

## Original article

## Evaluation of two commercial omalizumab/free IgE immunoassays: implications of use during therapy

Dana L. Baker  
Kun Peng  
Melissa Cheu  
Saloumeh Kadkhodayan Fischer  
Genentech, South San Francisco, CA, USA

**Address for correspondence:**

Saloumeh Kadkhodayan Fischer, Department of Assay Development and Technology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA.  
Tel: +1 6502254153; Fax: +1 6502251998;  
sallyk@gene.com

**Keywords:**

Dosing table – Enzyme-linked immunosorbent assay – IgE – Omalizumab – Pharmacodynamic

Accepted: 12 December 2013; published online: 24 January 2014  
Citation: Curr Med Res Opin 2014; 30:913–22

**Abstract****Background:**

The anti-IgE monoclonal antibody, omalizumab, is approved in the US as add-on therapy for patients  $\geq 12$  years of age with moderate-to-severe persistent allergic asthma. Omalizumab is administered according to the US Food and Drug Administration approved dosing table included in the prescribing information. The dosing table was developed using Genentech's free IgE assay and is designed to achieve free serum IgE levels of  $<50$  ng/mL, known to be associated with clinical benefit. Lack of clinical benefit in a subset of patients on omalizumab has prompted demand for commercial free IgE assays to guide omalizumab dosing. To date, two commercial free IgE assays marketed by ViraCor-IBT (no longer offered) and BioTeZ have been available to physicians.

**Objective:**

This study compares the results generated from the two commercial free IgE assays with the free IgE levels generated by the Genentech assay.

**Methods:**

Two serum sample sets were prepared using 20 samples from patients with a wide range of IgE and omalizumab from an omalizumab clinical trial and 36 samples from omalizumab-naïve patients. Different amounts of omalizumab were added to the 36 omalizumab naïve samples based on measured total IgE levels to ensure that a good range of IgE and omalizumab was represented in the study samples. Samples were randomized for blinded analysis of free IgE levels using the Genentech, ViraCor-IBT and BioTeZ free serum IgE assays. Analysis of samples in the ViraCor-IBT assay were conducted by ViraCor-IBT and the analysis of samples using the Genentech and BioTeZ assay methods were conducted by a third party contract research organization.

**Results:**

The ViraCor-IBT and BioTeZ free IgE assays demonstrated significantly higher free IgE levels than the Genentech free IgE assay. Twenty-nine of 56 samples tested  $<50$  ng/mL in the Genentech assay; of these, 12/29 (41%) and 20/29 (69%) tested  $>50$  ng/mL in the BioTeZ and ViraCor-IBT assays, respectively. In the BioTeZ free IgE evaluations, 11/20 samples that were re-tested had inter-assay differences ranging from 40–190%.

**Conclusions:**

Free ligand (such as IgE) measurements are challenging and dependent on the method and reagents used. The ViraCor-IBT and BioTeZ methods tend to over-estimate free serum IgE levels compared with the Genentech free IgE assay. Using these assays to monitor therapy and adjust omalizumab doses post treatment is considered off-label use and could lead to a potential risk for unnecessary treatment and/or risk to patient safety.

Introduction

Asthma has an allergic basis in an estimated two thirds of patients and the role of an IgE-mediated response in allergic asthma is well established<sup>1–5</sup>. The anti-IgE antibody omalizumab (Xolair®) has been used successfully for several years as an add-on treatment for patients who are 12 years of age and above with moderate-to-severe persistent allergic asthma and symptoms that are inadequately controlled with inhaled corticosteroids<sup>6</sup>. In the European Union (EU) omalizumab is used as add-on therapy for patients 6 years of age and above with severe persistent allergic asthma, whose symptoms are inadequately controlled with inhaled corticosteroids and long-acting  $\beta_2$ -agonists<sup>7</sup>.

Free IgE levels fall rapidly following omalizumab therapy and this reduction is maintained with continued therapy<sup>8</sup>. Reduction of free serum IgE levels to <50 ng/mL has been shown to be clinically efficacious<sup>8,9</sup>. In contrast, total IgE levels are elevated following initiation of omalizumab therapy due to formation and slow clearance of biologically inactive omalizumab-IgE complexes, and remain elevated for up to 1 year after discontinuation of treatment<sup>6,8</sup>.

The appropriate dose of omalizumab is determined using an approved dosing table that was derived using a free IgE assay developed by Genentech and is included in the prescribing information<sup>6</sup>. The approved dosing table was designed to ensure that free serum IgE is reduced to a clinically efficacious level of <50 ng/mL<sup>8,9</sup>. The dose and frequency of administration of omalizumab is determined by a patient's bodyweight (kg) and serum total IgE level (IU/mL) before the start of treatment<sup>6</sup>. The omalizumab prescribing information specifies that re-testing of total IgE levels during omalizumab treatment is not recommended and cannot be used as a guide for dose determination<sup>6</sup>.

However, not all asthma patients receiving omalizumab experience clinical benefits. Some treating physicians believe that it would be beneficial to know whether the prescribed omalizumab dose has reduced free serum IgE levels into the therapeutic range (<50 ng/mL). This has created a demand for an omalizumab specific free-IgE assay, to identify "patients who may have been under dosed and in whom non-complexed IgE is free to continue to arm Fc $\epsilon$ R1 on mast cells and basophils" as stated in one paper<sup>10</sup>. However, it is important to note that such post-treatment dose adjustments based on free IgE measurements have not been approved by health authorities and are considered off-label use.

