The Efficacy of Omalizumab Treatment in Chronic Spontaneous Urticaria is Associated with Basophil Phenotypes

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Abstract

Background—The mechanisms underlying disease pathogenesis in chronic spontaneous urticaria (CSU) and improvement with omalizumab are incompletely understood.

Objective: To examine if the rate of clinical remission is concordant with baseline basophil features or the rate of change of IgE-dependent functions of basophils and/or plasmacytoid dendritic cells (pDC) during omalizumab therapy.

Methods—Adults (n=18) with refractory CSU were treated with omalizumab 300 mg monthly for 90 days. Subjects recorded daily Urticaria Activity Scores (UAS), and clinical assessments with blood sampling occurred at baseline and on days 1, 3, 6, 10, 20, 30, 60, and 90 following omalizumab. At baseline, subjects were categorized by basophil functional phenotypes, determined by *in vitro* histamine release (HR) responses to anti-IgE antibody, as CSU-responder (CSU-R) or CSU-non-responder (CSU-NR), as well as basopenic (B) or non-basopenic (NB).

Results—CSU-R/NB subjects demonstrated the most rapid and complete symptom improvement. By day 6, CSU-R/NB and CSU-NR/NB had increased anti-IgE mediated basophil HR relative to baseline, and these shifts did not correlate with symptom improvement. In contrast, CSU-NR/B basophil HR did not change during therapy. The kinetics of the decrease in surface IgE/FcεRI was similar in all 3 phenotypic groups and independent of the timing of the clinical response. Likewise, pDC surface IgE/FcεRI decline and TLR-9 induced IFN-α responses did not reflect clinical change.

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Conclusions—Changes in basophil IgE based HR, surface IgE or FcεRI, bear no relationship to the kinetics in the change in clinical symptoms. Baseline basophil count and basophil functional phenotype, as determined by HR, may be predictive of responsiveness to omalizumab.

Capsule Summary:

Baseline basophil count and basophil functional phenotype, as determined in response to anti-IgE, may be predictive of responsiveness to omalizumab.

Keywords

Chronic Spontaneous Urticaria; Omalizumab; Basophil; FcεRI; IgE; Plasmacytoid Dendritic Cell; Histamine Release; Basophil Activation Test

Introduction

Chronic spontaneous urticaria (CSU) is a skin disease characterized by recurrent hives and/or angioedema for at least 6 weeks with an estimated prevalence of 1% [1]. Symptoms of pruritus and wheals result from skin mast cell (SMC) degranulation of histamine and other mediators, leading to vasodilation and infiltration by basophils, lymphocytes, monocytes, neutrophils and eosinophils [2]. IgG autoantibodies targeting either IgE or FcεRI are reported in approximately 30–40% of cases with an unclear role in pathogenesis [1]. Over the decades, a role for basophils in CSU pathophysiology has emerged [3]. Basopenia is a feature of active CSU[4], is related to disease severity, [5–7] and may reflect the recruitment of basophils to skin lesions[8]. In addition, blood basophils have altered IgE receptor-mediated histamine degranulation [9, 10] that has recently been classified into two basophil functional phenotypes [11]. These phenotypes are defined based on the magnitude of histamine release observed after optimal activation of their IgE receptor: CSU-responders (CSU-R) release ≥ 10% of cellular histamine content whereas CSU-non-responders (CSU-NR) demonstrate < 10% release [11]. A third group, termed CSU basopenics (CSU-B) are unable to be classified due to extreme basopenia [6, 11, 12]. These basophil functional phenotypes are stable during disease, however, in remission, enhanced degranulation mediated through FcεRI is observed along with normalized basophil counts [7, 13]. A longitudinal study of basophil functional subsets and disease outcomes revealed that CSU-B and CSU-NR experience more severe disease but of shorter duration as compared to CSU-R[6].

First-line therapy of CSU is second-generation H1 antihistamines up-dosed to fourfold approved dosing [14], however, this regimen is insufficient to control symptoms in half of patients [15]. Omalizumab, a monoclonal IgG anti-human IgE antibody, is approved for antihistamine-refractory patients at 150 mg or 300 mg subcutaneously every 4 weeks, an approach different from allergic asthma where dosing is based upon the patient's baseline serum IgE level and weight. By binding to free IgE, omalizumab prevents its attachment to FcεRI on FcεRI bearing mast cells and basophils, leading to down-regulation of FcεRI levels on these cells[16, 17]. Overall phase III clinical trials in CSU demonstrated nearly 40% of subjects treated with 300 mg of omalizumab achieved well-controlled urticaria (weekly urticaria activity score (UAS-7) ≤ 6) at 2 weeks[18]. However, an understanding of the IgE dependent pathways of CSU disease that underlie the rapid response to a fixed dose of

omalizumab is lacking[19]. To date, biomarkers associated with a poor response to omalizumab include low baseline serum IgE[20, 21], low basophil FcεRI expression [22] and positive BHRA and/or ASST results [23, 24], but an underlying basis for these findings is lacking.

In allergic disease, omalizumab induced reductions of basophil FcεRI expression and allergen responses occur more rapidly than for mast cells, and both cellular shifts contribute to the clinical effects [16, 25–27]. Similar changes have been noted in omalizumab treated CSU patients[28], however, the timing of cellular shifts relative to a rapid clinical response is unknown. Dendritic cells also express FcεRI which can modulate cellular function and can be rapidly altered by omalizumab therapy[29]. For the purposes of understanding the underlying mechanisms behind the therapeutic efficacy of omalizumab in CSU, a study of dendritic cell and basophil behavior during treatment is warranted. The primary objective of this study was to examine whether the rate of clinical remission with omalizumab therapy in CSU subjects would be concordant with baseline basophil features or the rate of change of IgE-dependent functions of basophils. Additionally, for the first time, we will explore the potential role of dendritic cell functional changes during omalizumab treatment.

