

Efficacy and safety of multiple doses of QGE031 (ligelizumab) versus omalizumab and placebo in inhibiting allergen-induced early asthmatic responses

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Background: Omalizumab is an established anti-IgE therapy for the treatment of allergic diseases that prevents IgE from binding to its receptor. QGE031 is an investigational anti-IgE antibody that binds IgE with higher affinity than omalizumab. **Objective:** This study compared the effects of QGE031 with those of omalizumab on clinical efficacy, IgE levels, and FcεRI expression in a clinical model of allergic asthma.

Methods: Thirty-seven patients with mild allergic asthma were randomized to subcutaneous omalizumab, placebo, or QGE031 at 24, 72, or 240 mg every 2 weeks for 10 weeks in a double-blind, parallel-group multicenter study. Inhaled allergen challenges and skin tests were conducted before dosing and at weeks 6, 12, and 18, and blood was collected until 24 weeks after the first dose.

Results: QGE031 elicited a concentration- and time-dependent change in the provocative concentration of allergen causing a 15% decrease in FEV₁ (allergen PC₁₅) that was maximal and approximately 3-fold greater than that of omalizumab ($P = .10$) and 16-fold greater than that of placebo ($P = .0001$) at week 12 in the 240-mg cohort. Skin responses reached 85% suppression at week 12 in the 240-mg cohort and were maximal at week 18. The top doses of QGE031 consistently suppressed skin test

responses among subjects but had a variable effect on allergen PC₁₅ (2-fold to 500-fold change). QGE031 was well tolerated. **Conclusion:** QGE031 has greater efficacy than omalizumab on inhaled and skin allergen responses in patients with mild allergic asthma. These data support the clinical development of QGE031 as a treatment of asthma. (*J Allergy Clin Immunol* 2016;■■■:■■■-■■■.)

Key words: Asthma management, allergy, immunology, IgE, ligelizumab, mAb, QGE031, biologics

Allergic diseases, including asthma, are dependent on the development of increased IgE levels.¹ Allergen-specific IgE plays a central role in the initiation and propagation of the inflammatory cascade in allergic responses. In susceptible subjects exposure to specific antigens results in production of allergen-specific IgE that binds to high-affinity IgE receptors (FcεRI) on the surfaces of mast cells or basophils.^{2,3} The binding of allergen to the specific cell-bound IgE results in degranulation of preformed mediators, such as proteases and vasoactive histamines, synthesis of lipid-derived eicosanoids, leukotrienes, and prostaglandins and

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consultant for Novartis and Boehringer; receives payments for lectures from Novartis, Boehringer Ingelheim, and AstraZeneca; and receives payments for development of educational presentations from Boehringer Ingelheim and Novartis. B. Dahlen receives grant support from Novartis, receives payments for lectures from Novartis, Merck, Sharp and Dohme. M. Lavolette serves as a consultant for GlaxoSmithKline and receives payment for lectures from Merck, AstraZeneca, GlaxoSmithKline, and Boston Scientific. P. J. Lowe is an employee of and owns stock options in Novartis Pharma AG. A. Skerjanec is an employee of and owns stock options in Novartis Pharma AG. S. Maahs is an employee of Novartis. A. Drollmann is an employee and owns stock options of Novartis Pharma AG. P. M. O'Byrne serves as a consultant for AstraZeneca, GlaxoSmithKline, Merck, Boehringer, and Novartis and receives grant support from AstraZeneca, Amgen, Genentech, Axikin, and Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used

Allergen PC₁₅: Provocative concentration of allergen causing a 15% decrease in FEV₁
 CV: Coefficient of variation
 Methacholine PC₂₀: Provocative concentration of inhaled methacholine to reduce FEV₁ by 20%

transcription of cytokines. Together, these mediators induce early- and late-phase allergic responses.^{2,3}

Omalizumab was the first anti-IgE therapy and humanized mAb licensed for the treatment of patients with moderate-to-severe persistent allergic asthma.⁴ Omalizumab binds to the constant region (Cε3) of the IgE molecule⁵⁻⁷ and prevents free IgE binding to IgE receptors. The reduction in serum free IgE levels leads to downregulation of FcεRI surface expression on effector cells,⁵⁻⁹ further dampening the effector cell response to allergen.¹⁰ Correlations between free IgE levels and asthma symptoms in controlled clinical studies suggest that greater IgE suppression leads to better clinical benefits.¹¹

QGE031 is a fully humanized IgG₁ mAb directed against human IgE, which binds to the Cε3 domain of IgE with higher affinity than omalizumab.¹² QGE031 is designed to obtain greater suppression of free IgE and IgE bound to mast cells and basophils compared with that observed with omalizumab; this might translate into superior interruption of the allergic cascade and greater clinical efficacy in asthmatic patients. The results from 2 phase I randomized, double-blind, placebo-controlled clinical trials investigating the pharmacokinetics, pharmacodynamics, and safety of QGE031 in atopic but otherwise healthy subjects have demonstrated greater suppression of free IgE, basophil FcεRI, and surface IgE and skin prick test responses to allergen by QGE031 when compared with omalizumab.¹² The current study investigated whether QGE031 also enhances suppression of allergen-induced responses in the airways.¹³

METHODS

Study design

This was a double-blind, randomized, placebo-controlled, parallel-group multicenter study in patients with mild allergic asthma. Subjects were recruited at 7 centers in 2 countries (6 sites in Canada and 1 site in Sweden), and the study was conducted between November 15, 2012, and October 28, 2013. The study consisted of a screening period to assess eligibility, followed by a baseline evaluation of responses to allergen (skin testing). Subjects who met the eligibility criteria were randomized to receive 6 treatment interventions of QGE031 (24, 72, or 240 mg), omalizumab per the dosing table,¹⁴ or placebo every other week for 10 weeks. Skin prick and bronchial provocation tests to assess the provocative concentration of allergen causing a 15% decrease in FEV₁ (allergen PC₁₅) were performed at baseline and 6, 12, and 18 weeks after the first dose, and blood samples were collected throughout the study for pharmacokinetics and serum IgE and basophil assessments (Fig 1). The study protocol was approved by local ethics committees and registered with ClinicalTrials.gov as NCT01703312.

Subjects

Eligible subjects were men and women aged 18 to 65 years with mild allergic asthma defined by having a provocative concentration of inhaled methacholine to reduce FEV₁ by 20% (methacholine PC₂₀) of 16 mg/mL or less, a positive skin prick test response to 1 or more common aeroallergens, and bronchoconstriction to inhaled allergen by demonstrating a decrease in

FEV₁ of 15% or greater within 2 hours of the challenge. Subjects were permitted to use occasional inhaled short-acting β₂-agonist therapy (withheld for ≥8 hours before each visit) on an as-needed basis; no other asthma medications were permitted. Current smokers and ex-smokers with a greater than 10 pack-year history were excluded. Subjects could not have poorly controlled asthma, FEV₁ of 70% of predicted value or less, or a respiratory tract infection within 6 weeks before their first screening visit. All subjects provided written informed consent.

Dosing

Subjects received subcutaneous injections over a period of 10 weeks; administration was every 2 weeks for QGE031 or matching placebo, whereas omalizumab and its matching placebo were administered according to the omalizumab dosing table (every 2 or 4 weeks).¹⁴ Subjects remained onsite for observation for a period of 2 hours for the first 3 injections of study drug and 30 minutes for subsequent injections. Subjects, investigative staff, persons performing assessments, and data analysts remained blind to the identity of the treatment (active drug vs placebo) from the time of randomization until database lock.

Allergen PC₁₅ inhalation challenge

Allergen inhalation was performed, as described previously, by means of tidal breathing from a Wright nebulizer (Roxon Medi-Tech, Montreal, Quebec, Canada).¹⁵ During the baseline period, the starting concentration of allergen extract for inhalation was determined from a formula by using results from allergen skin prick titration testing and responsiveness to methacholine.¹⁶

Skin testing

During the screening period, standardized aeroallergen extracts were used for skin prick tests, and subsequent skin titrations administered the selected extract over a range of dilutions. The area under the allergen dilution-response curves for skin wheal diameters was calculated by using the linear trapezoidal rule.

Methacholine inhalation challenge

Methacholine inhalation challenges were performed by means of tidal breathing from a Wright nebulizer,¹⁷ and methacholine PC₂₀ was calculated by using log-linear interpolation.

Blood measurements

Blood was collected before dosing and every other week until 24 weeks after the first dose for measurements of serum total QGE031, serum total IgE, serum free IgE, FcεRI on basophils and dendritic cells, CD23 (FcεRII) on B cells, and bound IgE on basophils.

See the [Methods](#) section in this article's Online Repository at www.jacionline.org for additional information about allergen PC₁₅, skin testing, methacholine challenge, and blood measurements.

Safety

Safety assessments included physical examinations, systems review, open-ended health inquiry, standard 12-lead electrocardiograms, vital signs, clinical laboratory evaluations (hematology; blood chemistry, including renal function; and urinalysis), anti-QGE031 antibodies, and adverse event and serious adverse event monitoring. On dosing days, the injection site was assessed for local reactions.