To date, two commercial free serum IgE assays, one from Viracor-IBT (no longer available), and the second from BioTeZ have been marketed to allow physicians to

measure free IgE in patients treated with omalizumab. Although neither of the two commercial assays has been validated for use during omalizumab therapy, they provide physicians with an opportunity to monitor post-baseline free serum IgE off-label. In principle, the amount of free IgE detected in any sample can differ between assays. This can be attributed to differences in formats, binding affinity of capture and detection antibodies, sample dilution and incubation times between assays, which influence the equilibrium of omalizumab/IgE complexes. Given that the commercial free IgE assays may potentially be used to make treatment decisions in conjunction with the dosing table and target free IgE levels that were derived from the free IgE assay developed by Genentech, it is important to fully understand whether there are differences between the IgE data obtained from these assays compared with the Genentech assay. The objective of this study was to evaluate human serum samples with a wide range of serum total IgE, free IgE, and omalizumab concentrations, and to compare the values obtained from the two commercial assays to values from Genentech's free IgE assay.

Methods

Collection and pooling of samples: human serum samples

Sera from patients diagnosed with allergic asthma were used to prepare a set of 56 human serum samples; including 20 in vivo and 36 in vitro samples. The 20 in vivo human serum samples were prepared using 44 clinical serum samples obtained from 32 allergic asthma patients who participated in an omalizumab clinical trial (Genentech, South San Francisco, CA, USA). To protect patient identity and also provide sufficient sample volume for the various assays, sera samples from patients with similar free IgE and omalizumab profiles based on the clinical trial assay data were combined to generate 20 'in-vivo' samples (Table 1).

The 36 'in-vitro' human serum samples were prepared using 15 individual human sera obtained from

**Table 1.** Summary of 20 'in-vivo' samples generated for assay comparison. Serum samples from patients with allergic asthma enrolled in omalizumab clinical studies were pooled together to generate 20 'in-vivo' samples. Each 'in-vivo' pooled sample was created from sera from two to three clinical samples with similar free IgE and omalizumab levels. 'In-vivo' pooled samples were targeted to have three levels of free IgE with varying levels of omalizumab.

| Free IgE Group | Free IgE range               | No. of samples |
|----------------|------------------------------|----------------|
| Low            | 0–15 ng/mL (0–6.2 IU/mL)     | 6              |
| Mid            | 16–50 ng/mL (6.3–20.7 IU/mL) | 7              |
| High           | 51–150 ng/mL (20.8–62 IU/mL) | 7              |
| Total          |                              | 20             |

\*Xolair is a registered trade name of Roche/Genentech, South San Francisco, CA, USA; and Novartis AG, Basel, Switzerland

Curr Med Res Opin Downloaded from informahealthcare.com by 212.66.126.223 on 07/14/14  
For personal use only.

**Table 2.** Summary of 36 ‘in-vitro’ samples with known omalizumab levels. Allergic asthma sera were obtained from 15 individuals, who had not been previously exposed to omalizumab, and grouped according to measured total IgE levels. For each sample in each total IgE group, omalizumab was added at approximately either 0, 3, 9, or 27× the molar level of total IgE to generate a total of 36 ‘in-vitro’ samples.

| Total IgE group | Total IgE range                | No. of individual sera | Approximate molar ratios of omalizumab: total IgE added to each serum | No. of samples generated per individual serum | Total no. of samples |
|-----------------|--------------------------------|------------------------|-----------------------------------------------------------------------|-----------------------------------------------|----------------------|
| Low             | 121–363 ng/mL (50–150 IU/mL)   | 3                      | 0, 3, 9, 27×                                                          | 4                                             | 12                   |
| Mid             | 364–847 ng/mL (150–350 IU/mL)  | 3                      | 0, 3, 9, 27×                                                          | 4                                             | 12                   |
| High            | 848–1694 ng/mL (350–700 IU/mL) | 1                      | 0, 3, 9, 27×                                                          | 4                                             | 4                    |
|                 |                                | 2                      | 0×                                                                    | 1                                             | 2                    |
|                 |                                | 2                      | 3×                                                                    | 1                                             | 2                    |
|                 |                                | 2                      | 9×                                                                    | 1                                             | 2                    |
|                 |                                | 2                      | 27×                                                                   | 1                                             | 2                    |
| Total           |                                | 15                     |                                                                       |                                               | 36                   |

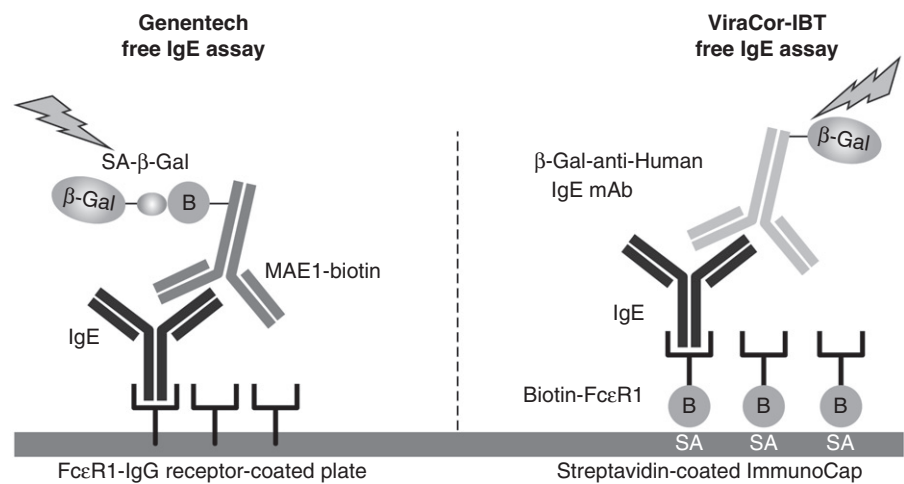


**Figure 1.** Free IgE ligand binding assays by Genentech and Viracor-IBT. The Viracor-IBT assay is a fluoro-enzyme immunoassay (FEIA) similar to the Genentech assay but utilizing an ImmunoCAP (a cellulose sponge) instead of a microtiter plate as the solid phase. Omalizumab and FcεR1 are both specific for the Fc-fragment of human IgE, thus any IgE bound to omalizumab is blocked from being captured and only free IgE is detected. B, biotin; β-Gal, beta galactosidase; mAb, monoclonal antibody; SA, streptavidin; SA-β-Gal, streptavidin-beta-galactosidase.

omalizumab-naïve individuals with known levels of total IgE (Table 2). The individual human sera were purchased from Bioreclamation (Hicksville, NY, USA). Total IgE levels were within the Food and Drug Administration (FDA) approved total IgE target-treatment range for omalizumab (30–700 IU/mL). Known levels of omalizumab were added to individual human sera to obtain a wide range of omalizumab/total IgE molar ratios (from 0 to 27).

