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Safety, Tolerability, Pharmacokinetics, and pharmacodynamics of YH35324, a novel Long-Acting High-Affinity IgE_{Trap}-Fc protein in subjects with Atopy: Results from the First-in-Human study

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ABSTRACT

Background: YH35324, a long-acting IgETrap-Fc fusion protein, is a novel therapeutic agent for immunoglobulin E (IgE)-mediated allergic diseases. This randomized, double-blind, placebo/active-controlled, single ascending dose Phase 1 study assessed the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of YH35324 in subjects with atopy.

Methods: Eligible subjects were healthy subjects or atopic adults with mild allergic rhinitis, atopic dermatitis, food allergy, or urticaria, and a serum total IgE level of 30–700 IU/mL (Part A) or > 700 IU/mL (Part B). In Part A, 35 subjects in 5 cohorts received YH35324 (0.3, 1, 3, 6, and 9 mg/kg), 8 received omalizumab (300 mg), and 9 received placebo. In Part B, 8 subjects received YH35324 and 8 received omalizumab.

Results: Twenty subjects (38.5 %) in Part A (YH35324: 37.1 %, omalizumab: 50.0 %, placebo: 33.3 %) and 10 subjects (62.5 %) in Part B (YH35324: 100 %; omalizumab: 25.0 %) experienced treatment-emergent adverse events (TEAEs). TEAEs were mostly grade 1/2; no serious AEs, AE-related treatment discontinuation, or anaphylaxis were reported. YH35324 exhibited dose-proportional increase in C_{max} and AUC_{last} over the dose range of 0.3–9 mg/kg. YH35324 rapidly suppressed serum-free IgE levels to a significant extent (< 25 and < 82.8 ng/mL, both $P < 0.05$) and with longer duration than omalizumab.

Conclusion: This study showed that YH35324 has a favorable safety profile and is effective in reducing serum-free IgE levels in subjects with atopic conditions.

1. Introduction

Chronic allergic diseases have a significant impact on health outcomes and quality of life [1–3]. Developing effective therapeutics for these diseases is critical as the number of affected patients continues to grow [4]. Immunoglobulin E (IgE) plays a central role in chronic allergic diseases and is an important target for intervention [5]. IgE mediates its effects via two Fcε receptors, i.e., the high-affinity IgE receptor (FcεRI) and the low-affinity IgE receptor (FcεRII, also known as CD23) [6,7]. FcεRI is expressed on the surface of immune effector cells, especially mast cells and basophils [8]. The binding of antigens to an IgE or allergen-IgE complex already bound to the FcεRI on these effector cells leads to degranulation and the release of inflammatory mediators such as histamine, arachidonic acid metabolites, and prostaglandin D2, as well as the secretion of inflammatory cytokines. FcεRII is known to play roles in the regulation of IgE production and antigen presentation

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[9,10].

IgE-targeting therapeutic agents such as omalizumab and ligelizumab have been developed to treat chronic allergic diseases when there is insufficient response to first-line treatments such as antihistamines [11]. These anti-IgE antibodies sequester serum-free IgE, thus preventing receptor binding and downstream activation of allergic inflammatory cascades [11]. Omalizumab has been approved by the United States (US) Food and Drug Administration (FDA) as a treatment for moderate to severe persistent asthma in patients 6 years of age and older with a positive skin test or *in vitro* reactivity to a perennial aeroallergen and symptoms that are inadequately controlled by inhaled corticosteroids [12]. It is also indicated for chronic spontaneous urticaria (CSU) in adults and adolescents 12 years of age and older who remain symptomatic despite H1 antihistamine treatment [12]. However, omalizumab has not been indicated for patients with asthma, 12 years of age and older, with pretreatment serum total IgE levels exceeding 700 IU/ml [12]. Serum sickness or vasculitis and anaphylaxis have also been known to occur with omalizumab treatment [12]. Ligelizumab, which has improved binding affinity for serum-free IgE compared with omalizumab is in late-stage clinical development as an IgE-targeting therapeutic agent [13]. However, Phase 2b and Phase 3 trials reported that only 30 %–51 % and 39 %–43 % of CSU patients treated with ligelizumab had complete resolution of their hives at Week 12 and Week 24, respectively [14,15]. In recent phase 3 trial, ligelizumab did not demonstrate superior efficacy over omalizumab in adolescents and adults with CSU [16]. Similarly, in a phase 3 trial involving asthmatic patients, ligelizumab did not significantly improve asthma control or reduce exacerbations compared to both omalizumab and placebo. As a result, it failed to demonstrate superiority over either placebo or omalizumab [17].