Study outcomes

The primary objective of this study was to compare the effects of treatment with 240 mg of QGE031 versus omalizumab per the dosing table¹⁴ on the allergen PC₁₅ shift at week 12 compared with baseline values. The secondary

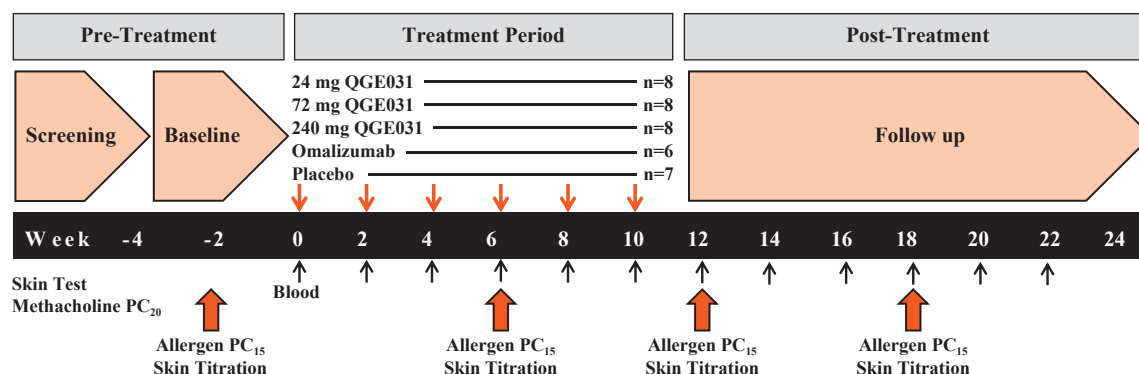


FIG 1. Study design.

objectives were to compare the effects of QGE031 (24 and 72 mg) versus omalizumab on allergen PC₁₅ shift at week 12 and to compare all 3 QGE031 doses (24, 72, and 240 mg) and omalizumab versus placebo on the allergen PC₁₅ shift at week 12; to analyze the pharmacokinetics of QGE031; to analyze pharmacodynamic effects on total IgE, basophil-bound IgE, and basophil FcεRI levels and skin prick test responses; and to evaluate safety and tolerability.

Statistical analysis

The intent-to-treat data were analyzed, unless otherwise indicated. Statistical analysis for allergen PC₁₅ and skin prick test titration area under the curve used a linear mixed-effects model for repeated measures with the log₂-transformed baseline (allergen PC₁₅) and baseline (skin titration area under the curve) as covariates, respectively; treatment, time, and interaction between treatment and time as fixed effects; and interaction between the baseline covariates and time. An autoregressive structured covariance matrix was used to model the correlation of values measured on the same subject. Treatment contrasts were estimated separately for each study day along with 95% CIs, and the contrasts were tested for statistical significance at the 1-sided 2.5% α level. The estimated differences and CIs from the model were back-transformed to obtain geometric means and geometric mean ratios and respective CIs. For basophil surface IgE measurement and expression of FcεRI, no inferential statistical analysis was performed.

Modeling methodology

The pharmacokinetics/pharmacodynamics model descriptive of drug pharmacokinetics (either omalizumab or QGE031) and total circulating IgE, basophil FcεRI, and surface IgE levels was implemented, as previously described by Lowe et al.¹⁸ The detailed pharmacokinetics/pharmacodynamics modeling approach is described in the [Methods](#) section in this article's Online Repository.

RESULTS

Thirty-five of 37 enrolled subjects completed the study as planned. There were no relevant differences in demographic or baseline data between the treatment groups ([Table I](#)). One subject (in the QGE031 24 mg group) withdrew consent because of logistically being unable to complete the study according to the protocol, and 1 subject (placebo group) discontinued trial procedures at the decision of the sponsor (dosing error). Three additional subjects received incorrect treatment allocations: 2 subjects received placebo instead of 24 and 72 mg of QGE031, respectively, for their first study treatment, and 1 subject received the second dose of omalizumab at 2 weeks rather than 4 weeks as intended per the dosing table.

Bronchial allergen challenge responses

The decrease in PC₁₅ varied widely between subjects and ranged from a less than 2- to greater than 500-fold change (0.85-9.2 double dilutions) at week 12 in subjects receiving 72 and 240 mg of QGE031 ([Fig 2, A](#), and see [Table E1](#) in this article's Online Repository at www.jacionline.org).

Both 72 and 240 mg of QGE031 elicited a concentration- and time-dependent change in allergen PC₁₅ that was maximal and approximately 3-fold greater than that for omalizumab at week 12 ($P = .14$ and $P = .10$, respectively) and was returning to baseline by week 18 ([Fig 2, B](#)). The effects on PC₁₅ were also assessed in a sensitivity analysis excluding the 4 misdosed subjects, which showed a positive effect for QGE031 versus omalizumab, reaching statistical significance at week 12 ($P = .03$). Compared with placebo at week 12, 240 mg of QGE031 improved allergen PC₁₅ by 16-fold ($P = .0001$), whereas omalizumab improved allergen PC₁₅ by 5-fold ($P = .02$; [Fig 2, C](#)). QGE031, 24 mg, had minimal effects on allergen PC₁₅.

Skin prick titration response

QGE031 elicited a dose- and time-dependent suppression of the allergen-induced skin test response ([Fig 3, A](#)); at week 12, there was a significant difference between QGE031 cohorts and placebo (all $P < .01$) versus no significant change in the omalizumab cohort ([Fig 3, B and C](#)). Both 72 and 240 mg of QGE031 showed more pronounced inhibition of skin prick test response than omalizumab ($P < .0001$ and $P = .002$), reaching 22%, 74%, and 85% suppression in the omalizumab and 72- and 240-mg QGE031 cohorts, respectively, at week 12. In contrast to the data for inhaled allergen PC₁₅, suppression of the skin prick test response in the 240-mg group was maximal at week 18, 8 weeks after the last dose of QGE031, and was consistently observed in all subjects.

Pharmacokinetics of QGE031

The average steady-state serum concentrations at week 10, before dose 6, and after the 24-, 72-, and 240-mg biweekly subcutaneous doses were 1.8 $\mu\text{g/mL}$ (coefficient of variation [CV], 34%), 5.6 $\mu\text{g/mL}$ (CV, 33%), and 13.2 $\mu\text{g/mL}$ (CV, 25%), respectively. Similar results were found at week 12, one dosing interval after the last dose: 2.3 $\mu\text{g/mL}$ (38%), 6.7 $\mu\text{g/mL}$ (36%), and 15.5 $\mu\text{g/mL}$ (28%), respectively. Exposure increased with dose, with pharmacokinetic steady state achieved at the end of the dosing period.

TABLE I. Demographics and baseline characteristics of study patients by treatment group

Parameters	QGE031, 24 mg (n = 8)	QGE031, 72 mg (n = 8)	QGE031, 240 mg (n = 8)	Omalizumab (n = 6)	Placebo (n = 7)
Age (y [range])	26.3 (24-29)	32.3 (22-59)	34.4 (18-55)	33.0 (22-48)	30.1 (18-50)
Male sex, no. (%)	2 (25.0)	3 (37.5)	4 (50.0)	2 (33.3)	1 (14.3)
Race, no. (%)					
White	3 (37.5)	8 (100.0)	7 (87.5)	5 (83.3)	7 (100.0)
Asian	4 (50.0)	0	0	0	0
Other	1 (12.5)	0	1 (12.5)	1 (16.7)	0
Weight (kg)	64.0 (14.60)	67.8 (18.20)	82.6 (18.64)	77.8 (16.43)	68.4 (14.73)
BMI (kg/m ²)	23.2 (4.45)	23.6 (3.91)	28.6 (6.22)	26.2 (4.17)	24.4 (3.17)
FVC (L)	3.93 (0.93)	4.28 (1.09)	4.43 (1.28)	4.50 (1.10)	4.06 (0.93)
FEV ₁ (L)	3.06 (0.62)	2.98 (0.62)	3.33 (1.00)	3.55 (0.88)	3.18 (0.96)
FEV ₁ (% predicted)	87.63 (7.96)	83.31 (9.93)	90.45 (17.57)	95.17 (11.34)	87.07 (10.75)
Screening total IgE (IU/mL), median (range)	163.00 (83.7-695.0)	117.00 (37.0-336.0)	98.90 (77.1-521.0)	97.95 (41.6-604.0)	97.10 (67.7-295.0)

Data are presented as means (SDs), unless otherwise specified.