Free IgE assays

Genentech

The Genentech assay is a fluorescent version of the free IgE enzyme-linked immunosorbent assay (ELISA) reported by Fahy *et al.* (1997) and Ådelroth *et al.* (2000)<sup>11,12</sup>. The Genentech free IgE assay detects only free IgE and has a reporting range of 2–150 ng/mL (0.83–62 IU/mL). (Figure 1, Table E1). This assay has four controls – a low, mid, high and a complex control. The low,

mid and high concentration controls were made by diluting a human plasma with high total IgE in fetal bovine serum (FBS) targeting concentrations of 4, 20, and 100 ng/mL human IgE. The complex control contains a mixture of omalizumab and human IgE in human serum and targets the middle of the calibration curve to produce a free human IgE level of approximately 20 ng/mL. The complex control is most relevant as it has complexes of omalizumab and IgE identical to in vivo conditions. The inter-assay variance on this control was 6.4% during validation.

Viracor-IBT

The Viracor-IBT free IgE assay uses the same capture reagent as the Genentech free IgE assay, but utilizes high binding ImmunoCAP instead of ELISA plates, a different detection reagent, sample incubation times and sample dilutions (Figure 1, Table E1). The reporting range is 12.3–2662 ng/mL (5.1–1100 IU/mL); however, this assay is no longer commercially available.

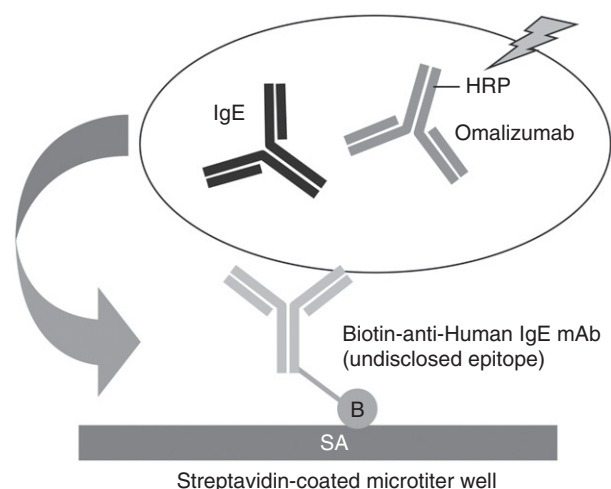


**Figure 2.** The BioTeZ assay (recovery ELISA IgE/omalizumab). The BioTeZ assay measures both free IgE and omalizumab levels in a single assay. Samples are treated with four concentrations of added IgE and incubated simultaneously with HRP-labeled omalizumab in a streptavidin-coupled microtiter plate coated with a biotin-labeled anti-human-IgE mAb. Only free IgE should be detected since the IgE/omalizumab complexes in the sample should not bind the HRP-omalizumab detection reagent. Standard curves containing different levels of IgE combined with different levels of unlabeled omalizumab are used with a complex equation to interpolate both free IgE and omalizumab levels in the samples. B, biotin; HRP, horseradish peroxidase; mAb, monoclonal antibody; SA, streptavidin.

### BioTeZ

The BioTeZ recovery ELISA uses the method reported previously by Strohner *et al.* (2012)<sup>13</sup> to simultaneously quantify total therapeutic antibody level, in this case omalizumab, and the free level of the target antigen (in this case IgE) in human serum samples. The free IgE reporting range is 2.4–4840 ng/mL (1–2000 IU/mL) in the absence of omalizumab and 2.4–1210 ng/mL (1–500 IU/mL) in the presence of omalizumab (Figure 2, Table E1).

### Total IgE assay

Total IgE was measured in all 56 human serum samples by Viracor-IBT laboratories (Lee's Summit, MO, USA) using a commercially available assay (ImmunoCAP 1000†) with a reporting range of 4.84–12,100 ng/mL (2–5000 IU/mL). The assay was verified by Genentech to have no interference from omalizumab.

### Total omalizumab assay

#### Genentech

The Genentech total omalizumab assay is a sequential ELISA with a reporting range  $\geq 28$  ng/mL<sup>14</sup>.

†ImmunoCAP 1000 is a registered trade name of Phadia AB, Uppsala, Sweden

### BioTeZ

The BioTeZ recovery ELISA uses the method reported previously by Strohner *et al.* (2012)<sup>13</sup> to simultaneously quantify total omalizumab and the free IgE; the omalizumab reporting range is 400–80,000 ng/mL (Figure 2, Table E2).

### Free IgE and omalizumab assay comparison

All 56 in vitro and in vivo serum samples were randomized with unique identification numbers and sent out for blinded evaluation to: 1) Viracor-IBT to determine free IgE using the Viracor-IBT assay; 2) a contract research organization (CRO) to determine free IgE using the Genentech free IgE ELISA and the BioTeZ recovery ELISA IgE/omalizumab assay; and 3) the CRO also obtained omalizumab levels using either the BioTeZ recovery ELISA or the Genentech total omalizumab ELISA.

### BioTeZ reproducibility

Based on the data from the first round of evaluation any samples in the BioTeZ recovery ELISA exhibiting free IgE levels of <30% or >300% of those obtained in the Genentech free IgE assay were considered extreme outliers. Twenty out of the 56 samples analyzed were considered outliers. To confirm the outlier data, these samples were re-randomized and submitted to a third party CRO for a second round of blinded analysis using the BioTeZ assay.