YH35324, a long-acting IgETrap-Fc fusion protein consisting of the extracellular domain of human FcεRIα and human IgD/IgG4 modified Fc region, is a novel therapeutic agent being developed for the treatment of various IgE-mediated allergic diseases [18]. It suppresses effector cell activation via a dual mechanism by binding to serum-free IgE with high affinity and eliminating anti-FcεRIα autoantibodies [18]. The IgD/IgG4 Fc region of YH35324 has been engineered so that it does not elicit FcγR-mediated or complement component 1q (C1q)-mediated immune effector functions, thus avoiding unwanted side effects such as anaphylaxis [18].

In *in vitro* studies, YH35324 showed superior binding affinity for human IgE compared with omalizumab. In a cell-based competitive binding assay, YH35324 showed more potent suppression of IgE binding to CD23 and mast cell degranulation than omalizumab [19]. In non-human primate (NHP) studies, single subcutaneous injections of 10, 30 and 60 mg/kg of YH35324 elicited potent and sustained suppression of serum-free IgE to below clinically meaningful levels (i.e., < 25 ng/mL) in NHPs with high baseline serum-free IgE levels, whereas omalizumab did not show such suppression [19]. In studies using various IgE-mediated allergic disease animal models, YH35324 also showed excellent therapeutic effects. In mouse models for house dust mite (HDM)-induced allergic asthma, airway hyper-responsiveness was improved, and both serum-free IgE and HDM-specific IgE were effectively suppressed. Inflammatory cytokines (IFN-γ, IL-4, IL-5, and IL-13) were also suppressed, and infiltration of macrophage, neutrophil, and eosinophil was reduced in the bronchoalveolar lavage fluid [19]. In an ovalbumin-induced food allergy mouse model, YH35324 completely suppressed the occurrence of diarrhea, effectively decreased serum-free IgE, and inhibited mast cell degranulation [19].

Based on these non-clinical results, this first-in-human (FIH) study was conducted to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) following subcutaneous injection of YH35324 in healthy subjects or atopic adults with mild allergic rhinitis, atopic dermatitis, food allergy, or urticaria.

2. Methods

This randomized, double-blind, placebo/active-controlled, single ascending dose (SAD) Phase 1 study (ClinicalTrials.gov identifier: NCT05061524) was conducted between September 2021 and January 2023 at 4 study sites in South Korea. The local independent ethics committee at each site reviewed and approved the study protocol and documents used for informed consent prior to study initiation. The study was conducted in accordance with Good Clinical Practice, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use guidelines, and the Declaration of Helsinki. All subjects provided written informed consent before any study-related procedures were performed.

2.1. Subjects

Key inclusion criteria were as follows: men and women aged 19–55 years who had confirmed atopy, defined as a positive result for at least 1 inhalant or food allergens (birch, timothy grass, ragweed, mugwort, cat, dog, *Alternaria*, house dust mites, shrimp, wheat, and peanuts) in a skin prick test and/or serum allergen-specific IgE test at screening; healthy subjects or subjects experiencing mild symptoms due to confirmed conditions of allergic rhinitis, atopic dermatitis, food allergy or urticaria without the use of any maintenance medication for ≥ 1 year before screening; a body weight of 45–100 kg (50–100 kg for males); and a serum total IgE level of 30–700 IU/mL (Part A) or exceeding 700 IU/mL (Part B) at the time of screening. Refer to the Supplementary Materials for the complete inclusion and exclusion criteria.

