. The patients randomised to omalizumab or placebo therapy were well matched in terms of distributions of age, sex, body mass index, serum total IgE concentrations, smoking history, lung function, asthma symptom scores and inhaled corticosteroid dosages (Table I). These dosages were reduced in both groups to 400 μg/day beclometasone equivalent between 12 and 16 weeks and further to 200 μg/day between 16 and 20 weeks. In the omalizumab *vs.* placebo treated groups, 7 *vs.* 8 patients were taking long-acting β2-agonist, 4 *vs.* 1 were taking oral leukotriene receptor antagonist and 2 *vs.* 2 were taking oral theophylline preparations. These medications were all stopped or substituted at Time A (Figure 1). One patient randomised to omalizumab and 3 randomised to placebo were taking oral prednisolone at dosages of 15 mg/day and 15, 10 and 5 mg/day respectively which were reduced to 7.5 mg/day and 7.5, 5 and 0 mg/day respectively according to the predetermined regimen (see Table E I in the online repository section).

Adverse events and withdrawals

Two patients randomised to omalizumab therapy withdrew from the study prematurely, one following an asthma exacerbation (an expected adverse

event) at week 5 and another who elected to withdraw after 16 weeks because of subjective deterioration of symptoms not confirmed by spirometry. There were no other adverse events.

Primary outcome measure (FEV_1)

Spirometry was performed at baseline and at Times A and B (Figure I). Compared with baseline, the median changes in absolute and % predicted FEV_1 by 20 weeks were positive in the omalizumab treated patients, despite substantial reduction of existing anti-asthma treatment, but negative in the placebo treated patients (median (SIQR) change 0.325 (0.09,0.66) *vs*. -0.06 (-0.14,0.27) litre, p=0.02; 12(3,18.75) *vs.* -2.0 (-10.5,15.0) % predicted, p=0.009: see Figure II).

Exploratory variables

As shown in Table I, the median PEF and ACQ scores between baseline and Time B improved by what is regarded as a clinically significant degree in the patients randomised to omalizumab therapy but not placebo, although the differences between the groups did not quite attain statistical significance in non-parametric testing. In contrast, ACD and mini-AQLQ scores improved to a similar extent in both groups despite reduction of conventional therapy.

Markers of airway inflammation

Median numbers of tryptase$^+$ mast cells, CD20$^+$ B cells, CD138$^+$ plasma cells, total IgE$^+$ cells, IgE$^+$/tryptase$^+$ mast cells, IgE$^+$/CD20$^+$ B cells and IgE$^+$/CD138$^+$ plasma cells per unit area of the bronchial biopsy sections just prior to commencement of omalizumab or placebo therapy are shown in Table II. Percentage changes in these variables between baseline and Time A (Figure I) are shown in Table II and Figure III. Omalizumab, compared with placebo therapy was associated with significant reduction in the numbers of total IgE$^+$ cells ($p<0.001$), tryptase$^+$ mast cells ($p<0.001$) and CD138$^+$ plasma cells ($p=0.005$). Tryptase$^+$/IgE$^+$ mast cells were also reduced with omalizumab treatment but the difference between the groups did not attain statistical significance. No significant difference was observed in changes in mucosal CD20$^+$ B lymphocyte numbers and BMK-13$^+$ eosinophils in the two groups. Very few of the B cells and plasma cells showed detectable IgE immunoreactivity as expected (Table II), so it was impracticable to evaluate changes following omalizumab therapy.

DISCUSSION

In this study, treatment of symptomatic, moderate/severe non-atopic asthmatics with omalizumab stabilised and indeed improved lung function in the face of substantial reduction of existing therapy which resulted in (predictable) deterioration of patients treated with placebo. All patients treated with omalizumab improved their FEV_1 under these circumstances within a time period used to gauge the outcome of omalizumab therapy in routine clinical practise. We contend that this provides proof of concept that omalizumab therapy can stabilise asthma and possibly improve lung function, reduce disease exacerbations –and spare anti-inflammatory therapy in non-atopic asthma, outcomes which are congruent with similar findings in atopic asthmatics such as in the landmark INNOVATE study.[7] Unlike that study, however, the present study was neither designed nor powered to detect reductions in exacerbations and improvements in quality of life; nevertheless we observed some encouraging, if non-significant improvements in some of these outcome measures, notably the ACQ score in the patients treated with omalizumab. This perhaps reflects the fact that ACQ is more reflective of short term asthma stability, whereas mini–AQLQ, which improved in both the active and placebo treated groups, is more influenced by tight asthma monitoring and reassurance by the study investigator. Our data are also congruent with a previous, placebo controlled 16 week study[31] designed primarily to examine the effects of

omalizumab on the expression of the high-affinity IgE receptor FcεRI on blood leukocyte subsets in non-atopic asthmatics which also showed an improvement in FEV_1 in patients treated with omalizumab (ongoing therapy was not altered).