Methods

Study Design

This phase IV, single site, open-label clinical trial approved by the Johns Hopkins Institutional Review Board was conducted between October 2017 and February 2020. A total of 11 visits occurred over 15 weeks, and consisted of a screening period (day −21 to −14), a run-in period (day −14 to day 0) and a treatment period of omalizumab 300 mg every 4 weeks for a total of 3 doses (day 0 to day 90) (Figure 1). Frequent visits on days 1, 3, 6, 10, 20 and 30 after omalizumab initiation aimed to capture the rate of clinical changes as they related to IgE bearing cell measures. Subjects recorded twice daily urticaria activity score (UAS) scores using electronic and paper diaries and weekly UAS-7 scores were calculated. The UAS is a validated measure of CSU disease activity calculated from the average of twice daily recorded itch (0 – 3; 0= no itch, 1= mild itch, 2= moderate itch, 3= severe itch) and hives (0 – 3, 0= no hives, 1= 1–6 hives, 2= 7–12 hives, 3= > 12 hives in the past 12 hours) scores with a daily maximum of 6. The UAS-7 is the sum of 7 daily average UAS scores with a maximum value of 42 [30]. Subjects with a UAS-7 ≥ 16 during screening or run-in and a clinic visit UAS ≥ 2 were eligible for enrollment. Blood was collected at each visit. Non-lesional skin punch biopsies were obtained at day 0, at the time of 50% symptom reduction as determined by the UAS-7 between days 6 to 30, and again at day 90. For subjects that did not achieve 50% symptom reduction by day 30, a non-lesional skin biopsy was obtained at day 30. The biopsy results will not be part of this report.

Subjects

Subjects ≥ 18 years of age with at least a 3-month history of CSU were recruited from allergy and dermatology clinics. Subjects were required to have active symptoms despite the use of 6 weeks of a daily 2nd generation H1-antihistamine up to 4-fold of the approved dose and maintained a stable dose of antihistamines throughout the study. Exclusion criteria

included active atopic dermatitis, inducible urticaria, use of doxepin within 14 days of screening, or use of omalizumab within 6 months of screening. Subjects were not permitted to use systemic steroids or immuno-suppressants during the study or within 4 weeks prior to enrollment. Use of H2-antihistamines and/or leukotriene receptor antagonists was not permitted within 7 days of screening or during the trial unless these medications were previously indicated for treatment of GERD, asthma or allergic rhinitis.

Basophil Histamine Release (BHR) Assay

Single Percoll density gradient sedimentation was used to enrich blood basophils of patients from venous samples [11]. Polyclonal goat antihuman IgE antibody (0.001 – 1.0 μg/mL; DACI Lab, Baltimore, MD) was used to stimulate basophils for HR in calcium-containing buffers for 45 minutes at 37° C. Basophils were also stimulated using fMLP (10^{-6} M) as a non-IgE receptor pathway stimulus for basophil degranulation. Supernatants were collected and analyzed via automated fluorometry[11]. Results are presented as the percentages of total histamine content of cell lysates of leukocyte aliquots after spontaneous HR was subtracted. Based on previous studies, maximum *in vitro* induced HR to an optimal concentration of anti-IgE antibody, was used to classify basophil functional phenotypes with CSU-R releasing 10% of cellular histamine content, and CSU-NR releasing <10% of cellular histamine content[11]. Patients with blood basophil counts less than 8000 cells per ml blood were classified as basopenic (CSU-B) based on Ward hierarchical clustering (see below, baseline basophil phenotype, Figure E1 and Table 1).

Basophil Activation Test (BAT)

At the time of blood sampling for BHR, samples were also collected into heparinized tubes (BD vacutainer) for BAT studies. Heparinized blood was incubated with either buffer, doses of anti-IgE antibody or FMLP identical to those used in the BHR assay for 30 minutes at 37° C. Samples were then washed and labeled with CD123 APC (ThermoFisher) and either CCR3 PE (ThermoFisher) or CD63 PE (Beckman Coulter). Cells were subsequently lysed and fixed (Beckman Coulter) and then analyzed on a BD FACS Calibur flow cytometer. Basophils were gated using CCR3+, CD123+ cell subset and results expressed as CD63 % positive relative to buffer.

Basophil surface IgE, FcεRI Quantification

A separate aliquot of blood was used for receptor analysis. After Percoll isolation, cells were fixed in 2% paraformaldehyde for 20 minutes at room temperature, and the cell suspension was stored at 4°C. Cells were blocked with 0.2 mg/ml non-specific human IgG and labeled first with either nonspecific goat IgG, nonspecific mouse IgG1 isotype control, goat anti-IgE antibody, 22E7, or 15A5 mIgG1 antibodies (gift from Hoffman-LaRoche) at concentrations previously determine to be optimal for flow cytometry (25 minute incubation). A second 25 minute incubation with anti-mouseIgG1-alexa647, or anti-goat-alexa647 with anti-CD123-FITC and CCR3 (PE-labeled) (1/30 dilution from eBioscience stock solution) was followed by washing and analysis. When receptor expression was high enough, it was possible to alternatively gate the samples to derive the plasmacytoid dendritic cell IgE surface expression and determine the kinetics of change in these two parameters.

Dendritic Cell Surface IgE, FcεRI and Function

Additional blood samples (~50 ml drawn in EDTA, 10mM final) were obtained at baseline and at the time of second skin biopsy. These specimens were subjected to 2-step Percoll density centrifugation, which produces both basophil-depleted cell (BDC) and basophil-enriched cell suspensions [31]. The BDC suspensions were washed 4 times to remove platelets and then immediately depleted of monocytes using $CD14^+$ immunobeads with two passes over LS columns (both from Miltenyi-Biotec, Gaithersburg, MD). The column flow-thru cells, collected during monocyte depletion, were then subjected to $BDCA4^+$ (CD304) selection (Miltenyi-Biotec). Cells retrieved from these LS columns were enriched for pDC and underwent testing for TLR9-dependent IFN-α production using stimulation with CpG-2216 oligonucleotide (TriLink Biotechnologies, San Diego, CA), as described [32]. Supernatants were harvested after 20 hours and assayed for IFN-α protein by ELISA (R&D Systems, Piscataway, NJ). Surface IgE and FcεRIα were detected on pDC, as previously described[29]. In brief, 1×10^7 washed BDCs were fixed in 1 ml buffered 4% paraformaldehyde and stored at −80°C, which allowed for pre and post treatment preparations to be concurrently analyzed by flow cytometry. Staining for pDC was done by using anti-BDCA-2/allophycocyanin (Miltenyi Biotec, Auburn, Calif.). Co-expression of cell surface IgE and FcεRIα was individually assessed using goat anti-human IgE/ fluorescein isothiocyanate (KPL, Gaithersburg, MD), and anti-FcεRIα/phycoerythrin (clone AER-37; ThermoFisher, Grand Island, NY), respectively. Analyses were performed using a Becton-Dickinson (BD) Accuri C6 Plus flow cytometer, with normalized mean fluorescence intensity (nMFI) determined after subtracting isotype control values.