2.2. Study design

The study design is shown in Fig. 1. The rationale for dose selection is described in the Supplementary Materials. A randomization list was generated according to the randomization ratio of treatment groups in each cohort, using a block randomization method. In Part A, eligible subjects with a serum total IgE level of 30 to 700 IU/mL were randomized in a stepwise manner to 5 cohorts (0.3, 1, 3, 6, and 9 mg/kg of YH35324). In Cohort 1, 4 subjects were randomized in a 3:1 ratio to receive either YH35324 or placebo. For Cohorts 2 to 5, 12 subjects per cohort were randomized in a 4:1:1 ratio to YH35324, omalizumab (active comparator, Xolair®, 300 mg), or placebo. As this was a FIH study, the first 2 subjects in Cohort 1 were administered YH35324 in an open-label manner with an interval of ≥ 24 h between administration before the other 2 subjects in the same cohort and subjects in the subsequent cohorts were administered the investigational product (IP) in a double-blinded manner. After all subjects in a cohort were administered the IP, the dose escalation committee (DEC) conducted a blinded review and assessment of all available safety and tolerability data. PK data were collected until the Day 15 visit of the last subject to decide whether to escalate the dose. In Part B, 16 subjects (Cohort 6) who had a baseline serum total IgE level of > 700 IU/mL were randomized to receive either YH35324 (8 subjects) or omalizumab (8 subjects) in a double-blinded manner. The YH35324 dose (6 mg/kg) was determined after reviewing the safety, tolerability, PK, and PD data in Part A. All subjects in both Part A and Part B were followed up till Day 113.

2.3. Outcome measures

The primary outcome measure was the occurrence and severity of adverse events (AEs). Safety and tolerability were assessed following a single subcutaneous administration of YH35324. Secondary outcome measures included PK profile, serum-free IgE levels, and serum total IgE levels following a single administration of YH35324. The PK profile included the following parameters: area under the curve (AUC) from time 0 h to the last quantifiable level (AUC_{last}), maximum serum level (C_{max}), AUC from zero extrapolated to infinity (AUC_{inf}), time to