In defining "non-atopic" for the purposes of this study we are confident that we have exceeded the rigour of the definition used in standard clinical practice. While it might be argued that it is theoretically impossible to exclude the presence of IgE responses to obscure aeroallergens in any individual, and while indeed non-conventional "allergens" such as Staphylococcal enterotoxins have been implicated in asthma pathogenesis as aforementioned, our argument that omalizumab may benefit non-atopic patients *as conventionally clinically defined* remains sound.

We also demonstrate for the first time the anti-inflammatory effects of omalizumab therapy within the mucosa of the target organ in non-atopic asthmatics. Having recently shown that IgE is increased in the bronchial mucosa of non-atopic, as well as atopic asthmatics[22], we here additionally show that omalizumab therapy substantially reduced the numbers of cells expressing IgE immunoreactivity (most of these cells were likely mast cells). In a subset of these patients omalizumab, but not placebo therapy also invariably reduced total IgE concentrations in biopsy tissue extracts (data not shown). We speculate that this reflects dissociation of IgE from its receptors following blockade of receptor binding, which is followed by down-regulation of –FcεRI on target

cells such as mast cells and basophils[32-34]: this, as aforementioned, is understood to be *prima facie* the mechanism by which omalizumab exerts its anti-asthma effect. –Interestingly, omalizumab therapy also reduced the numbers of bronchial mucosal mast cells and plasma cells. We speculate that this is because IgE influences mast cell survival and activation to produce cytokines required for B cell proliferation, switching to IgE synthesis and plasma cell differentiation[35]. Omalizumab does not induce apoptosis of B cells, although it has been postulated that it inhibits IgE synthesis[36, 37], consistent with our data. One other study addressing the effects of omalizumab on bronchial mucosal inflammation[38] also showed that treatment of a group of mild, stable atopic asthmatics with omalizumab for 16 weeks was associated with reduced numbers of bronchial mucosal FcεRI$^+$ cells and IgE$^+$ cells compared with placebo. We speculate that, by mechanisms yet to be fully defined, these IgE-dependent phenomena can effect destabilisation of asthma in a subset of patients regardless of their conventional atopic status. We further speculate that other mediators, for example eosinophil products, may be responsible for destabilisation of asthma in other subsets of patients. Thus, when defining endotypes of disease in order to identify potential responders to new anti-asthma biologicals it might be as fruitful to identify endotypes of disease *exacerbation* as it is to identify endotypes of stable disease.

~~It is of interest that the clinical effects of omalizumab in this study were observed in the absence of changes in the numbers of bronchial mucosal eosinophils. Like omalizumab, anti-eosinophil biologicals such as mepolizumab also stabilise asthma in subsets of patients, but do not necessarily normalise lung function. We speculate that there may exist a variety of mechanisms for inflammatory destabilisation of asthma, including IgE-dependent mechanisms, eosinophil-dependent mechanisms and possibly others. Thus the only other study of which we are aware addressing the effects of omalizumab on bronchial inflammation in mild, atopic asthmatics[38] found that therapy with omalizumab was associated with reductions in bronchial mucosal eosinophils, T cells, FcεRI^{+} cells and IgE^{+} cells, a small but significant reduction in B cells and a trend for reduction in mast cells. These were patients with stable disease, in whom lung function (methacholine PC_{20}) was not altered. Clearly these questions merit further contemplation and research.~~

In summary, the present data support our hypothesis that omalizumab has the potential to stabilise asthma and reduce bronchial mucosal inflammation in asthma regardless of atopic status as conventionally defined, with the corollary that restricting treatment to patients with the "endotype" of atopy may miss potential responders.