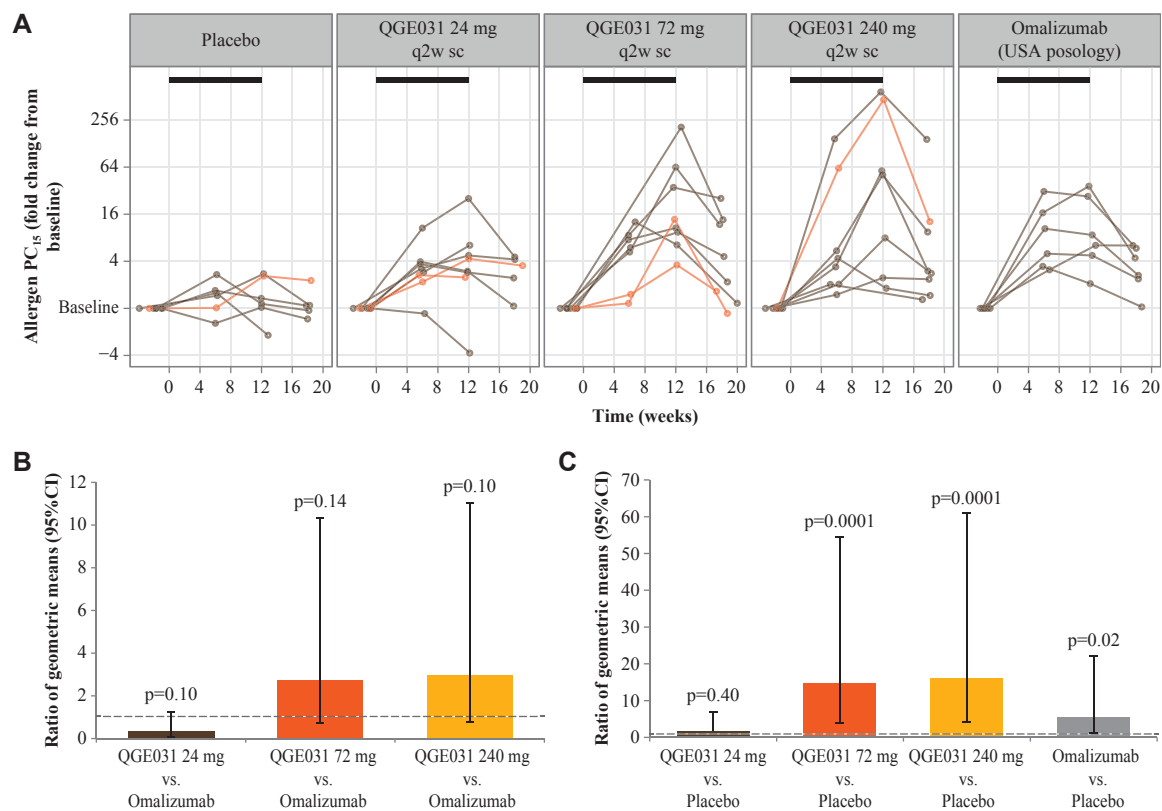


FIG 2. Effect of different doses of QGE031 and omalizumab on allergen PC₁₅ in the airways. **A**, Individual subject time-course data grouped by cohorts for placebo, the 3 dose levels of QGE031, and omalizumab. Data are presented as the fold change from baseline. A 10- or 100-fold decrease in sensitivity indicates that a 10- or 100-fold higher dose of allergen was required to elicit the same 15% change in FEV₁. Red-colored data represent subjects with baseline IgE levels of greater than 700 IU/mL. **B** and **C**, Statistical analyses of the week 12 geometric mean ratios of required allergen concentrations versus omalizumab (Fig 2, B) and placebo (Fig 2, C). Error bars represent 95% CIs.

Effects on total serum IgE levels

Total IgE levels (sum of free and drug-complexed levels) increased from baseline across all treatment groups, except the placebo group. The magnitude and duration of IgE capture was dependent on QGE031 dose (Fig 4, A), with omalizumab also demonstrating the same effect. Although measurements of free IgE were undertaken, all QGE031 doses suppressed IgE levels less than the assay lower limit of quantification in the majority of subjects. Because the downstream basophil surface IgE

(Fig 4, B) and FcεRI (Fig 4, C) biomarkers showed clear responses, no further analysis of free IgE was carried out.

Surface IgE and expression of FcεRI

Although no inferential statistical analysis was performed, we observed numeric dose-dependent changes in basophil surface IgE levels by QGE031. There were consistent reductions in levels of basophil surface IgE in all subjects receiving the 2 higher

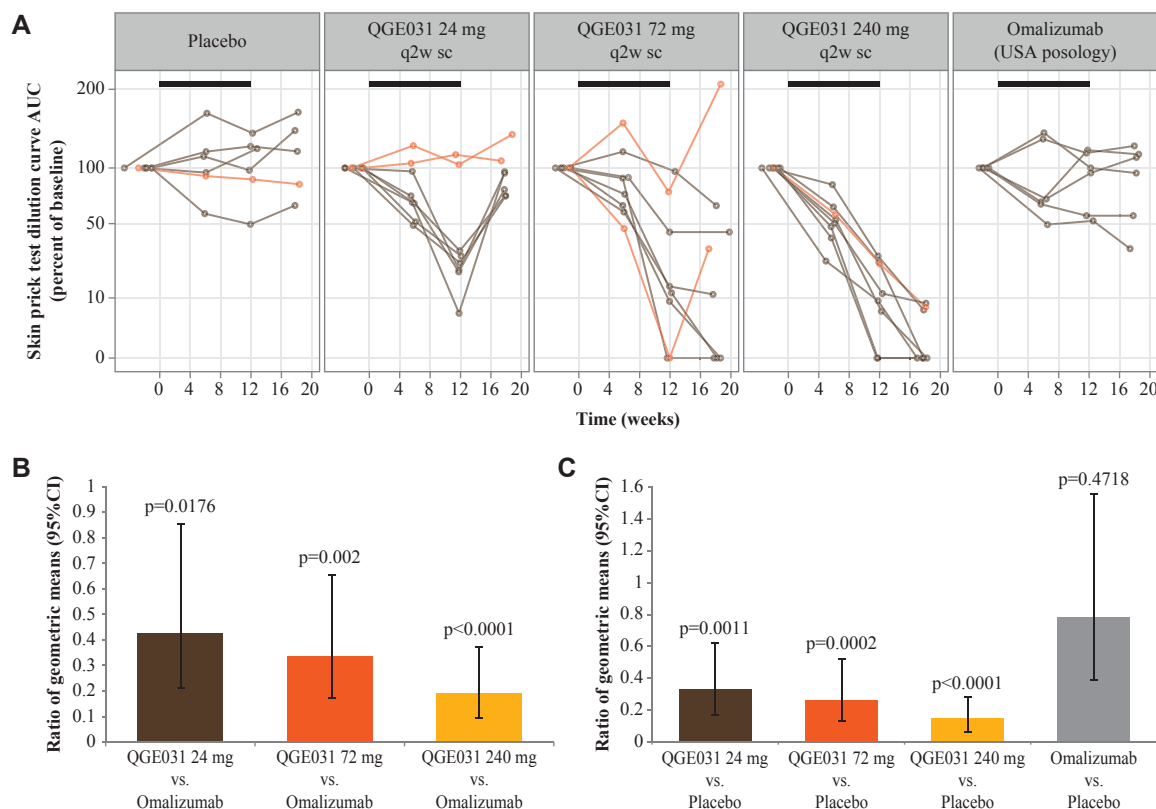


FIG 3. Effect of different doses of QGE031 and omalizumab on the area under the curve (AUC) of the wheal response to allergen skin prick titration with allergen. **A**, Individual subject time-course data grouped by cohorts for placebo, the 3 dose levels of QGE031, and omalizumab. Data are presented as the percentage change in dilution curve AUC from individual patient baseline. Red-colored data represent subjects with baseline IgE levels of greater than 700 IU/mL. **B** and **C**, Statistical analyses of the week 12 geometric mean ratios of AUC of wheal size across all dilutions versus omalizumab (Fig 3, B) and placebo (Fig 3, C). Error bars represent 95% CIs. Lower ratios indicate lower sensitivity to allergen and therefore greater inhibition by the drug.

QGE031 doses, whereas the response was less pronounced with omalizumab and 24 mg of QGE031 (Fig 4, B). The suppression at week 12 from baseline was 66%, 99%, 99%, and 95% for 24, 72, and 240 mg of QGE031 and omalizumab, respectively. Surface IgE levels were reduced by 71%, 99%, 99%, and 95% for 24, 72, and 240 mg of QGE031 and omalizumab, respectively, when compared with placebo and by 86% and 87% with 72 and 240 mg of QGE031 compared with omalizumab.

Descriptive analyses demonstrated that basophil expression of FcεRI at week 12 was reduced from baseline by 27%, 82%, 85%, and 77% with 24, 72, and 240 mg of QGE031 and omalizumab, respectively. Reduction over expression in placebo-treated subjects was 22%, 81%, 84%, and 75% for 24, 72, and 240 mg of QGE031 and omalizumab, respectively, and 21% and 34% compared with the value in omalizumab-treated subjects in the 72- and 240-mg QGE031 cohorts, respectively. There was no suppression achieved in the 2 subjects with the highest IgE levels in the 24-mg QGE031 cohort. After stopping treatment, the longest duration of suppression of FcεRI was seen with the 240-mg dose (Fig 4, C).