### Data analysis

In all, six assays were performed in two CROs to determine free IgE (using three different assay formats), total IgE (by Phadia's ImmunoCap assay) and omalizumab (by the Biotez and Genentech assay formats) levels in 56 in vitro and in vivo samples. Data obtained from each of the 56 samples at Viracor-IBT laboratories/CRO(s) were unblinded and compiled at Genentech. Samples that yielded less than reportable (LTR) or greater than reportable (GTR) results in any of the six assays were entered as either half the LTR or twice the GTR limits for the specific assay. Among the 56 samples, 12 samples, (three in-vivo samples plus nine in-vitro samples) were categorized as omalizumab-negative; the remaining 44 samples (17 in-vivo samples plus 27 in-vitro samples) were categorized as omalizumab-positive. The molar ratio of omalizumab/total IgE was calculated for each sample using the results from Genentech's total omalizumab assay and Phadia's ImmunoCap total IgE assay.

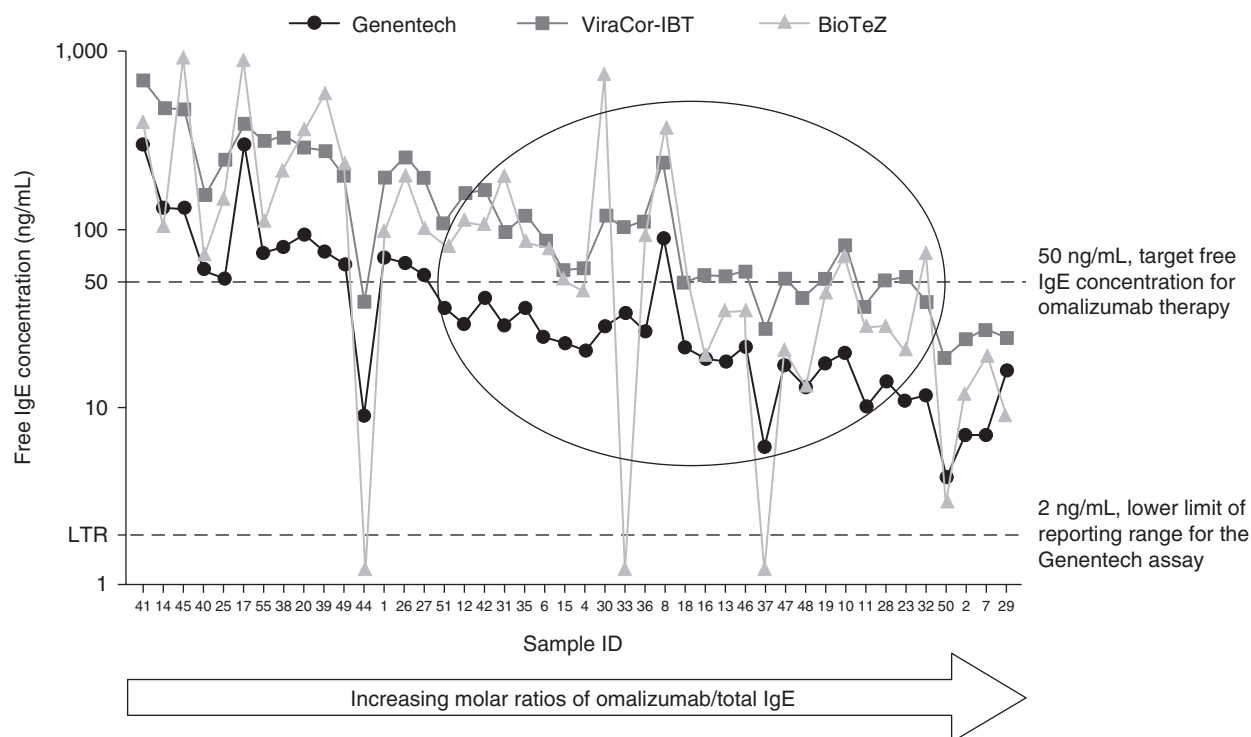


**Figure 3.** Comparison of free IgE levels measured using the Genentech, Viracor-IBT, and BioTeZ free IgE assays in the 44 omalizumab-positive samples sorted by increasing molar ratios of omalizumab/total IgE. Free IgE levels decreased with increasing omalizumab/total IgE molar ratios. Several samples screening at  $\leq 50$  ng/mL free IgE in Genentech assay exhibited  $>50$  ng/mL free IgE in commercial assays. The oval highlights a subset of omalizumab-positive samples determined to have  $<50$  ng/mL free IgE in the Genentech assay, but in general to have  $>50$  ng/mL free IgE in the commercial assays. LTR, lower than reportable.

## Results

### Evaluation of free IgE levels

All three assays showed a trend of decreasing free IgE with increasing omalizumab/total IgE molar ratios (Figure 3). However, both the Viracor-IBT and BioTeZ assays tended to measure significantly higher levels of free IgE compared with the Genentech assay in the omalizumab-positive samples. Twelve (41%) and 20 (69%) of the 29 omalizumab-positive samples that had free IgE levels  $<50$  ng/mL in the Genentech assay were  $>50$  ng/mL in the BioTeZ and Viracor-IBT free IgE assays, respectively (Table 3). Two of 44 omalizumab-positive samples (numbers 17 and 41) were GTR in the Genentech free IgE assay (Figure 3). However, three of the 44 omalizumab-positive samples (numbers 33, 37, and 44) with detectable free IgE levels in both the Genentech and Viracor-IBT assays were LTR or false negatives in the BioTeZ assay (Figure 3); and three of the 12 omalizumab-negative samples (numbers 5, 52, and 56) had significantly lower levels of free IgE detected in the BioTeZ assay compared with the Genentech and Viracor-IBT methods (data not shown). Forty-six of the original 56 samples had free IgE data in the measurable range of the Genentech free IgE assay ( $<150$  ng/mL; data not shown). For these 46 samples the

BioTeZ free IgE assay had an average percentage change of  $191 \pm 392$  (%CV = 205) and the Viracor-IBT free IgE assay had an average percentage change of  $230 \pm 95$  (%CV = 41) compared with the Genentech free IgE assay.