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Characteristics & Outcomes	Randomisation	Baseline	Time A	Time B
Age (yr)	Omalizumab	47 (22, 66)		
	Placebo	53.50 (25, 59)		
Sex (M/F)	Omalizumab	5/3		
	Placebo	3/5		
Weight (Kg)	Omalizumab	84 (55, 133)		
	Placebo	87.50 (61, 112)		
Height (cm)	Omalizumab	172 (155, 178)		
	Placebo	166 (152, 177)		
Never smokers (n/8)	Omalizumab	3		
	Placebo	4		
Serum total IgE (IU/ml)	Omalizumab	75 (8, 264)		
	Placebo	49 (2, 284)		
FEV_1 (L)	Omalizumab	2.44 (1.81, 3.43)	0.225 (-0.32, 1.24)	0.325 (0.03, 1.16)*
	Placebo	2.34 (1.16, 3.66)	0.005 (-0.30, 0.45)	-0.06 (-0.63, 0.39)
FEV1 % predicted	Omalizumab	75 (50, 98)	6 (-13, 36)	12 (1, 29)**
	Placebo	80 (47, 114)	-4 (-11, 14)	-2 (-23, 15)
PEF (L/min)	Omalizumab	364 (233, 480)	10.50 (-3, 55)	17.50 (-34, 104)
	Placebo	308 (174, 455)	8.50 (-41.00, 92.00)	4 (-83, 111)
ACD score	Omalizumab	1.75 (0.86, 2.84)	0.00 (-1.15, 0.45)	-0.28 (-1.21, 0.65)
	Placebo	1.97 (0.39, 2.80)	-0.26 (-1.45, 1.10)	-0.24 (-1.52, 2.00)
ACQ score	Omalizumab	2.28 (1.43, 3.43)	-0.50 (-1.85, 0.72)	-0.71 (-1.14, 0.14)
	Placebo	2.42 (0.71, 3.28)	-0.35 (-1.00, 0.57)	-0.28 (1.71, 1.14)
AQLQ score	Omalizumab	4.33 (2.53, 5.27)	0.18 (-1.00, 2.26)	0.46 (-0.40, 2.06)
	Placebo	4.60 (3.73, 5.80)	0.37 (-1.34, 1.87)	0.67 (-1.60, 2.30)
Inhaled corticosteroid dosage (BDP equivalent: µg/day)	Omalizumab	2000 (800, 4000)	No change from baseline	Reduced to 200 µg BDP equivalent/day
	Placebo	1800 (500, 2000)		

Table I

Table I

Baseline demographics and clinical characteristics of non-atopic asthmatics randomised to omalizumab or placebo therapy and absolute changes from Baseline at Times A and B (see Figure 1). All variables are shown as the median and range. ACD: Juniper Asthma Control Diary. ACQ: Juniper Asthma Control Questionnaire. AQLQ: Juniper mini-Asthma Quality of Life Questionnaire. *p = 0.02, **p = 0.009 (Mann-Whitney U Test).

Table II

Cell Type	Treatment Group	Absolute numbers/mm^2 at Baseline	% change between Baseline and Time A
Mast Cell	Omalizumab	7.2 (0.0, 124.8)	-80 (-100, -37)***
	Placebo	29.9 (4.3, 75.6)	22 (-37, 183)
B Cell	Omalizumab	1.5 (0.00, 9.7)	0 (-100, 460)
	Placebo	2.1 (0.3, 9.4)	-11 (-100, 82)
Plasma Cell	Omalizumab	0.0 (0.0, 36.6)	-75 (-100, -13)**
	Placebo	1.7 (0.0, 6.8)	32 (-45, 315)
IgE$^+$ Cell	Omalizumab	12.4 (1.2, 175.9)	-69 (-83, -28)***
	Placebo	16.5 (2.9, 60.9)	40 (-39, 111)
IgE$^+$ Mast cell	Omalizumab	9.2 (0.0, 124.5)	-56 (-100, 316)
	Placebo	26.2 (4.3, 50.2)	22 (-44, 181)
IgE$^+$ B cell	Omalizumab	0.0 (0.0, 0.3)	0 (0, 112)
	Placebo	0.0 (0.0, 0.0)	0 (0, 0)
IgE$^+$ Plasma cell	Omalizumab	0.0 (0.0, 6.0)	0 (-100, 0)
	Placebo	0.0 (0, 0.5)	0 (-100, 0)
Eosinophils	Omalizumab	9.03 (2.92, 17.20)	-7.80 (-83.72, 37.62)
	Placebo	4.26 (0.31, 21.35)	17.75 (-45.16, 2491.78)

Table II

Bronchial mucosal inflammatory cells: absolute counts (median, range) per mm^2 at baseline and % change following treatment with omalizumab/placebo at Time A (see Figure I).

Figure I

Clinical trial flow chart outlining interventions. **Baseline:** Time from screening visit to first bronchoscopy and commencement of omalizumab/placebo (Weeks -4 to 0). **Time A:** Time span during which the patients had a second bronchoscopy (Weeks 12 to 14) after which therapy was reduced. **Time B:** End of the trial 20 weeks from the first injection of omalizumab/placebo.

Figure II

Comparison of effect of treatment with omalizumab and placebo on changes in absolute and % predicted FEV_1 between Baseline and Times A and B (see Figure I for definitions). Bars represent the median and interquartile range; Mann-Whitney U Test.

Figure III

Effects of omalizumab and placebo treatment on numbers (% change from baseline) of bronchial mucosal (A) total IgE^+ cells; (B) $tryptase^+$ mast cells; (C) $IgE^+/tryptase^+$ mast cells; (D) $CD138^+$ plasma cells; (E) $CD20^+$ B cells. Median with interquartile range; Mann-Whitney U Test. (F) Typical immunofluorescence images of a bronchial biopsy section stained with anti-CD138 (green), anti-IgE (red) and nucleoprotein (blue).