Safety

Nasopharyngitis and worsening asthma were the most common adverse events in subjects who received QGE031 and

omalizumab. There was no QGE031 dose response in the occurrence of these 2 adverse events. The rates of both events were lower in the 240-mg QGE031 group. All adverse events were reported as mild to moderate in nature. A total of 24% of the adverse events were suspected to be related to study drug, mainly being injection-site tolerability issues (see Table E2 in this article's Online Repository at www.jacionline.org).

Eight subjects randomized to QGE031 reported injection-site reactions that were mostly of a mild nature. Injection-site erythema was reported as an adverse event in 3 subjects randomized to receive QGE031.

There were no serious adverse events and no withdrawals because of adverse events in the active treatment groups. No cases of anaphylaxis or generalized urticaria were reported. In addition, there were no clinically meaningful changes in hematology, blood chemistry, urinalysis, vital signs, or electrocardiographic recordings.

Mathematic modeling and simulation

The mathematic model curves fitted the observed patient data well, as shown by the individual predicted curves and patient data in Fig E1 in this article's Online Repository at www.jacionline.org. For this analysis, deviations in patient assignment and dosing were taken into account. The model was capable of simulating

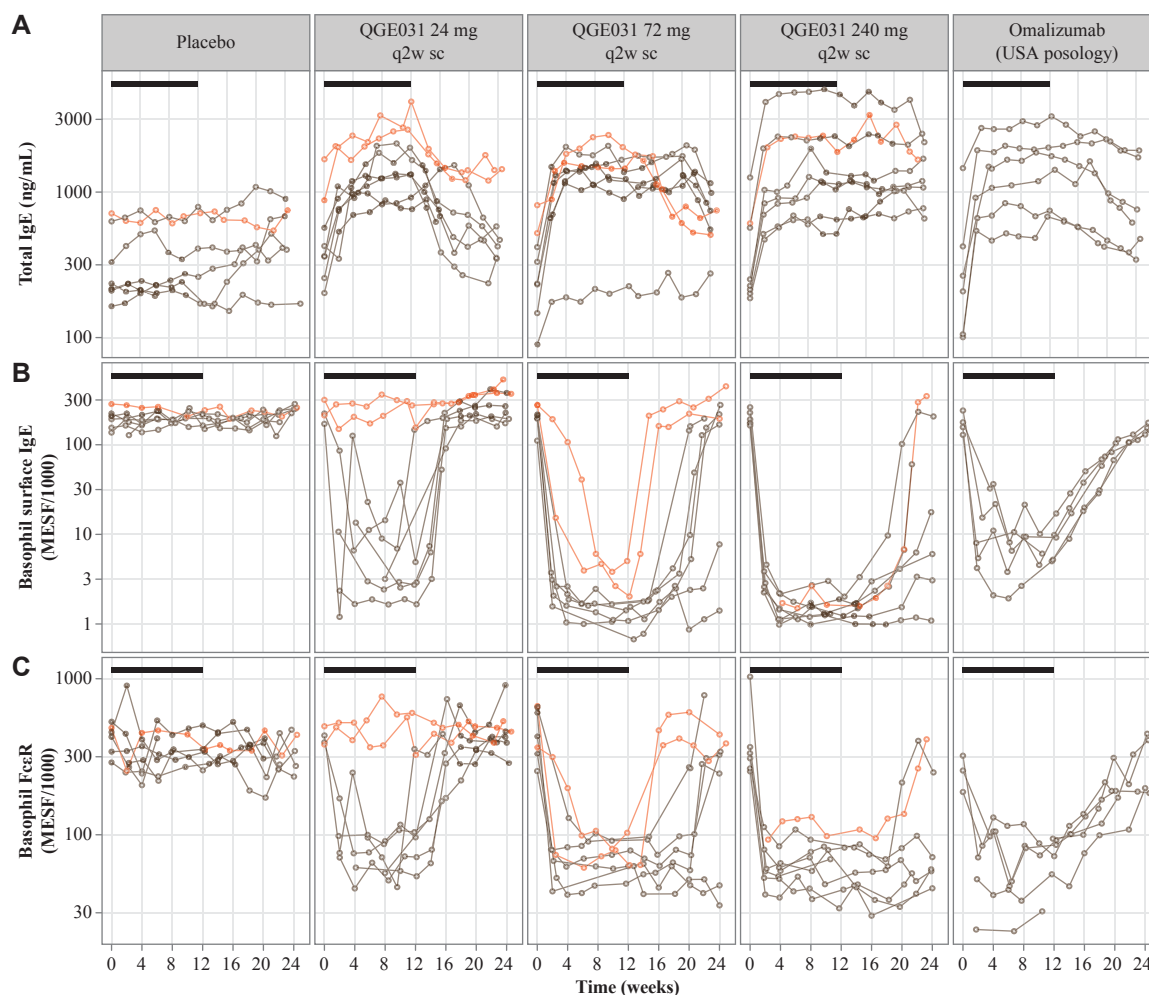


FIG 4. Effect of different doses of QGE031 and omalizumab on IgE and IgE receptor expression. Shown are the individual subject time course of circulating total IgE (**A**), basophil surface IgE levels (**B**), and basophil expression of FcεR (**C**) grouped by cohorts for placebo, the 3 dose levels of QGE031, and omalizumab. Red-colored data represent subjects with baseline IgE levels of greater than 700 IU/mL. Units for the basophil FACS assay were molecules of equivalent soluble fluorophore (MESF) divided by 1000.

both biomarker and clinical responses, with concordance between simulated and observed data (see Fig E2, A, in this article's Online Repository at www.jacionline.org), normal distributions of random variation between patients (see Fig E2, B), and accurate and unbiased predictions in quantile-quantile plots (see Fig E2, C). These, together with the adequately precise pharmacokinetics, biomarker, and allergen challenge pharmacodynamic parameter estimates (see Tables E3 and E4 in this article's Online Repository at www.jacionline.org), were such that the model was considered valid and acceptable for providing predictions through simulation.

Simulations were subsequently carried out for approximately 1000 virtual patients with demographics matching the patients studied herein. The simulations predicted the biomarker and allergen test response time courses from pretreatment to 6 months for a series of doses from zero to 600 mg of QGE031 administered every 4 weeks or omalizumab dosed as per the US dosing table.¹⁴

These mathematic simulations showed that maximum inhibition of the skin allergic response should occur after about 12 weeks of treatment. Inhibition of the bronchial airway allergen PC₁₅ response was predicted to occur earlier, after about 8 weeks

of treatment (see Fig E3 in this article's Online Repository at www.jacionline.org, showing selected QGE031 doses). The biomarker and allergen challenge responses from the 168th day were taken to plot QGE031 dose responses. These suggested that for those patients with baseline IgE levels of less than the median value of 250 IU/mL, QGE031 at a regimen of 36 mg every 4 weeks should provide equivalent inhibition of allergen-induced skin and bronchial responses to omalizumab when dosed according to the US dosing table (Fig 5). Maximal QGE031-induced responses were projected to exceed those of omalizumab and to occur with every 4 weeks of doses of between 120 and 240 mg. Dividing the simulated population by the 250 IU/mL median baseline IgE level suggested that patients with lower baseline values should achieve maximum benefit with 120 mg every 4 weeks, whereas those with higher baseline IgE levels might require higher doses, such as 240 mg every 4 weeks.

DISCUSSION

In this human model of allergic asthma, QGE031 inhibited allergen-induced airway responses in patients with mild allergic

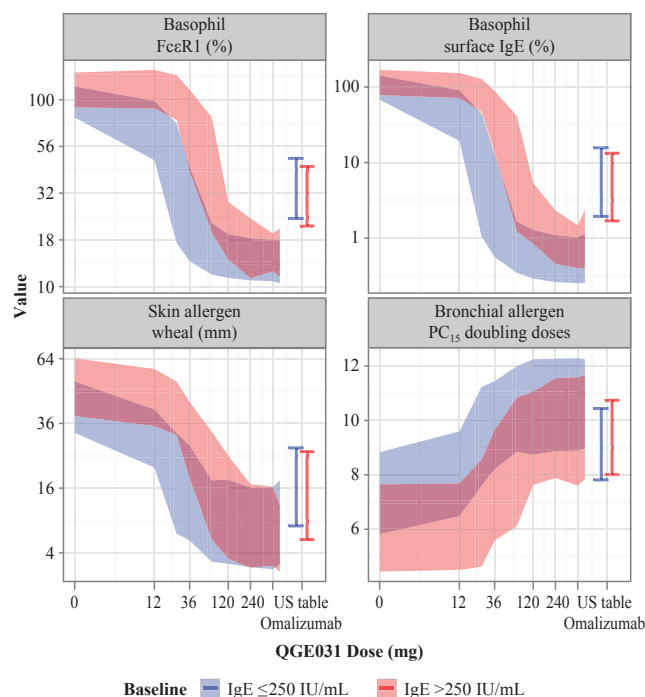


FIG 5. Simulation of steady-state (week 24) dose responses to QGE031 for a patient population split by the median baseline IgE level (>250 IU/mL or ≤ 250 IU/mL). Simulated doses on the x-axis are those for QGE031 administered every 4 weeks. Omalizumab error bars refer to simulated doses administered according to the US dosing table. Wheal measurements are for the area under the complete dilution curve. PC₁₅ values represent the concentration of allergen required to elicit a 15% decrease in FEV₁ on the log₂ scale. On the original scale, log₂(6) would be a concentration (inverse of dilution) of 64, whereas log₂(12) is 4096.