### Evaluation of total IgE levels

Total IgE data generated by Phadia's ImmunoCAP was used to calculate the omalizumab/total IgE molar ratio of each sample as described in *Data analysis*. As noted by Hamilton *et al.*<sup>15,16</sup>, all commercial, FDA-approved total IgE assays, including Phadia's ImmunoCAP assay, are calibrated to the WHO IRP 75/502 total IgE standard and exhibit accuracy within  $\sim 10\%$  of each other. The precision of the different total IgE assays are all  $\sim 9\%$ , indicating no advantage to one particular total IgE assay method over another for samples without any omalizumab. However, we chose to use the Phadia ImmunoCAP assay for our analysis because it has been shown (internally at Genentech) to exhibit no interference from omalizumab in samples.

### Evaluation of omalizumab levels

The overall accuracy and precision of the Genentech total omalizumab assay was confirmed during assay validation at

the CRO (inter-assay accuracy of 94.5% to 102.6% and inter-assay precision of 6.1% to 11.2% throughout the reportable range of the assay). The spread of BioTeZ data around the Genentech data shows that the BioTeZ

**Table 3.** Comparison of free IgE results for the 44 omalizumab-positive samples. The omalizumab dosing table was designed to define doses that decrease patient free IgE levels to  $\leq 50$  ng/mL as determined using the Genentech free IgE assay. The 44 omalizumab-positive samples were therefore subdivided using the Genentech free IgE assay into two groups with  $\leq 50$  ng/mL or  $>50$  ng/mL free IgE (29 and 15 samples, respectively). The number of omalizumab-positive samples exhibiting free IgE levels above or below 50 ng/mL in the BioTeZ and Viracor-IBT free IgE assays are compared within the two Genentech free IgE sample subgroups.

| Comparison of free IgE results for the 44 omalizumab-positive samples | Genentech Free IgE    |                   |
|-----------------------------------------------------------------------|-----------------------|-------------------|
|                                                                       | $\leq 50$ ng/mL<br>29 | $>50$ ng/mL<br>15 |
| BioTeZ Free IgE                                                       |                       |                   |
| $\leq 50$ ng/mL                                                       | 17                    | 0                 |
| $>50$ ng/mL                                                           | 12                    | 15                |
| Viracor-IBT Free IgE                                                  |                       |                   |
| $\leq 50$ ng/mL                                                       | 9                     | 0                 |
| $>50$ ng/mL                                                           | 20                    | 15                |

Twelve (41%) and 20 (69%) of the 29 samples with  $\leq 50$  ng/mL free IgE in the Genentech free IgE assay tested as  $>50$  ng/mL in the BioTeZ and Viracor-IBT free IgE assays respectively. Fifteen (100%) of the 15 samples with  $>50$  ng/mL free IgE in the Genentech free IgE assay tested as  $>50$  ng/mL in both the BioTeZ and Viracor-IBT free IgE assays.

omalizumab assay was highly variable, detecting approximately 20% to 3000% of omalizumab reported by the Genentech assay (Figure 4). In addition, the BioTeZ assay generated false-positive results in three of the twelve omalizumab-negative samples (numbers 5, 52, and 56) and false-negative results in eight of the 44 omalizumab-positive samples (numbers 8, 17, 30, 31, 33, 39, 45 and 49) compared with the results from the Genentech assay (Figure 4).

Reproducibility of the BioTeZ assay

Twenty samples that exhibited free IgE levels as  $<30\%$  or  $>300\%$  of the corresponding Genentech free IgE results, when first measured using the BioTeZ assay, were re-randomized and reanalyzed using the BioTeZ assay (Table 4). In repeat free IgE evaluations with the BioTeZ assay, more than half of the samples (11/20) had inter-assay differences ranging from 40% to 190%. The remaining nine samples had free IgE results with a  $\leq 30\%$  difference between the two evaluations. Similar inconsistencies between BioTeZ assays were observed for total omalizumab measurements (Table 4). Seventy percent of the in vivo samples (14/20) had inter-assay differences ranging from 33% to 183%. The remaining six samples had omalizumab levels that differed by less than 30% between two evaluations.