Statistical Analysis

Generally, ANOVA was the statistical test applied to the results. A primary design categorization was based on the basophil phenotype. As noted below, the 3 basophil phenotypes were not necessarily independent. Therefore, the statistical analysis required independent comparisons of non-releasers/releasers and baso-normal/basopenic as two dichotomous categories. Tukey-Kramer analyses were utilized for comparing post-hoc group characteristics. Chi-square was used for comparison of categorical values. To determine the $T_{1/2}$ metric for changes in daily UAS scores, the daily score values were first smoothed using a smoothing window of ±1 day. Once the data was normalized to the average of day −14 to 0, the crossing point for a value of 0.5 was extrapolated for the $T_{1/2}$ of the UAS metric. Without the smoothing step, a similar procedure derived the $T_{1/2}$ results for basophil surface IgE and FcεRI.

Results

Subjects

Of 27 subjects recruited, 8 failed to meet screening criteria, 1 was lost to follow-up and 18 subjects completed treatment with omalizumab (Figure E2) with demographics shown in Table 1. Two-thirds of subjects were females with an average age of 44 years with 7 years of CSU disease. The average baseline UAS-7 was 29 despite the use of antihistamines. All subjects received omalizumab 300 mg every 4 weeks, yielding a broad range in omalizumab dose (mg/kg/IgE) given the wide range of IgE levels (<2 to 1558 kU/L).

Baseline Basophil Phenotype and Symptom Response

Subjects were next categorized by two baseline basophil characteristics, circulating numbers of basophils and IgE-mediated histamine release profile (Table I). These then were examined for their clinical symptom response to omalizumab. As noted, Ward hierarchial clustering was used to set the threshold for basopenia (< 8000 basophils/mL). There were two clear clusters (non-basopenic (n=9) average basophil count 28600 basophils/mL, basopenic (n=9) average basophil count 2670 basophils/mL) separated by a 10-fold difference in basophil counts (Figure E1). Basopenics were more likely to be males (p =0.038, Chi-square), and received a higher average monthly omalizumab dose (p = 0.039, ANOVA) whereas no difference was found in age, baseline UAS, disease duration or total IgE. Daily UAS scores from Day −7 to 50 after omalizumab declined slower in basopenic subjects leading to a later day for a skin biopsy at the time of 50% symptom decline (23 days basopenic subjects vs. 12 days non-basopenic subjects, p = 0.043 ANOVA) (Figure 2A).

The second baseline basophil characteristic examined was the functional phenotype (CSU-R vs. CSU-NR) [11]. For the responder/non-responder dichotomy, there were 10 basophil CSU-NR, 7 CSU-R and one subject that could not be classified due to extreme basopenia (subject 10, Table 1). With this classification, CSU-NR subjects had a significantly higher baseline UAS-7 (33 vs. 23, p < 0.01, ANOVA) but no other differences were found (i.e. age, IgE, omalizumab dosing, gender, or duration of disease). UAS symptom reduction was faster and more robust for CSU-R as compared to CSU-NR (Figure 2B) with an earlier average day of 50% UAS-7 reduction (day 12. 5 vs day 20 CSU-NR). However, it became apparent that there was a strong association between the non-responder status and the presence of basopenia (Table E1, chi-square p= 0.004 shown in companion paper as Table 2C). Therefore, we next used two-parameter categories and examined 3 sub-groups defined as a CSU-NR, non-basopenic (n=3, CSU-NR/NB) vs. CSU-NR with basopenia (n=7, CSU-NR/B) vs. CSU-R non-basopenic (n=6, labeled CSU-R/NB) for the remainder of the analyses. Two subjects were excluded from this classification, one due to extreme basopenia (subject #10) and another as the sole basopenic CSU-responder (Subject #8).

CSU-R/NB had the lowest mean baseline UAS-7 score of 23 of all 3 groups, indicative of the mildest symptoms; this was significantly different from the other two groups (p=0.016, Table 1). Of the three groups, NR/B received the highest dose of omalizumab, 0.807 mg/kg/IgE (ANOVA comparison of 3 groups, p = 0.0473, Tukey-Kramer HSD post-hoc analysis). Daily UAS scores from −7 to 50 days are shown in Figure 2C whereas weekly UAS-7 for the entire study are shown in Figure 2D. Classifying subjects according to both basophil characteristics, it is apparent that the distinguishing phenotype is the responder status; both CSU-NR/B and CSU-NR/NB were similar in their response to treatment (Figure 2C). Of interest, the CSU-R/NB group had the fastest and most complete symptom decline in response to omalizumab with an average UAS-7 reduction of 61% from baseline at week 1 and a 92% reduction at week 4 (Figure 2D). In contrast, CSU-NR/NB (43% week 1, progressing to 63% at week 4) and CSU-NR/B (17% week 1, progressing to 38% at week 4) experienced slower and less complete symptom decline on treatment with omalizumab. By the end of week 12, the overall reduction from baseline UAS-7 symptoms was 98% for CSU-R/NB, 64% for CSU-NR/NB, and 51% for CSU-NR/B. The sole patient with CSU-R

and basopenia status followed the clinical response profile of CSU-R/NB subjects (data not shown).

Basophil Histamine Release and Basophil Activation Test Measures

Using the dual parameter classifications, we next examined the shifts in basophil anti-IgE triggered histamine response during omalizumab treatment relative to symptom changes. By day 6 of omalizumab therapy, both CSU-R/NB (Figure 3A) and CSU-NR/NB (Figure 3B) demonstrated an elevation in their anti-IgE mediated BHR as compared to baseline. The greater increase occurred in subjects with the CSU-NR/NB profile, but did not correspond to the greatest symptom improvement. This elevation persisted throughout the remainder of the study, through day 90. The similar timing for a rise in basophil histamine release in the corresponding CSU-R/NB and CSU-NR/NB groups and lack of concordance with the timing of clinical changes or changes in surface IgE or FcεRI suggests that this change is the result other variables in the basophil response. The notable lack of change in the CSU-NR/B anti-IgE antibody triggered response during omalizumab therapy is presented and discussed in a companion manuscript (Figure 3C and companion paper). However, no difference in HR was identified between the three groups when stimulated by fMLP, a non-IgE mediated pathway during treatment (Figure E3A). Similar shifts in anti-IgE induced profiles were noted by day 6 using BAT measures along with no change in BAT FMLP induced response (Figure E4 and E3B).