asthma in a dose-dependent manner compared with placebo. When QGE031 was dosed at 240 mg every 2 weeks, there was a nominal 3-fold greater shift in allergen PC₁₅ compared with that after omalizumab. The attenuated airway bronchoconstriction to inhaled allergen was accompanied by suppression of skin prick test responses to allergen likely mediated, at least in part, through reductions in expression of FcεRI and surface IgE on FcεRI-bearing cells. Omalizumab, which was included as a comparator in the present study, increased the concentration of allergen required to elicit an acute bronchoconstrictor response of about 9.0-fold from baseline (3.2 doubling dilutions) and approximately 5-fold compared with placebo (2.3 doubling dilutions), as previously described.¹⁹⁻²¹

The immediate bronchoconstriction observed after allergen challenge is initiated by activation of mast cells and basophils to release potent constrictors of airway smooth muscle, namely histamine and cysteinyl leukotrienes. Treatment with a combination of antihistamines and leukotriene inhibitors has been shown to completely inhibit both the early and late asthmatic responses to inhaled allergen.^{22,23} As such, blocking the allergen-induced IgE-mediated activation of mast cells and basophils with QGE031 should inhibit early and late responses, as previously demonstrated with omalizumab.¹⁹

Similar to findings in the airways, inhibition of skin prick test titration responses to allergen in subjects administered 72 and 240 mg of QGE031 every second week was more pronounced compared with that seen in those dosed with omalizumab. Development of the skin wheal to allergen can be completely

blocked by antihistamines, suggesting that QGE031 effectively prevents release of histamine in the skin. It is notable that doses of QGE031 providing the greatest inhibition of allergen PC₁₅ and skin wheals also provided the longest duration of inhibition after stopping treatment. The extended window of efficacy observed after QGE031 treatment compared with omalizumab supports the potential adoption of less frequent dosing.

Total IgE levels increased in all active treatment groups, including omalizumab. The level of total circulating IgE is determined by several variables, including cellular synthesis, elimination of free IgE and drug-IgE complexes, binding affinity to FcεRI, and occupancy of IgE receptors. Inhibition of the early asthmatic response and skin wheal size by QGE031 is accomplished through a reduced opportunity for antigen to cross-link IgE bound to IgE receptors. Reduced basophil FcεRI and surface IgE levels after QGE031 treatment have been reported in phase 1 studies.¹² These results were confirmed in the current study.

For all outcomes measured, the response duration was longest for patients receiving the highest dose of QGE031; this effective period was longer than that for omalizumab. However, the temporal relationship to QGE031 varied considerably across outcomes. The effect of QGE031 on circulating total IgE levels, basophil FcεRI expression, and surface IgE levels was rapid, sometimes achieving a maximal change by the first blood draw 2 weeks after treatment initiation. The efficacy on clinical allergen challenge responses lagged behind, with submaximal responses when first measured at 6 weeks after dosing. At all doses of QGE031, allergen PC₁₅ peaked at 12 weeks, 2 weeks after the last dose, returning to pretreatment levels by week 18, a pattern similar to that seen with omalizumab. Mean peak efficacy in the skin was also observed at 12 weeks in the 24- and 72-mg QGE031 cohorts. However, at week 18, those who received 72 mg of QGE031 either had no worsening of skin responses or continued to improve. The 240-mg cohort continued to show further improvement from the week 12 challenge, unlike for PC₁₅. These observations of different kinetics across biomarkers and tissue responses were carried over to the pharmacokinetics/pharmacodynamics model simulations, which was logical given that the model was derived from the data. Simulations of continuous treatment every 4 weeks to steady state predicted maximum inhibition of the skin allergic response should occur after 12 weeks of treatment. Inhibition of the bronchial response, on the other hand, should occur slightly earlier, after about 8 weeks of QGE031 treatment.

These findings highlight fundamental differences in the responses of airway mucosal surfaces compared with the skin. Skin prick test responses were assessed 10 minutes after allergen exposure by measuring the size of wheal developing from acute histamine release and were markedly suppressed in all subjects treated with the highest dose of QGE031. On the other hand, the maximum decrease in FEV₁ in the airways is detected between 10 and 30 minutes after inhalation and is manifested through release of both histamine and cysteinyl leukotrienes. Although cross-linking of IgE on mast cells and basophils initiates immediate airway responses to inhaled allergen, activation of other cells in the airways bearing FcεRI, such as dendritic cells and B cells, can support and prime type 2 inflammation in the airway microenvironment.²⁴⁻²⁶ We were unable to assess FcεRI expression on mast cells, but we did observe reduced FcεRI expression on basophils, as well as reduced surface IgE levels

on basophils. Alternate pathways, such as protease activation of epithelial cells through protease-activated receptor 2, can lead to IgE-independent mast cell and basophil activation by mediators, such as the alarmins thymic stromal lymphopoietin and IL-33.²⁷⁻²⁹ Furthermore, responsiveness to inhaled allergen can be regulated by airway cells and mediators independent of the IgE pathway.^{30,31} Thus anti-IgE treatment does not directly target all of the pathways contributing to development of the early asthmatic response and might explain the heterogeneity of suppression of the early asthmatic response to allergen in patients treated with QGE031.

Stratification of subjects during randomization distributed those with high IgE levels (>700 IU/mL) equally across each of the QGE031 treatment groups. At lower doses of QGE031, it was generally observed that high IgE levels equated with poorer responses to QGE031. This was most evident with effects on FcεRI and surface IgE levels on basophils. At 240 mg of QGE031, this difference was no longer apparent in any of the efficacy measurements. The dose-response simulations suggested that not all patients would achieve the same degree of benefit for any given dose, with 25th to 75th percentile bands representing between-patient variation in response. Some patients, especially those with higher serum IgE levels, might require higher QGE031 dosages.

Although studies of omalizumab have shown correlations between free serum IgE levels and clinical responses during treatment,^{11,32,33} these were based on statistical correlations across relatively large populations of patients. For individual patients, it is unlikely that reductions in levels of biomarkers, such as free serum IgE or blood cell FcεRI alone, will be sufficient to predict responses to anti-IgE treatment.³⁴ There are many other factors involved, including cellular IgE receptor expression (FcεRI and FcεRII/CD23), specific/total IgE ratios, and cellular sensitivity,³⁵ even before considering local tissue IgE concentrations and permeation of the drug to the tissue site.

In conclusion, QGE031 was well tolerated in adults with mild allergic asthma. In this model of allergen-induced bronchoconstriction, 10 weeks of treatment with QGE031 at 240 mg effectively shifted the allergen PC₁₅ to nominally 3-fold greater than that achieved with omalizumab, which showed efficacy similar to that seen in previous reports. The degree of suppression of allergen-induced airway responses was variable among subjects. The time course observed after dosing was different from that of omalizumab, with markedly delayed or incomplete reappearance of predosing levels of FcεRI or IgE on basophils in those treated with the highest dose of QGE031. Although optimum dose and duration of therapy were not evaluated in this study, mathematic modeling of differences in PC₁₅ among omalizumab and QGE031 data predicted that for patients with lower baseline IgE levels 36 mg of QGE031 every 4 weeks might provide the same level of protection against allergen-induced responses as the currently approved omalizumab administered per the US dosing table, with 120 mg of QGE031 administered every 4 weeks being superior. Larger doses, such as 240 mg every 4 weeks, might be required to overcome higher levels of baseline serum IgE and allow sufficient free drug to permeate tissues. This small data set supports the clinical development of QGE031 as an alternative treatment to omalizumab with an improved dosing frequency and potential for greater efficacy against allergic responses in the airways and skin.