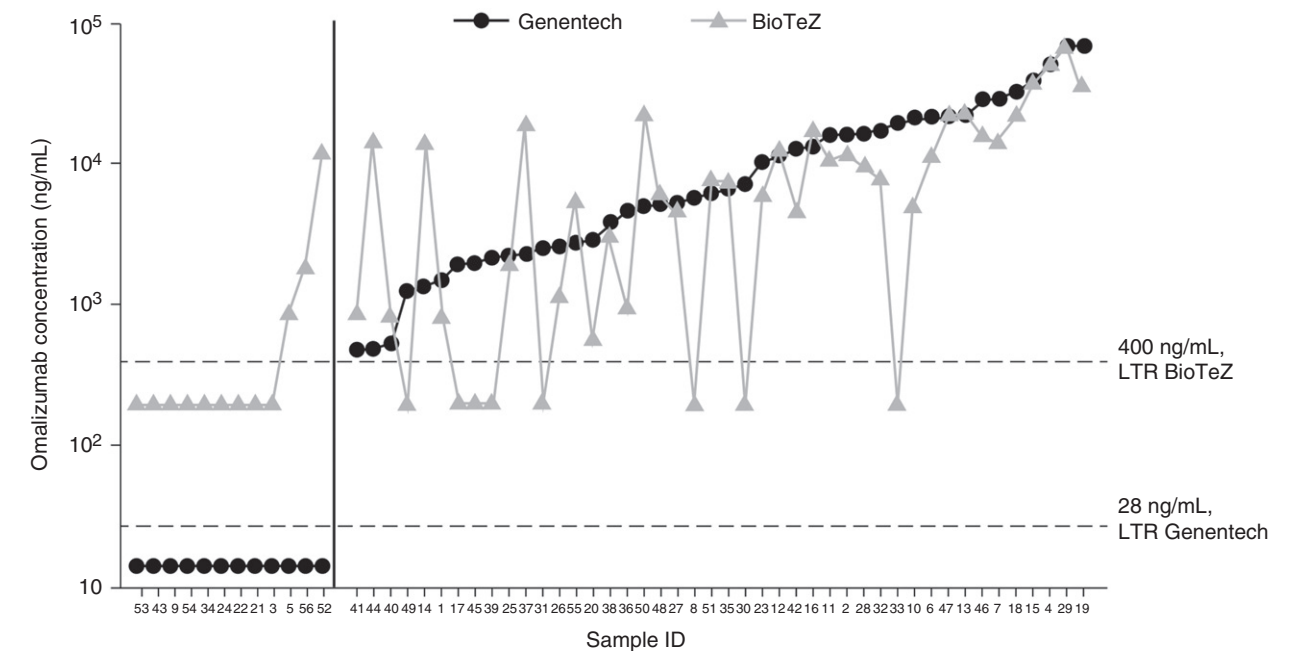


**Figure 4.** Comparison of omalizumab levels in all 56 samples measured using the Genentech and BioTeZ omalizumab assays and sorted by increasing omalizumab levels detected by using the Genentech assay. The vertical line divides the 12 omalizumab-negative (left-side) and 44 omalizumab-positive (right-side) samples. The BioTeZ assay detected three false-positives (samples above the 400 ng/mL lower limit of reporting for the BioTeZ assay) in the 12 omalizumab negative samples and seven false-negatives (samples below the 400 ng/mL lower limit of reporting for the BioTeZ assay) in the 44 omalizumab-positive samples. LTR, lower than reportable.

**Table 4.** Repeat BioTeZ assay of free IgE and omalizumab levels in 20 samples showing large discrepancies between the Genentech assay and the BioTeZ assay on first testing in the BioTeZ IgE assay. Reproducibility of the BioTeZ free IgE and omalizumab assay was tested by repeat analysis of 20 samples determined to have <30% to >300% of the corresponding Genentech free IgE results in the first analysis.

| Sample ID | Free IgE level (ng/mL) |          | % Difference <sup>a</sup> | Omalizumab level (ng/mL) |          | % Difference <sup>b</sup> |
|-----------|------------------------|----------|---------------------------|--------------------------|----------|---------------------------|
|           | 1st test               | 2nd test |                           | 1st test                 | 2nd test |                           |
| 5         | 15                     | 39       | 89                        | 875                      | <400     | 126                       |
| 6         | 83                     | 41       | 68                        | 11,787                   | 16,419   | 33                        |
| 8         | 385                    | 74       | 136                       | <400                     | 875      | 126                       |
| 10        | 71                     | 54       | 28                        | 4991                     | 4061     | 21                        |
| 12        | 117                    | 136      | 15                        | 12,743                   | 4043     | 104                       |
| 17        | 917                    | 686      | 29                        | <400                     | <400     | 0                         |
| 20        | 357                    | 182      | 65                        | 558                      | 949      | 52                        |
| 26        | 212                    | 534      | 86                        | 1153                     | 200      | 141                       |
| 30        | 755                    | 502      | 40                        | <400                     | <400     | 0                         |
| 31        | 208                    | 129      | 47                        | <400                     | 2915     | 174                       |
| 32        | 75                     | 56       | 30                        | 7866                     | 13,661   | 54                        |
| 33        | <2.4                   | 49       | 190                       | <400                     | 4372     | 183                       |
| 36        | 93                     | 22       | 124                       | 967                      | <400     | 131                       |
| 37        | <2.4                   | <2.4     | 0                         | 19,285                   | 12,927   | 39                        |
| 39        | 602                    | 514      | 16                        | <400                     | <400     | 0                         |
| 44        | <2.4                   | <2.4     | 0                         | 14,476                   | 9487     | 42                        |
| 45        | 941                    | 829      | 13                        | <400                     | <400     | 0                         |
| 49        | 237                    | 213      | 11                        | <400                     | <400     | 0                         |
| 52        | 12                     | 5        | 82                        | 12,054                   | 7756     | 43                        |
| 56        | 369                    | 779      | 71                        | 1854                     | <400     | 161                       |

% Difference =  $(|Value1 - Value2| / ((Value1 + Value2) / 2)) * 100$ .

<sup>a</sup>Eleven out of 20 samples (see shaded cells) had free IgE results with greater than 30% difference between two evaluations, with differences ranging from 40% to 190%.

<sup>b</sup>Fourteen out of 20 samples (see shaded cells) had omalizumab levels with greater than 30% difference between two evaluations, with differences ranging from 33% to 183%.

Free IgE values of 1.2 ng/mL and omalizumab values of 200 ng/mL were used to calculate % differences for samples with free IgE levels of <2.4 ng/mL and omalizumab levels of <400 ng/mL respectively.

## Discussion

Asthma is a common chronic inflammatory disease of the airway that results in considerable morbidity and mortality. The positive response to IgE-targeted therapy with omalizumab in persistent allergic asthma is well established<sup>2-5</sup>. The omalizumab dosing table was developed using results from Genentech's free IgE assay from multiple clinical studies. Subsequently, the dosing table was confirmed by other investigators to be close to achieving the optimal <50 ng/mL (20.8 IU/mL) free IgE which correlates with improved clinical symptoms<sup>14</sup>. Two commercial free IgE assays have been developed which allow physicians to measure free IgE levels in patient samples post omalizumab treatment. Compared to the Genentech free IgE assay, on which the dosing table in the product label is based, both commercial assays generally overestimated free IgE levels by an appreciable amount. Notably, 12 (41%) and 20 (69%) of the 29 omalizumab-positive samples shown to have IgE levels <50 ng/mL in the Genentech assay returned levels >50 ng/mL in the BioTeZ and Viracor-IBT assays respectively. In addition, the BioTeZ assay reported extreme values (<30% or >300% of the corresponding Genentech data) for approximately one third of the total 56 samples. Repeat evaluation of these samples demonstrated poor reproducibility of the BioTeZ assay for both free IgE and omalizumab levels.