Basophil Surface IgE and FcεRI data

Basophil surface IgE and FcεRI levels were reduced in all subjects following treatment with omalizumab. The kinetics of the decrease in surface IgE/FcεRI was similar in all 3 phenotypic groups (CSU-R/NB, -NR/NB, -NR/B) and did not follow the different timelines for the clinical response (Figure 4A and 4B). Although the serum IgE levels for the 3 groups did not show a statistically significant difference, differences were apparent in the starting surface IgE levels; the CSU-NR/B group had lower surface IgE at baseline (ANOVA, p<0.0001, Tukey-Kramer HSD). In the figure 4A and B insets, the percentage of baseline surface IgE and receptor levels are shown. While there was a similar percentage reduction of surface IgE in all groups, the percentage of baseline receptor in the CSU-NR/B group displayed a rising curve after an initial fall on therapy. This pattern of change in FcεRI in low receptor expressing basophils at baseline has been noted by Deza et al[22]. However, a strong correlation was noted between serum IgE and the baseline cell surface IgE level for the entire study group (see companion report). It is also worth noting that the CSU-NR/B group experienced minimal change in *in vitro* anti-IgE antibody responses either by HR or BAT and showed the greatest expression of auto-antibodies (presented in a companion report).

Our interest in the relationship between the loss of basophil surface IgE or FcεRI led to analyzing the rate of change in UAS daily symptom scores expressed as $T_{1/2}$ of daily UAS (see statistical methods, i.e, the day at which symptoms were reduced by 50%,) vs. the $T_{1/2}$ for reduction in either basophil surface IgE or surface FcεRI. Figures 5A&B show these relationships and the results suggest that there is no correlation between changes in symptoms and the rate of change in basophil IgE or FcεRI. Indeed, anecdotally, we could

find individuals whose symptoms were reduced to 25% of their baseline with no changes yet occurring in the cell surface IgE or FcεRI. The online repository (Figure E5A and E5B) presents these results a different way, showing the kinetic curves for groups assembled on the basis of the rate of clinical change and the conclusion is similar.

Basophil Counts

From the perspective of the starting levels of circulating basophils, treatment with omalizumab did not, on average, increase basophil numbers in blood (Figure E6). In addition, there were no consistent changes in the subgroups (CSU-R/NB, -NR/NB, -NR/B). There were, however, significant increases among individual subjects that were consistent across multiple measurements during treatment. The most notable changes in some individual statistics occurred in the basopenic subjects but the increases still did not rise above the thresholds set for being classified as basopenic.

Dendritic Cells

Like basophils, other IgE-bearing cells are known to down-regulate FcεRIα following omalizumab administration, including pDCs from CSU subjects[33]. In this recent study, there were no apparent changes in pDC frequency before and after treatment; we also observed a similar stability in the frequency of pDCs (determined by flow cytometry, Figure 6E) and isolated numbers of pDCs before and after therapy (data not shown). To extend these observations, we examined pDC for functional responses before and after omalizumab treatment by focusing on their capacity to secrete IFN-α in response to TLR9-dependent activation. When classified according to the dual parameter basophil phenotypes, we observed that pDCs of the CSU-R/NB subjects had greater baseline TLR9 induced IFN responses as compared to the other 2 groups, and this pattern was maintained at the time of second biopsy on omalizumab (Figure 6A). When simply comparing IFN-α levels between the CSU-R and the CSU-NR using a dichotomous basophil R/NR classification, we observed significant IFN-α differences between these 2 groups both pre- and post-therapy, but no significant changes within groups (Figure 6B). We further confirmed a decline in FcεRI and surface IgE and at the indicated timepoints for functional assays (Figure 6C, 6D). Similar to basophils, in a subset of subjects, we found surface IgE on pDC decreased rapidly in those samples where there was confidence in the FcεRI expression with re-gating. In these cells, the loss of surface IgE was very rapid, a $T_{1/2}$ of 0.9±0.3 days (Figure E7A). Total FcεRI decreased with a slightly slower time course (1.8 days) and unoccupied receptors increased (Figure E7B and E7C).

Discussion

At present, an understanding of disease pathways in CSU is limited to a role for skin mast cell activation via unknown triggering mechanisms. Among the proposed triggers for SMCs are IgG auto-antibodies to IgE and FcεRI in a subset of patients. A role for basophils in CSU disease has emerged given the detection of these cells at CSU skin lesions, linkage of basopenia to disease severity, and altered basophil IgE pathway phenotypes[1, 3]. These altered basophil features, basopenia and altered IgE pathways improve in disease remission[7, 13]. The rapid efficacy of omalizumab in mitigating CSU symptoms has focused

attention on the contributions of basophils to CSU disease as well as other IgE bearing cell types such as pDCs. Past studies of omalizumab in allergic subjects have noted a faster reduction in IgE receptor levels and allergen activation of basophils as compared to mast cells, highlighting the possible contributions of basophils in clinical symptom relief in CSU [25, 26].

In this study, we confirmed the two expected basophil characteristics in CSU: basopenia and distinct anti-IgE functional phenotypes. A primary goal of the study was to determine if these well-defined basophil phenotypes would predict the clinical response to omalizumab. Furthermore, we also compared the onset of clinical symptom relief during omalizumab treatment relative to changes in basophil features to identify factors relevant to disease expression. When comparing the clinical responses to omalizumab, basopenic subjects had slower responses (Figure 2A) as did those with a CSU-NR profile (Figure 2B). Given the large overlap in these 2 basophil characteristics (basopenia with CSU-NR functional status), we examined the clinical response in subjects classified according to both parameters. We found that the functional phenotype was the dominant parameter and that the CSU subjects with a responder phenotype had the most rapid and complete clinical response to omalizumab (Figure 2A–D). The underlying disease mechanisms for the basophil CSU-R phenotype are not fully understood but appear to reveal a CSU subset that is dependent on IgE pathways for disease expression. This is the first description of this baseline basophil characteristic as a positive predictor for an omalizumab response. We have previously established the stability of this phenotype over time in active CSU patients[13], and the association of this phenotype with milder but more prolonged CSU disease course, profiles that were as observed in this study.[6]

Among the CSU-NR subjects, we found a large overlap with basopenia and both of these features are associated with more severe CSU disease[6]. Prior studies have also suggested that low serum IgE [20, 21] and basophil surface FcεRI[22] levels are negative predictors for omalizumab response. As the basopenic group in this study also had the lowest serum IgE and basophil surface FcεRI expression, our study supports these prior observations. Due to the small group numbers, the statistical analysis suggested no difference in the serum IgE levels among the 3 groups (Table I) but the trend was evident and there was a significant reduction in surface IgE at baseline on CSU-NR/B (Figure 4A). At baseline, a correlation between serum IgE and surface IgE was indeed present when analyzing all study participants (see companion paper).