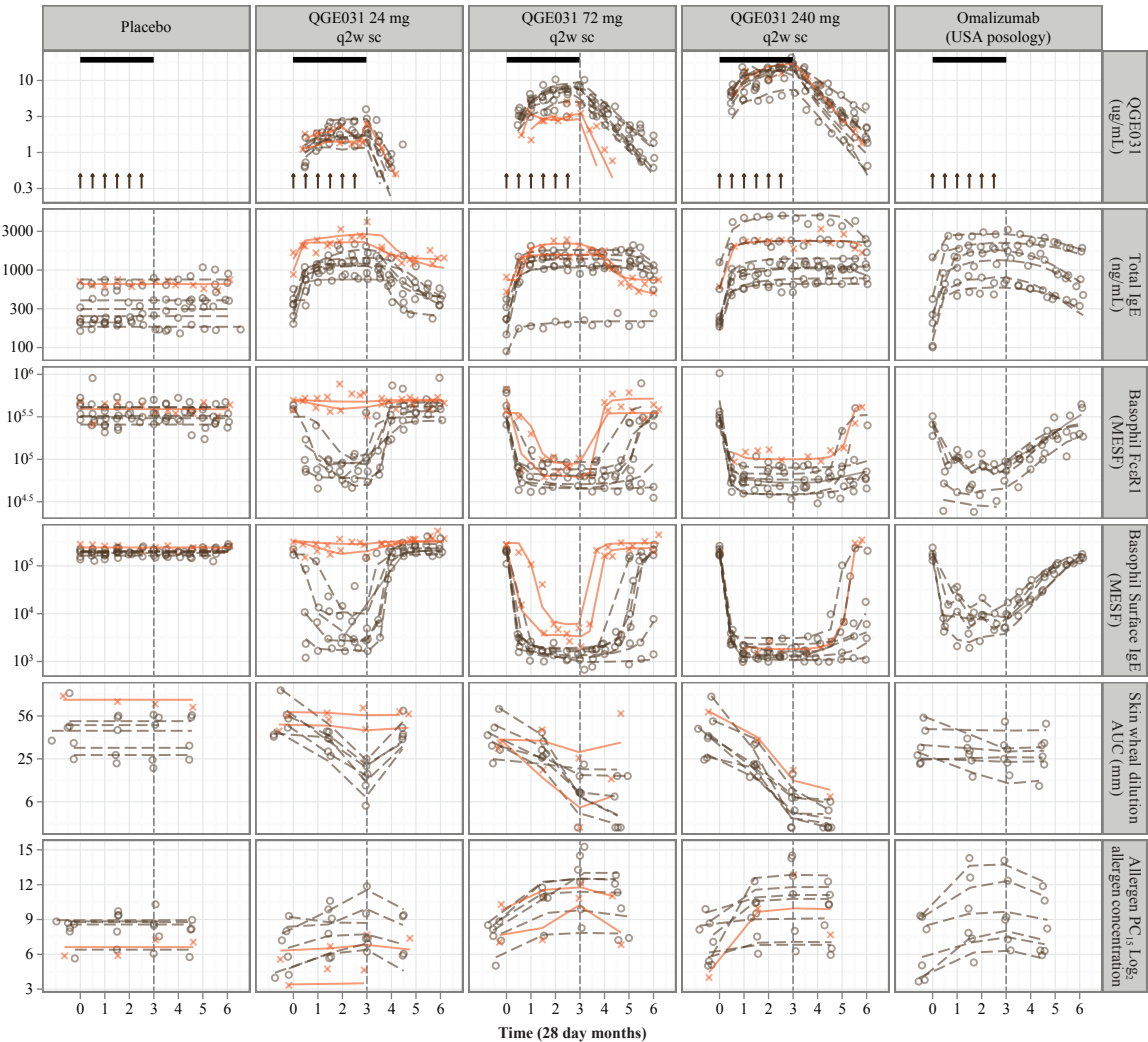
We gratefully acknowledge the help and support of Christina Vasalou (Novartis Pharma AG, Basel, Switzerland) for modeling some of the pharmacokinetics/pharmacodynamics data. Medical writing support was provided by Farid Khalfi (Novartis Ireland, Dublin, Ireland).

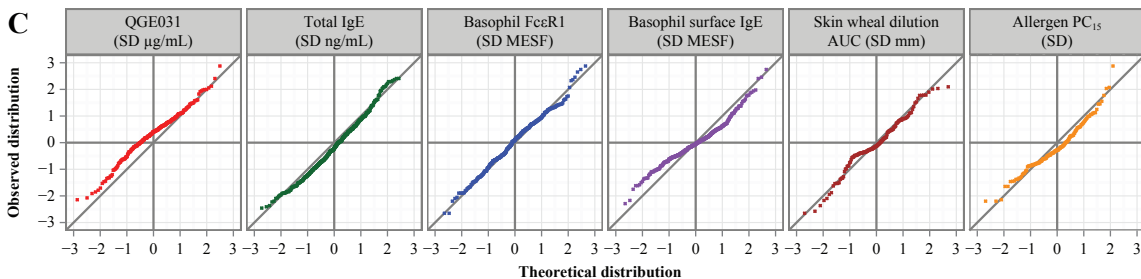
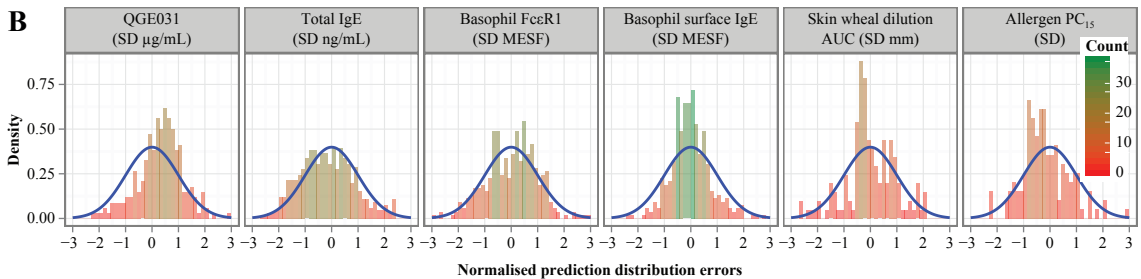
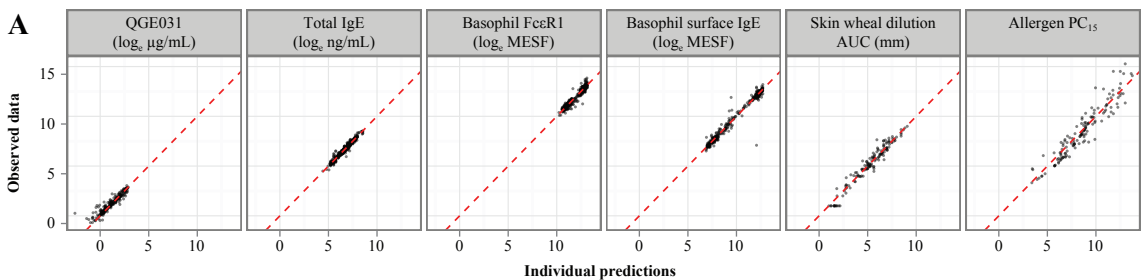
Clinical implications: QGE031 is a humanized antibody that binds IgE with higher affinity than omalizumab. QGE031 demonstrated a nominally 3-fold greater inhibition than omalizumab on allergen PC₁₅ responses in patients with mild allergic asthma.

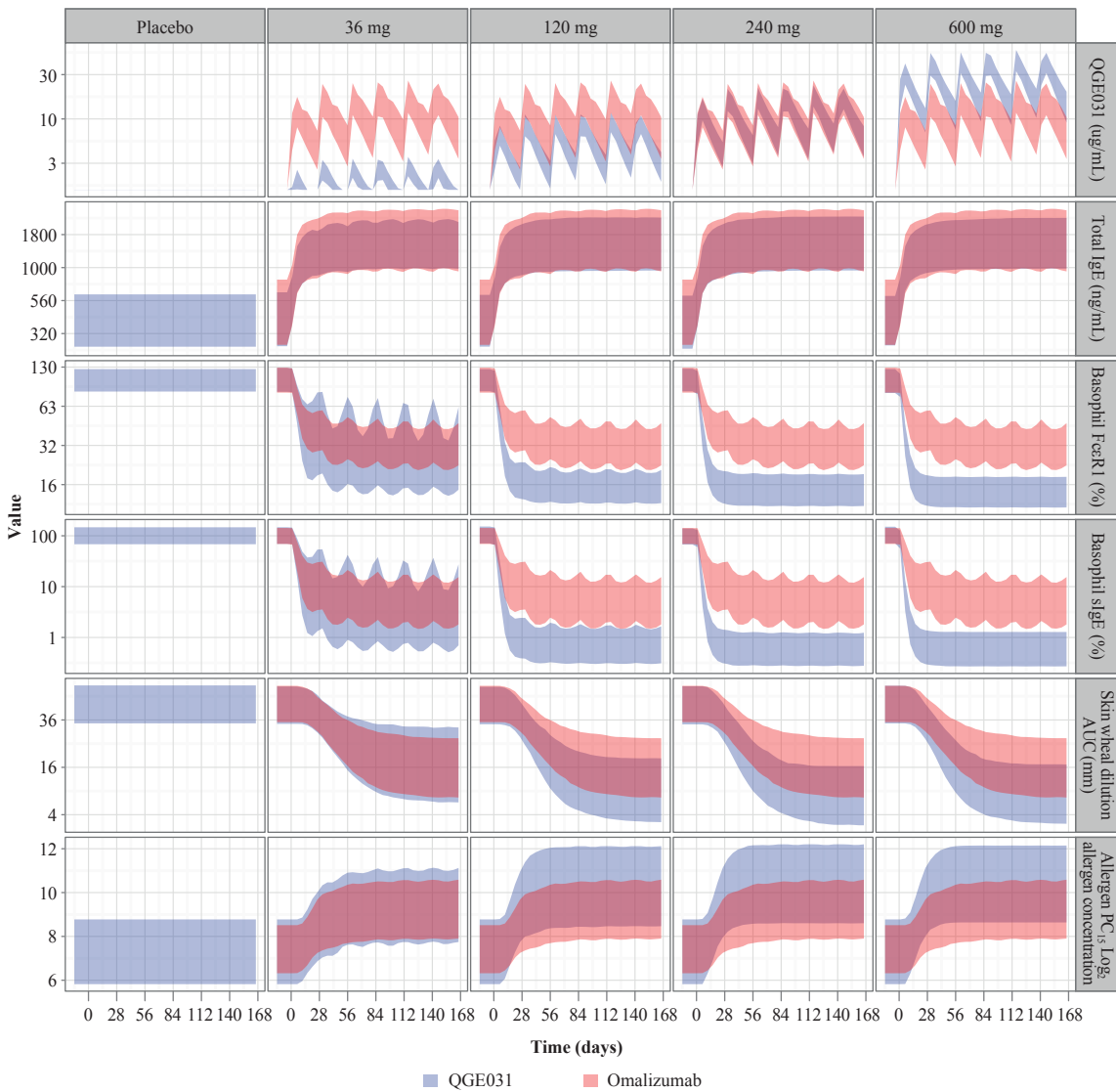
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Efficacy and safety of multiple doses of QGE031 (ligelizumab) versus omalizumab and placebo in inhibiting the allergen-induced early asthmatic response