The results reported here thus demonstrate that the commercial assays evaluated are not reliable for monitoring free IgE and, if used during omalizumab treatment, might overestimate free IgE levels. Over-estimation of free IgE levels could have two potential consequences: 1) physicians might unnecessarily increase the omalizumab dose to achieve further reductions in free IgE, thereby incurring additional cost without providing additional benefit to the patient, further potentially administer off-label doses of omalizumab; or 2) they might prematurely discontinue treatment on the mistaken assumption that the treatment is not effectively reducing free IgE.

Genentech does not intend to claim that their method of free IgE quantification is superior to that of the commercial assay formats evaluated in this study. Rather they wish to re-emphasize that the Genentech free IgE assay is the clinically relevant assay because it was used to generate the FDA-approved dosing table for omalizumab. The doses recommended in the dosing table aim to achieve serum levels of free IgE (i.e. IgE not complexed with omalizumab) of <50 ng/mL (20.8 IU/mL), levels known to deliver clinical benefit<sup>8</sup>. The omalizumab product label clearly states that on-treatment re-testing of serum total IgE levels cannot be used to guide dosing, and re-initiation of treatment after a period of treatment of less than 1 year should be based on the original pre-treatment serum levels of IgE<sup>6</sup>.

Furthermore, the product label does not state that free IgE levels should be monitored after treatment is initiated.

The stability of the IgE/therapeutic antibody complex can be affected by multiple factors, resulting in different free IgE measurements depending on the assay format, reagents used, sample dilution, and incubation times. The over-estimation of free IgE levels by the commercial assays evaluated relative to the Genentech assay may arise from use of methodologies that promote disruption of omalizumab-IgE complexes. The Viracor-IBT free IgE method used a very similar format to the Genentech method (Figure 1), but used the Phadia ImmunoCap technology versus the ELISA format. One reason for over-recovery in the Viracor-IBT method is the use of the Phadia technology. The high binding capacity of the ImmunoCap cellulose sponge in addition to the high IgE binding affinity of the receptor compared with omalizumab for IgE can lead to disruption of the omalizumab-IgE complex resulting in over-recovery of free IgE. The unusually long sample incubation times and high sample dilution could be responsible for over-recovery of free IgE in the BioTeZ method. The complexity of free ligand assays underscores the importance of adhering to the approved dosing table that was developed based on data from the Genentech assay.

From a clinical perspective, although omalizumab rapidly reduces free IgE (within 1 hour post-dose)<sup>8</sup>, responses to omalizumab treatment, and the timing of these responses, vary between individuals. Hamilton *et al.* (2005) suggested that analysis of the free and total IgE levels 1 month post omalizumab treatment may aid physicians in confirming adequate dosing for patients who do not experience the anticipated clinical response<sup>10</sup>. However, response to omalizumab is typically not observed until 12 to 16 weeks after dosing<sup>7</sup>, and in some patients can take longer<sup>17–19</sup>. In an analysis of two clinical studies of patients with moderate-to-severe allergic asthma, it was noted that, among patients who responded to 16 weeks of treatment with omalizumab, only 61% responded at 4 weeks whereas 87% had responded at 12 weeks<sup>18,19</sup>. Furthermore, another study shows that among 71 allergic asthmatics who did not exhibit a clinical response within the first 16 weeks of treatment with omalizumab added to optimized asthma therapy, 27 responded to omalizumab after a further 16 weeks of treatment<sup>20</sup>. This suggests that, by prolonging the initial observation time, it could be possible to extend the therapeutic benefits of omalizumab to a larger subgroup of potential 'late responders' who were prematurely classified as non-responders on the basis of the 16 week period of clinical evaluation.

It should, however, be acknowledged that some patients do not respond to omalizumab and there are many potential underlying factors affecting response other than free IgE levels<sup>21</sup>.

As specified by the prescribing information, the most effective method for evaluating continued need for

omalizumab is still periodic assessment of the patient's disease severity and level of asthma control (as specified in the prescribing information)<sup>6</sup>.

## Conclusion

There are clearly multiple factors that should be considered in addition to the absolute free IgE levels of patients, as they can impact the efficacy of omalizumab in non-responders. Given the variability in the disease and mechanisms of non-responsiveness to omalizumab, achieving the same target free IgE level for every individual may not be adequate to improve the overall response rate to omalizumab treatment. Moreover, it is important to note that the omalizumab dosing table was developed based on data measured by the free IgE assay developed by Genentech. Our findings demonstrate that commercially available assays do not provide a reliable assessment of free IgE levels and indicate that use of these assays to monitor omalizumab therapy and adjust omalizumab doses off-label could have negative consequences for patients. The prescribing information for omalizumab states that dosing should be determined based on total IgE at baseline; total IgE levels are elevated during omalizumab treatment due to circulating IgE-omalizumab complexes. Accordingly re-testing IgE levels cannot be used to determine omalizumab doses<sup>6</sup>. Our findings demonstrate that commercially available assays do not provide a reliable assessment of free IgE levels and indicate that use of these assays to monitor omalizumab therapy and adjust omalizumab doses off-label could have negative consequences for patients.

## Transparency

### Declaration of funding

This study was funded by Genentech Inc., South San Francisco, CA, USA.

Dana L. Baker

Kun Peng

Melissa Cheu

Saloumeh Kadkhodayan Fischer

### Declaration of financial/other relationships

D.L.B., K.P., M.C., and S.K.F. have disclosed that they are all employees of Genentech Inc., South San Francisco, CA, USA.