A second goal of the study was to determine if changes in the basophil compartment, notably with respect to cell surface IgE or FcεRI density, or functional phenotypes, would explain the rapidity of the response of CSU patients to omalizumab. This latter goal was predicated on characteristics of omalizumab to rapidly reduce FcεRI on human basophils (while causing a much slower decrease on mast cells) and the published observations that symptom reduction occurs rapidly in CSU patients being treated[18]. Our data suggests that the answer is that the two outcomes (rate of surface IgE reductions and symptom decreases) are not related and consistent with the recent observations of Aghdam[33]. The rate that cell surface IgE or FcεRI decreases during treatment is approximately the same for all subjects regardless of how one groups their basophil phenotypes (Figure 4A&B) or their rate of

symptom change (Figure 5A&B, E5A–B). Notably, the rate of receptor decrease is consistent with prior studies in asthma or peanut allergy[26, 34].

With regard to functional basophil changes, increased IgE receptor reactivity was noted in both the basophil CSU-R/NB and CSU-NR/NB groups, with the latter having the greater increase. The timing of change did not fit the timing of clinical response and may be explained by underlying changes in IgE pathway regulators such as SYK (see companion manuscript). In these non-basopenic subjects, these changes are consistent with the behavior we have found in allergics and asthmatics. Our findings agree with the Aghdam study which noted greater increase in basophil IgE reactivity in CSU subjects with modest to limited clinical response, whereas more modest basophil change was seen in those with the greatest clinical benefits[33]. Using the BAT assay, we were able to mirror basophils functional phenotypes based on histamine release profiles and reflect timing of omalizumab shifts. In contrast to other groups[12, 33, 35], we did not include IL-3 in BAT assay buffers as this may impact the functional readouts of basophils.

Additionally, we explored the effect of omalizumab on alternative IgE bearing cell types such as pDCs. Prior literature demonstrates that innate immune responses are impaired in pDCs first exposed to IgE cross-linking stimuli, with these pDCs exhibiting a reduced capacity to secrete IFN-α upon subsequent stimulation with TLR9 agonists [32]. pDC from CSU subjects are likewise reported to have impaired capacity to make IFN-α[36]. Although clinical trials demonstrate that omalizumab administration protects allergic asthmatics from viral-induced exacerbations[37], the impact of omalizumab in CSU subjects in regards to this IFN-α production defect has not been previously reported in relation to symptom relief of urticaria. In our study, following treatment with omalizumab, enhanced IFN-α secretion was observed, although this did not reach statistical significance. Furthermore, this innate immune response was predicted by basophil phenotypes both at baseline and following treatment with omalizumab, with the pDCs from CSU-R/NB secreting greater levels of IFN-α compared to those of CSU-NR/NB and CSU-NR/B. CSU-NR subjects have reductions in TLR9 induced IFN-α response, which may be related to the known down-regulation of this pathway with IgE receptor cross-linking. An explanation for impaired TLR-9 response linked to IgE pathway profiles between two independent IgE bearing cell types is not obvious. The potential for IgG auto-antibodies targeting and limiting pDC functions such as for basophils (see related manuscript) has not been previously reported and requires further study. The clinical implication of blunted pDC interferon response in CSU subjects has also not been explored. Like the recent Aghdam study [33], pDC IgE/FcεRI receptors declined and no differences in isolated pDC numbers were noted pre and post omalizumab.

Previous studies of CSU have noted an increase in basophil counts during remission and that treatment with omalizumab may also cause increases[7, 13, 38]. The underlying basis for basal basophil circulating numbers is not well understood. It is known that an acute response to systemic glucocorticoids include a marked reduction in circulating basophils but the mechanism underlying that response is also unknown[39]. As a leukocyte that marginates into tissues in response to stimulation, it stands to reason that processes that activate the cell may lead to its margination. Previous larger phase III clinical studies have observed that circulating basophil numbers increase during treatment of CSU patients with omalizumab

after 12 and 24 weeks of therapy[38]. In this study, the increases were inconsistent and in no group were the increases statistically significant, however, like Aghdam et al we observed an increase in the early timeframe post initiation of therapy[33]. When examined as a baseline characteristic, basopenic subjects had higher symptom scores and a more gradual symptom improvement as compared to non-basopenic subjects. This is consistent with previous studies noting basopenia as a marker of disease severity[6]. There were changes in some individuals in the basopenic group but the increases were never sufficient to change the basopenic status of the patient.

The potential clinical implications of our study should be mentioned. At present, basophil phenotyping via histamine release or BAT is only available in research centers. With the potential for more widespread use of the BAT assay, basophil functional phenotyping may be completed with greater ease, enabling the expansion of personalized medicine. Although we report CSU-R had the most rapid and complete clinical response to omalizumab, we would like to emphasize that all subjects experienced some degree of benefit, although without a placebo arm, it is unclear whether these responses were related to treatment. This data should therefore be utilized to inform providers and patients of potential timing and degree of response to omalizumab. Future studies are necessary to confirm if basopenia is a negative predictor for response to omalizumab.

In summary, these results demonstrate that changes in basophil surface IgE or FcεRI bear no relationship to the kinetics in the change in clinical symptoms. Whether the results were analyzed in the context of the 3 basophil phenotypes or the general relationships among the kinetics of decreases in symptoms, surface IgE or FcεRI, the results do not suggest a linkage between the receptor changes and clinical behavior. However, the study did identify a potential predictor of highly successful and rapid response to treatment, and that is the basophil functional responder phenotype. Two measurements, a basophil count and response of a subject's basophils to an *in vitro* stimulation with crosslinking anti-IgE antibody would predict the rapidity of the response and its efficacy. It is unclear why this phenotype is predictive because it doesn't appear to be related to what is happening to surface IgE or FcεRI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations:

CSU	Chronic Spontaneous Urticaria

SMC	Skin mast cell
FcεRI	High-affinity IgE receptor
UAS	Urticaria Activity Score
UAS-7	Urticaria Activity Score 7 day
BHR	Basophil histamine release
HR	Histamine release
BAT	Basophil activation test
CSU-R	Chronic spontaneous urticaria responder
CSU-NR	Chronic spontaneous urticaria non-responder
CSU-B	Chronic spontaneous urticaria basopenic
CSU-R/NB	Chronic spontaneous urticaria responder/non-basopenic
CSU-NR/NB	Chronic spontaneous urticaria non-responder/non-basopenic
CSU-NR/B	Chronic spontaneous urticaria non-responder/basopenic
pDC	Plasmacytoid dendritic cell
nMFI	Normalized mean fluorescent intensity
BDC	Basophil depleted cells