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Supplementary Materials

All study measurements were performed according to standardized procedures shared across all study centers.

Methods

Allergen PC₁₅ Inhalation Challenge

Doubling concentrations of aqueous allergen extracts were inhaled orally through a Hans Rudolph valve for 2 minutes each. The FEV₁ was measured at 10 minutes post-inhalation and the percent fall was calculated from the baseline FEV₁ value. The test was terminated when a fall in FEV₁ of at least 15% occurred, and the allergen PC₁₅ was calculated using linear interpolation. Single point extrapolation of the PC₁₅ was used when FEV₁ fell by 8 to 14% at the highest concentration of allergen, or >15% on the first concentration. If FEV₁ fell less than 8% at the highest concentration of allergen, the value of the next allergen concentration was assigned. PC₁₅ values were log₂ transformed prior to statistical analyses.

Skin Testing

During the screening period, standardized aero-allergen extracts including ragweed, tree and grass pollen, dog, cat or horse dander, and dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) were used for skin prick tests and compared to a positive control (1 mg/mL histamine) and a negative control (diluent). The size of the wheal was measured in the horizontal and vertical directions after 10 minutes and the allergen for inhalation was selected on the basis of the largest skin response. Subsequent skin titrations administered the selected extract over a range of dilutions. The area under the allergen dilution-response curves for the skin wheal diameters was calculated using the linear trapezoidal rule.

Methacholine Inhalation Challenge

Doubling concentrations of methacholine chloride (Methapharm, ON) were inhaled orally through a Hans Rudolph valve for 2 minutes each. The FEV₁ was measured post-inhalation at 30 seconds and 90 seconds or until it stopped falling, and the percent fall was calculated from the lowest post-diluent FEV₁ value. The test was terminated when a fall in FEV₁ of at least 20% of the lowest post-saline value occurred, and the methacholine PC₂₀ was calculated using linear interpolation.

Measurements in Blood

Serum total QGE031 concentrations were determined by ELISA with a lower limit of quantification of 200 ng/mL and total IgE was determined on the Phadia 100 platform by ImmunoCAP® total IgE assay with a lower limit of quantification of 2.0 IU/mL in human serum. Free IgE in serum was determined using a sandwich ELISA with a quantification range from 2.50 ng/mL to 250 ng/mL in human serum. FcεRI on basophils and dendritic cells, CD23 (FcεRII) on B cells and bound IgE on basophils, and B cells were measured by flow cytometry. Basophils were identified as lineage cocktail negative with CD203c (CD203c-PerCPCy5.5), FcεRI (FcεRI-APC) and IgE (IgE-PE) staining on their surface. Dendritic cells were identified as lineage cocktail negative, HLA-DR positive (HLA-DR-V450) and FcεRI (FcεRI-APC) staining on their surface. B cells were identified as CD19 positive (CD19-FITC), and surface expressions of FcεRII (FcεRII - APC) and IgE (IgE-PE) were then assessed.

Statistical analysis

Six evaluable subjects per group provided 80% power to detect a 6.5-fold treatment difference, the reported difference between placebo and omalizumab-treated subjects, using a one-sided test at 2.5% alpha level. Under the assumption that not more than two subjects in a treatment group would drop out, 40 (8 each group) subjects were planned to be randomized. Since the drop-out rate was smaller than expected enrolment was stopped after randomization of 37 patients.

Modelling methodology

The PKPD model descriptive of drug pharmacokinetics (either omalizumab or QGE031), total IgE, basophil FcεR1 and surface IgE (sIgE) was implemented as previously described (18). Essentially, the PK was described using a two compartment model with clearance of the drug from both compartments, in keeping with the known properties of IgG1 monoclonal antibodies. QGE031 and omalizumab could bind available IgE to form complexes, thereby reducing the concentrations of unbound free IgE in both central and peripheral compartments. In the model

the production of basophil Fc R1 was driven indirectly by the peripheral free IgE levels, whereas basophil surface IgE varied as a function of Fc R1. Fixed effect, covariate and residual error parameters for the PK, IgE and basophil part of the PKPD model were estimated using the values from the previous analysis (18) as Bayesian priors. Inter-patient variabilities were not estimated but were fixed to those of the previous analysis.

Allergen induced skin wheal and bronchial PC₁₅ were preceded by basophil Fc R1 and surface IgE, themselves following suppression of free serum IgE in the peripheral body compartment (tissues). These observed dynamics were best described using a delay compartment model structure, most likely representing signal transduction processes and cell lifetimes in the respective tissues. The bronchial and skin allergen responses had different response times and correlation between the two was investigated.

The components of a population model are i) the structural model, which accounts for the systematic trends in the data and, to the extent possible, the mechanisms generating those trends; ii) the random effects model, which accounts for variability about those trends; iii) the covariate model which describes how patient factors affect the outcome. In this analysis, the model components were selected and assembled based on a combination of prior knowledge and data-driven decision-making guided by statistical and heuristic rules. There was only a limited search for covariates to explain between patient variability in the parameters; many of the previously studied subjects were healthy and therefore of limited applicability (18), and there were only 37 asthma patients in this analysis, a proportion of which were placebo controls.

NONMEM VII version 2 software system was used (Icon Development Solutions, Ellicott City, MD, USA), in a high performance Red Hat Linux computing environment. The models were run using the message passing interface (MPI) parallel computation, with approximately equal numbers of individuals passed to each compute core, the exact number varying according to the load on the core at that time. Parameter estimation was performed using stochastic approximation expectation maximisation with interaction (SAEM), since the model was relatively complex, with manual selection of 10000 burn-in iterations to ensure stable convergence, followed by 6000 averaging iterations to obtain maximum likelihood parameters. The default two chains were specified (ISAMPLE), this having been found optimal based on preliminary work. Both fewer and more chains gave less stable results when replicate runs were compared. For the PK, IgE and basophil part of the PKPD model, parameter estimation used the Bayes method with 20000 burn-in followed by 30000 random sampling iterations.

109 The first part of the model describing PK, IgE, basophil Fc R1 and surface IgE then allergen
110 skin prick wheal size was identical to that previously reported in (18). The bronchial PC₁₅
111 response was driven by the same biomarker as the skin prick wheal, basophil Fc R1. The
112 following coupled algebraic and differential equations were added to the previously described
113 model to describe the bronchial allergen PC₁₅ response:

114 $PCS = STIMP * (FCER / FCER0) ** ExpP(1)$

115 $PC1' = (PCS - PC1) / (KPC / 4)$

116 $PC2' = (PC1 - PC2) / (KPC / 4)$

117 $PC3' = (PC2 - PC3) / (KPC / 4)$

118 $PC4' = (PC3 - PC4) / (KPC / 4)$

119 $PC15 = PC4$

120 Where FCER divided by FCER0 was the relative change from time zero in the basophil Fc R1
121 marker driving the PC₁₅ test response (PCS) at time t, parameterised with a scale parameter
122 STIMP and exponent ExpP. The equations with the prime notation, PC1' to PC4', were the
123 differentials for the allergen bronchoprovocation response with KPC the mean response time for
124 the PC₁₅ drug induced changes. The differential equations were integrated using the ADVAN13
125 routine of Nonmem with a tolerance of 6.

126 All subject level parameters in the model had a typical value for the population with random
127 effects which were either log-normal distributions for KPC and STIMP, or normal distributions
128 for parameters which were powers, namely ExpP. Covariates were added as in (18). The final
129 individual prediction output was then given by addition of residual error (EPS) with square root
130 transformation, plus one, for the skin prick test data and log(2) transformation for the bronchial
131 doubling allergen dose data which was on the log(2) scale.