CMRO peer reviewers may have received honoraria for their review work. The peer reviewers on this manuscript have disclosed that they have no relevant financial relationships.

### Acknowledgments

Medical writing support was provided by professional medical writer Christine Arris PhD (CircleScience), funded by Genentech Inc., South San Francisco, CA, USA.

## References

- Global Initiative for Asthma (GINA). From the global strategy for asthma management and prevention. Available at: <http://www.ginasthma.org> [Last accessed 25 November 2013]
- Burrows B, Martinez FD, Halonen M, et al. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271-7
- Holgate S, Casale T, Wenzel S, et al. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005;115:459-65
- Ishizaka K, Ishizaka T. Identification of gamma-E-antibodies as a carrier of reaginic activity. *J Immunol* 1967;99:1187-98
- Johansson SG, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967;13:381-94
- Genentech, Novartis US. Xolair FDA Prescribing Information. Available at: <http://www.gene.com/gene/products/information/pdf/xolair-prescribing.pdf> [Last accessed 25 November 2013]
- European Medicines Agency (EMA). Xolair EPAR – product information. Available at: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Product\\_Information/human/000606/WC500057298.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000606/WC500057298.pdf) [Last accessed 25 November 2013]
- Hochhaus G, Brookman L, Fox H, et al. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003;19:491-8
- Casale TB, Bernstein IL, Busse WW, et al. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;100:110-21
- Hamilton RG, Marcotte GV, Saini SS. Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy. *J Immunol Methods* 2005;303:81-91
- Fahy JV, Fleming HE, Wong HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828-34
- Ädelroth E, Rak S, Haahtela T, et al. Recombinant humanized mAb-E25, an anti-IgE mAb, in birch pollen-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;106:253-9
- Strohner P, Staatz A, Sarrach D, et al. The recovery ELISA – a newly developed immunoassay for measurement of therapeutic antibodies and the target antigen during antibody therapy. *Clin Chem Lab Med* 2012;50:1263-9
- Lowe PJ, Tannenbaum S, Gautier A, Jimenez P. Relationship between omalizumab pharmacokinetics, IgE pharmacodynamics and symptoms in patients with severe persistent allergic (IgE-mediated) asthma. *Br J Clin Pharmacol* 2009;68:61-76
- Hamilton RG. Proficiency survey-based evaluation of clinical total and allergen-specific IgE assay performance. *Arch Pathol Lab Med* 2010;134:975-82
- Hamilton RG. Accuracy of Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). *J Allergy Clin Immunol* 2006;117:759-66
- Bousquet J, Wenzel S, Holgate S, et al. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. *Chest* 2004;125:1378-86
- Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184-90
- Soler M, Matz J, Townley R, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 2001;18:254-61
- Bousquet J, Siergiejko Z, Swiebocka E, et al. Persistency of response to omalizumab therapy in severe allergic (IgE-mediated) asthma. *Allergy* 2011;66:671-8
- Heaney LG, Robinson DS. Severe asthma treatment: need for characterising patients. *Lancet* 2005;365:974-6

## Supplementary tables

Table E1. Overview of free IgE assays.

| Free IgE assays     |                                        |                                                                      |                                                                                                  |
|---------------------|----------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
|                     | Genentech                              | ViraCore-IBT                                                         | BioTeZ                                                                                           |
| Assay format        | Sequential ELISA                       | Sequential ImmunoCap                                                 | Simultaneous ELISA                                                                               |
| Capture reagent     | FcεR1-IgG                              | SA-ImmunoCap/Biotin-FcεR1                                            | SA-well/Biotin-anti-hu IgE mAb                                                                   |
| Detection reagent   | Biotin-anti-hu IgE mAb (with SA-β-Gal) | β-Gal-anti-hu IgE mAb                                                | HRP-omalizumab                                                                                   |
| Sample dilution     | Neat (1:2 in well)                     | Neat                                                                 | BioTeZ 1:20–1:40 (1:80–1:160 in well)                                                            |
| Sample incubation   | 1.5 hrs at RT                          | 0.5 hrs at RT                                                        | Overnight at 2–8°C                                                                               |
| IgE reporting range | 0.83–62 IU/mL (2–150 ng/mL)            | 5.1–1100 IU/mL (12.3–2662 ng/mL)                                     | Without omalizumab 1–2000 IU/mL (2.4–4840 ng/mL)<br>With omalizumab 1–500 IU/mL (2.4–1210 ng/mL) |
| Data reported       | Free IgE                               | Free IgE, total IgE, free/total IgE ratio (total IgE separate assay) | Free IgE and omalizumab                                                                          |

β-Gal, beta-galactosidase; C, centigrade; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; hu, human; mAb, monoclonal antibody; SA, streptavidin; SA-β-Gal, streptavidin-beta-galactosidase; RT, room/ambient temperature.

Table E2. Overview of omalizumab assays.

| Omalizumab assays          |                                              |                                |
|----------------------------|----------------------------------------------|--------------------------------|
|                            | Genentech                                    | BioTeZ                         |
| Assay format               | Sequential ELISA                             | Simultaneous ELISA             |
| Capture reagent            | Human-myeloma-derived IgE from the U266 cell | SA-well/Biotin-anti-hu IgE mAb |
| Detection reagent          | HRP-anti-omalizumab mAb                      | HRP-omalizumab                 |
| Sample dilution            | ≥1:100                                       | 1:20–1:40 (1:80–1:160 in well) |
| Sample incubation          | Overnight at RT                              | Overnight at 2–8°C             |
| Omalizumab reporting range | ≥28 ng/mL                                    | 400–80,000 ng/mL               |
| Data reported              | Omalizumab                                   | Omalizumab and free IgE        |

C, centigrade; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; hu, human; mAb, monoclonal antibody; RT, room/ambient temperature; SA, streptavidin.

Curr Med Res Opin Downloaded from informahealthcare.com by 212.66.126.223 on 07/14/14  
For personal use only.