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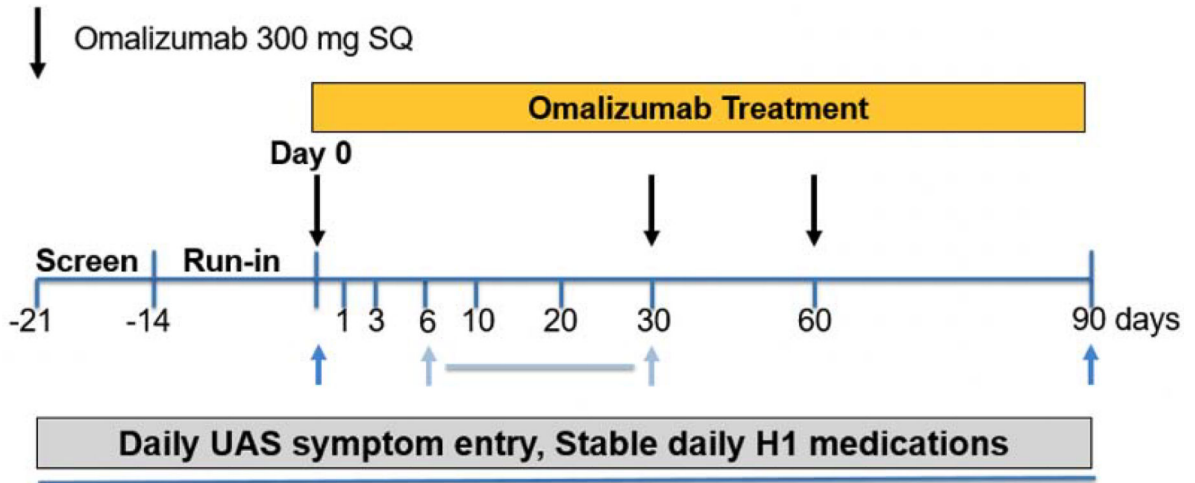
Clinical Implications or Key Messages

Baseline basophil count and basophil functional phenotype, as determined in response to anti-IgE, may be predictive of responsiveness to omalizumab.
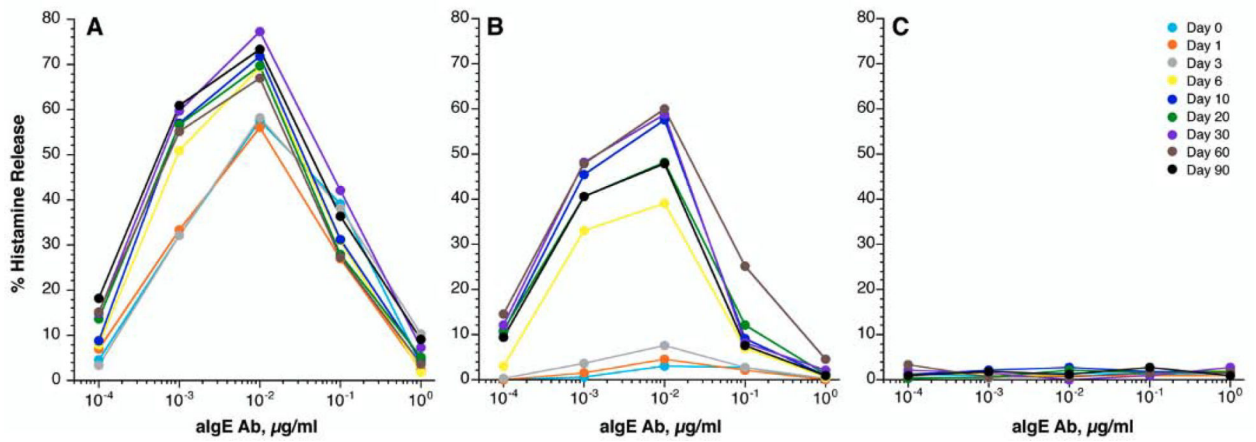
Figure 1:

Study design. Depicted days indicate visits with laboratory studies. Black arrows indicate days of omalizumab dosing. Blue arrows indicate timing of skin biopsies. The second skin biopsy occurred between day 6 and 30 at the time of 50% symptom reduction relative to baseline UAS-7. If 50% symptom reduction was not achieved by day 30 then biopsy performed at day 30.
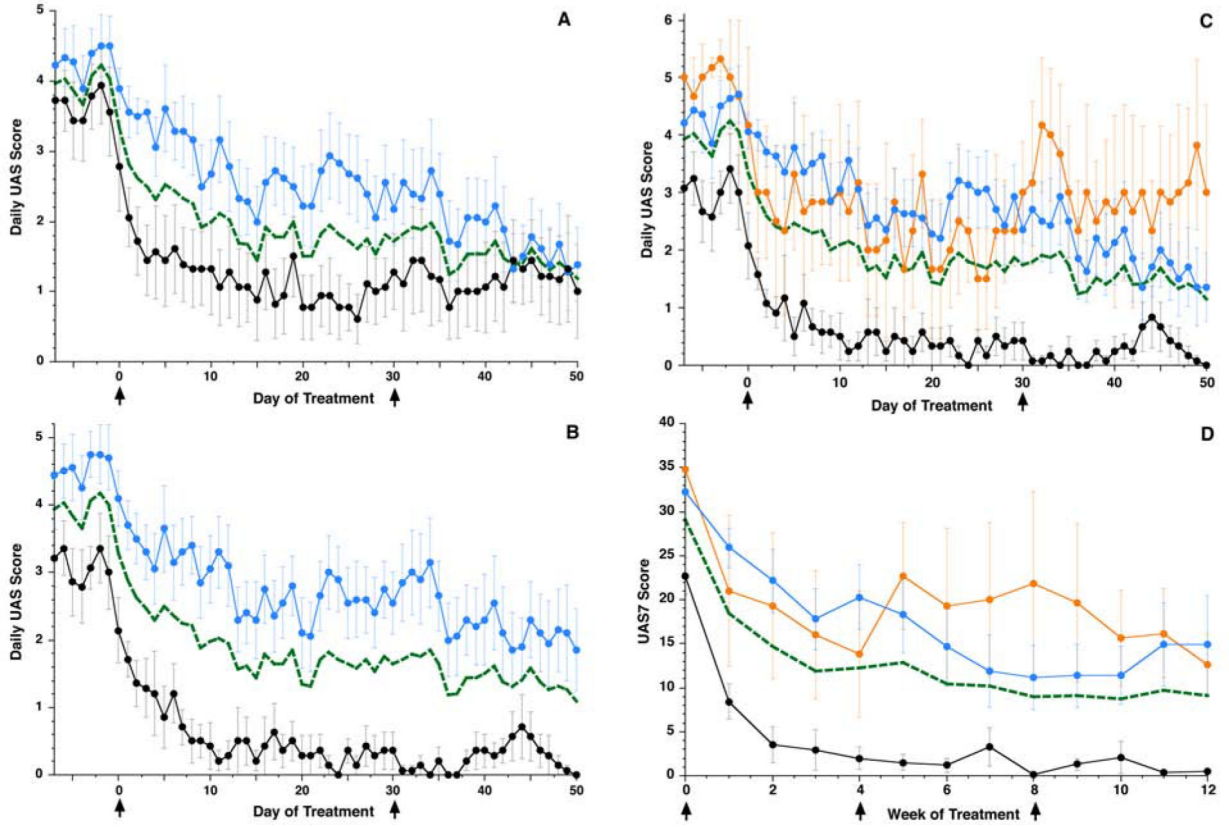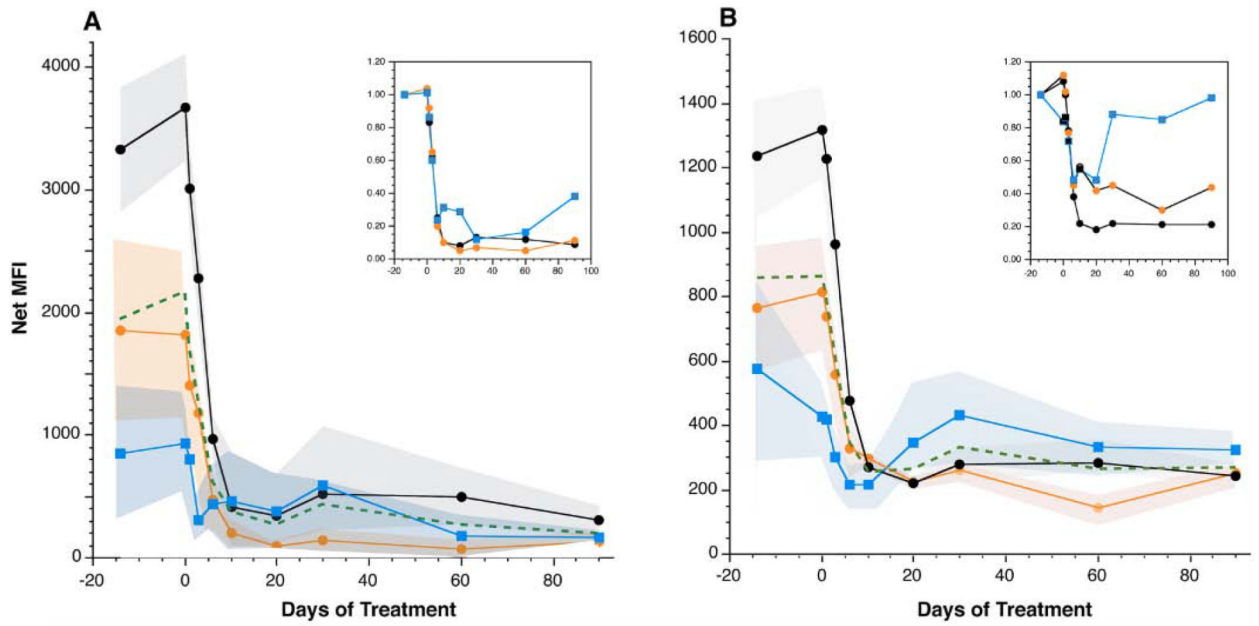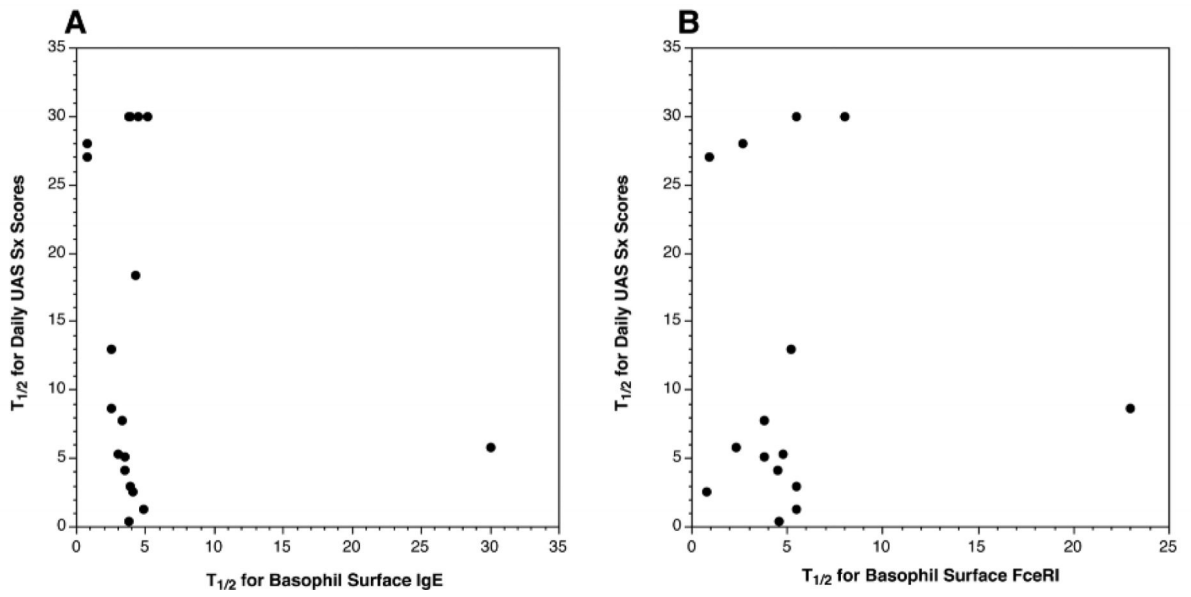
Figure 2.
Clinical response during treatment represented by daily UAS scores for subjects. Black arrows indicate days of omalizumab dosing. (A) Single parameter classification based on basopenic status: Blue line (n=9) – basopenics average, Black line (n=9) – non-basopenics average, Green dashed line (n=18) – Average. (B) Single parameter classification based on basophil histamine response: Black line (n=7) – responders average, Blue line (n=10) – non-responders average, Green line (n=17) – Average. (C) Daily UAS scores during treatment with omalizumab according to the dual parameter basophil categorization of basopenia and responder status. (D) Weekly UAS7 scores during treatment. Black arrows indicate days of omalizumab dosing. For both panels 2C and 2D, Black line (n=6) – CSU-R/NB average, Orange line (n=3) – CSU-NR/NB average, Blue line (n=7) – CSU-NR/B average, Green line (n=16) – Average.
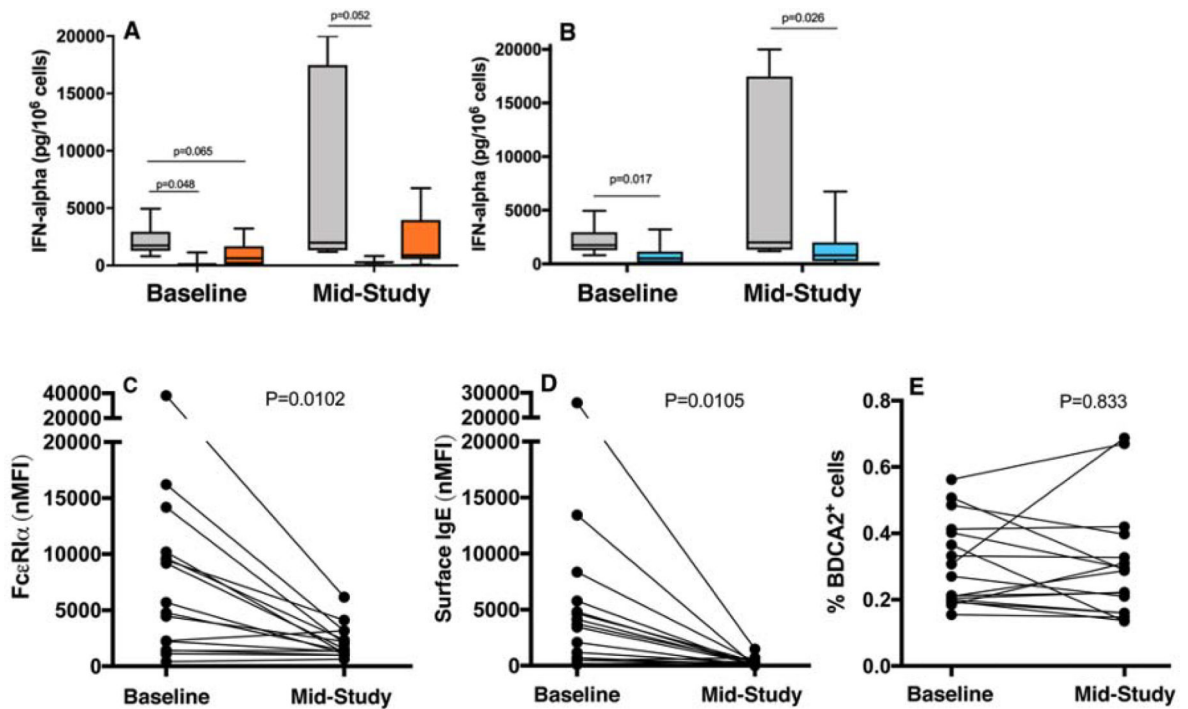
Figure 3:

In vitro basophil histamine release in response to anti-IgE antibody stimulation at the indicated day of study. (A) Responder/Non-basopenics (CSU-R/NB), (n=6); (B) Non-responder/Non-basopenics (CSU-NR/NB), (n=3); (C) Non-responder/basopenics (CSU-NR/B) (n=7). The colored lines represent day of study.

Figure 4.
Omalizumab-induced reductions in basophil surface IgE (A) and FcεRI (B) were similar amongst basophil subsets. Inset figures represent change relative to baseline for the 3 categories (2-parameter categories). For both panels, Black line – CSU-R/NB average, Orange line – CSU-NR/NB average, Blue line – CSU-NR/B average, Green line – Average. Colored regions surrounding each line represent the SEM bands for the averages.

Figure 5:

Relationship between rates of reduction ($T_{1/2}$) in basophil surface IgE (**A**) or FcεRI (**B**) versus the rate of reduction ($T_{1/2}$) in clinical symptoms. Each dot represents one subject.

Figure 6:

TLR9-mediated secretion of IFN-α, *in vitro*, from isolated pDCs at baseline and at mid-study measurement. Grouped by dual parameter basophil classifications (A):CSU-R/NB (n=6, grey bar), CSU-NR/NB (n=3, blue bar), NR/B (n=6, orange bar). (B): CSU-R (n=6, grey bar), CSU-NR (n=9, blue bar). (C) FcεRI (D) surface IgE, and (E) percent BDCA2^{+} pDC by flow at baseline and mid-study timepoints.

Table 1:

Study population baseline characteristics.

Subject	Age	Gender	Duration	IgE	Dosing	BslUAS7	MidPtDay	BasoPheno	BasoCount
1	62	F	5	191	0.019	24.5	20	NR	B
2	34	F	<1	347	0.013	16.5	10	R	NB
3	50	F	4	133	0.024	23.5	20	NR	B
4	32	F	<1	21.4	0.238	32	6	R	NB
5	62	F	4	78.6	0.027	22	10	R	NB
6	39	F	<1	5.3	0.821	32	10	NR	B
7	31	M	2	610	0.004	20.5	6	R	NB
8	33	F	16	1558	0.002	25.5	20	R	B
9	39	M	1	< 2	2.054	42	30*	NR	B
10	52	M	50	153	0.020	30.5	30*	Unclassified	B
11	35	M	<1	< 2	1.586	35.5	20	NR	B
12	54	M	1	13.5	0.289	36	30*	NR	B
13	67	F	10	70.8	0.059	29	6	NR	NB
14	36	F	20	146	0.025	21.5	6	R	NB
15	71	F	2	149	0.037	41.5	30	NR	NB
16	29	M	8	4.2	0.854	32	30*	NR	B
17	36	F	1	197	0.016	34	6	NR	NB
18	22	F	6	56.3	0.084	23.5	30	R	NB
Average	44	67% F	7.4	207	0.340	29	17.7		
R/NB	36	83% F	5.6	210	0.065	23^	11.3		
NR/NB	58	100% F	4.3	139	0.037	35	14.0		
NR/B	44	43% F	2.9	50	0.807	32	22.9		

*
Indicates subjects that did not reach 50% UAS-7 reduction by day 30.

^
ANOVA p=0.0473

Duration = years of disease

Dosing = calculated dose of omalizumab in mg, per Kg body weight per IU IgE

BslUAS-7 = baseline UAS-7 score

MidPtDay = day at which symptom drop was greater than 50%

BasoPheno = anti-IgE functional category for the subject (NR = non-responder, R = responder)

BasoCount = Basophil count (B = basopenic, NB = non-basopenic)