132 Model selection was primarily based upon examination of deviations between observed data and
133 simulations. Toward this end the following diagnostics were utilised: a) normalised prediction
134 distribution of errors, NPDE, b) expected conditional weighted residuals, ECWRES, c)
135 individual residuals, IRES, d) the QQ plots of the observed with the predicted distribution and e)
136 the objective function which was used to decide between models which had the same numbers of
137 parameters but different functional forms.

138 Simulations from the fitted model were used to assess the clinical implications of the knowledge
139 contained within the model structure and parameter estimates. The simulations used the same
140 Nonmem code save for the exchange of the estimation for a simulation command. Input datasets
141 for the design of future studies used the patient demographics from the current asthma study,
142 replicated sufficient times to give approximately 1000 virtual patients and therefore reproducible
143

Supplementary Figure Legends

Figure S1: Mathematical model prediction of QGE031 steady-state dose response

Symbols are the observed data, with lines being the individual patient model predictions. Black dashed lines with circles are for patients with baseline screening IgE ≤ 700 IU/mL, whereas red solid lines with X symbols indicate baseline IgE > 700 IU/mL. The dosing events are shown in the upper panel; please note that for omalizumab, there was a mixture of four and two-weekly dosing as per the US dosing table.

MESF, Molecules equivalent soluble fluorophore.

Figure S2: Mathematical model validated for simulation

A Correlations between simulation-based predictions and observed data.

B Normalised prediction distributions of errors (NPDE, i.e. SD, standard deviations) between predictions and observed data.

C Quantile-quantile (QQ) plots of NPDE, with units being SD, standard deviations.

FcεR1, Basophil Fcε receptor type 1; *MESF*, Molecules of equivalent soluble fluorochrome; *sIgE*, Surface bound IgE.

Figure S3: Simulation of dose-response over time for 1000 new experimental scenarios with 0-6 months of dosing with zero to 600 mg QGE031 or omalizumab per US dosing table

The simulated doses refer to QGE031. Omalizumab was administered according to the US dosing table with results replicated across all panels of each row. The wheal measurements are for the area under the complete wheal size versus allergen concentration curve for all dilutions. The PC_{15} values represent the concentration of allergen required to elicit a 15% decrease in FEV_1 on the $\log_2$ scale. On the original scale, $\log_2(6)$ would be a concentration (inverse of dilution) of 64, whilst 12 is 4096.

1 **Table E1: Change from baseline of log2 PC₁₅ (number of doubling dilutions) at week 12**

Change from baseline of log2 PC₁₅ at week 12	QGE031 24 mg (n=8)	QGE031 72 mg (n=7)	QGE031 240 mg (n=8)	Omalizumab (n=6)	Placebo (n=6)
Mean (SD)	1.78 (1.82)	4.23 (1.92)	4.96 (3.39)	3.17 (1.56)	0.39 (0.96)
CV% mean	102.6	45.5	68.2	49.2	245.2
Min-Max	[-1.90, 4.66]	[1.84, 7.70]	[0.85, 9.19]	[1.05, 5.19]	[-1.14, 1.47]

2 CV, Coefficient of variation; SD, Standard deviation

3

4 **Table E2: Incidence of adverse events by preferred term (>1 AE in any treatment group)**

	QGE031 24 mg (n=8)	QGE031 72 mg (n=8)	QGE031 240 mg (n=8)	Omalizumab (n=6)	Placebo (7)	Total (n=37)
Subjects with AEs	8 (100.0)	5 (62.5)	7 (87.5)	3 (50.0)	6 (85.7)	29 (78.4)
Nasopharyngitis	5 (62.5)	2 (25.0)	1 (12.5)	3 (50.0)	0 (0.0)	11 (29.7)
Asthma	2 (25.0)	1 (12.5)	1 (12.5)	1 (16.7)	0 (0.0)	5 (13.5)
Oropharyngeal pain	1 (12.5)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)	4 (10.8)
Diarrhoea	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)	1 (14.3)	3 (8.1)
Headache	0 (0.0)	0 (0.0)	2 (25.0)	1 (16.7)	0 (0.0)	3 (8.1)
Injection site erythema	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	0 (0.0)	3 (8.1)
Nasal congestion	0 (0.0)	1 (12.5)	2 (25.0)	0 (0.0)	0 (0.0)	3 (8.1)
Upper respiratory tract infection	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	1 (14.3)	3 (8.1)
Urinary tract infection	2 (25.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.1)

5 AE, Adverse event

6

7 **Table E3: Model parameters for the PK, IgE, FcεR1 and surface IgE parameters**

	Estimate	95% CI*	
		Lower	Upper
Drug related parameters			
Clearance of drug, CL _X (L.d ⁻¹)	0.50	0.43	0.58
Volume of central compartment, V _C (L)	3.0	2.6	3.4
Volume of peripheral compartment, V _P (L)	16	15	18
Inter-compartmental flow, Q (L.d ⁻¹)	0.78	0.68	0.88
Absorption rate constant, k _a (d ⁻¹)	0.13	0.11	0.15
Bioavailability, BAV (%)	100	97	100
IgE related parameters			
IgE production rate, EPR (μg.d ⁻¹)	1500	1200	1800
Clearance of IgE, CL _E (L.d ⁻¹)	1.1	0.95	1.3
Reflection coefficient retaining drug-IgE complexes in central, SR (%)	84	77	91
QGE031 apparent <i>in vivo</i> binding constant for IgE, K _D (nM)	0.043	0.024	0.078
Omalizumab apparent <i>in vivo</i> binding constant for IgE, K _D (nM)	1.7	0.45	6.3
Basophil related parameters			
Mean residence time for basophils/FcεR1 receptor, k _{BASO} (days)	3.5	3.2	3.8
Baseline basophil FcεR1, BFcεR1 (MESF×10 ⁻³) [†]	50	43	58
Baseline basophil surface IgE, BSIgE (MESF×10 ⁻³) [†]	220	180	270
Half-max free IgE concentration for stimulating FcεR1, EC _{50R} (nM)	0.53	0.25	1.2
Free IgE stimulation of FcεR1 slope power coefficient, STIMC	3.2	2.8	3.6
FcεR1 to surface IgE slope or power coefficient, S _{IgES}	2.7	2.4	2.9
Further covariates (baseline predictors)**			
Baseline IgE on its production	0.70	0.65	0.76
on drug clearance	0.10	0.04	0.17
on K _D	0.17	0.02	0.32
on EC _{50R}	0.42	0.30	0.55
on S _{IgES}	-0.22	-0.32	-0.13
on IgE BFcεR1 & BSIgE	0.087	0.023	0.15
Residual errors, within patient (standard deviations, log scale)			
QGE031	0.23	0.21	0.25
Total IgE	0.12	0.12	0.13
Basophil FcεR1	0.21	0.20	0.23
Basophil surface IgE	0.32	0.30	0.34

8 Definitions for the parameters and their abbreviations are described in (18). [†]MESF, molecules of equivalent soluble
9 fluorephore. *CI, confidence interval. **A body weight to the power 0.9 covariate was applied to clearances, inter-
10 compartmental flow and IgE production rate, to the power 1.0 for volumes. For those parameters with covariates,
11 estimates are for 70 kg bodyweight and a baseline IgE of 365 ng/mL (150 IU/mL). The between-patient random
12 effects were set to be the same as for the healthy atopic subjects in (18).

13

Table E4: Structural and random effect parameters for the skin prick test and allergen PC₁₅ part of the model

Parameter	Structural parameters (theta)			Random effects, inter-patient variance (eta)		
	Estimate	95% CI*		Estimate	95% CI*	
		Lower	Upper		Lower	Upper
Skin mean response time, KSPT (d)	34	28	42	0.095	(zero sem)	
Intercept FcεR1 vs wheal, STIMW (mm)	51	46	56	0.0075	(zero sem)	
Slope of FcεR1 vs wheal, ExpW	1.2	0.78	1.5	0.49	0.031	0.94
Covariate of baseline wheal on ExpW	1.8	-0.27	3.8		-	
Covariate of baseline wheal on STIMW	1.8	1.2	2.5		-	
Bronchial mean response time, KPC (d)	17	sem 5.4e-06			-	
Intercept FcεR1 vs PC ₁₅ , STIMP	197	sem 8.8e-06			-	
Slope of FcεR1 vs PC ₁₅ , ExpP	-1.1	-0.75	-1.5		-	
Covariate of baseline PC ₁₅ on ExpP	0.24	-0.75	1.2	0.48	0.091	0.98
Covariate of baseline PC ₁₅ on STIMP	3.9	sem 2.8e-05			-	
Residual within patient variation (epsilon)						
Wheal	0.33	0.23	0.42			
PC ₁₅	0.88	0.66	1.1			

Definitions for the skin prick test parameters and their abbreviations are described in (18).

*CI, Confidence interval.