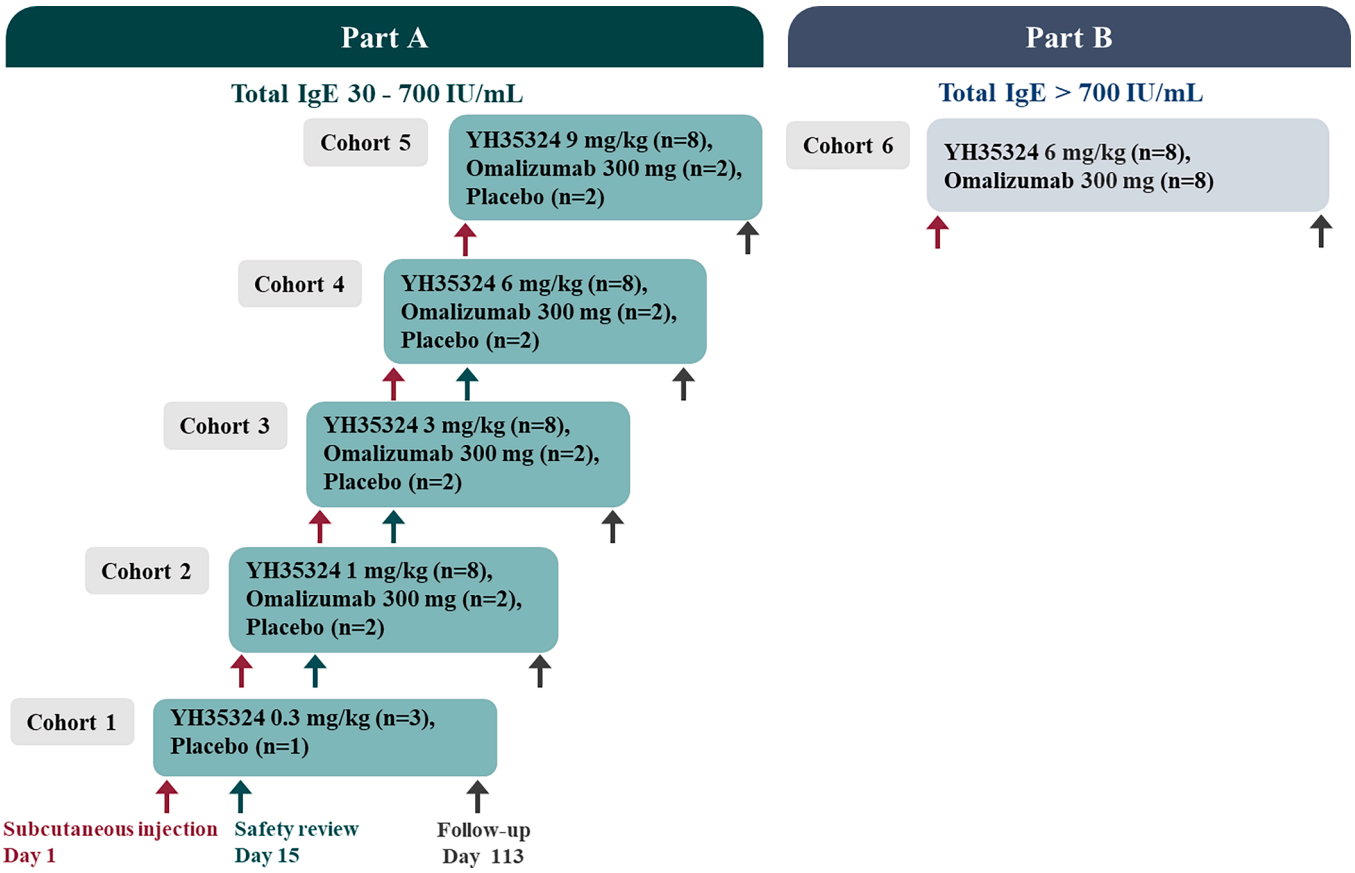

Fig. 1. Study design.

maximum level (T_{max}), elimination half-life ($t_{1/2}$), apparent serum clearance (CL/F), and apparent volume of distribution (V_z/F). These were calculated from the YH35324 serum level data using actual sampling times. Serum-free IgE levels were measured using enzyme-linked immunosorbent assays (ELISA) as described previously [20]. Serum total IgE levels were measured using the ImmunoCAP^{TM} (Thermo Fisher, Waltham, MA, US). Anti-YH35324 antibody levels (immunogenicity) were measured as an exploratory outcome. Study assessments and bioanalytical methods are described in the Supplementary Materials.

2.4. Statistical analyses

The sample size for this study was not determined based on statistical power calculations. It was estimated that administration of YH35324 to 8 subjects per cohort in Part A and Part B would provide an 83 % probability of observing any AEs with an expected incidence of 20 % or higher.

The safety set included all subjects who received the IP after randomization. The PK analysis set included all subjects who completed YH35324 administration and had quantifiable YH35324 levels. All subjects who received the IP and had ≥ 1 PD assessment at baseline and subsequent visits were included in the PD analysis set. The immunogenicity analysis set included all subjects who received YH35324 and had ≥ 1 immunogenicity assessment at baseline and subsequent visits.

AEs that occurred during the first 28 days after IP administration were summarized. AEs that occurred afterwards were listed separately. All recorded AEs were classified into standardized medical terminologies using the Medical Dictionary for Regulatory Activities (MedDRA) version 25.1. YH35324 serum levels at scheduled blood sampling times and other PK endpoints were summarized by YH35324 dose groups. Dose-proportionality of YH35324 was assessed in all cohorts except for Cohort 6 (Part B), using a power model with the log-transformed $C_{max}$and AUC_{last} as dependent variables and log-transformed doses as independent variables. Serum-free IgE and total IgE levels at each time point were summarized by treatment group. The maximum percentage decrease and the duration for which the serum-free IgE level was maintained at < 25 ng/mL or < 82.8 ng/mL for each subject was also summarized for each treatment group. Between-treatment comparisons (i.e., YH35324 versus placebo and YH35324 versus omalizumab) of the duration of serum-free IgE suppression were performed. In addition, the percentage of subjects in each treatment group who achieved a serum-free IgE level of < 25 ng/mL or < 82.8 ng/mL at least once during the study period was assessed. The number of subjects in the immunogenicity analysis set who tested positive for serum anti-YH35324 antibodies and neutralizing antibodies were summarized by YH35324 dose group.

3. Results

In Part A, 94 subjects were screened. Of these, 33 were deemed ineligible based on the inclusion/exclusion criteria, 5 withdrew consent, and 4 dropped out for other reasons (e.g., extra subjects, COVID-19 positive). The remaining 52 subjects were enrolled and randomized. One subject in Cohort 4 dropped out due to pregnancy, and another subject in Cohort 5 withdrew consent and dropped out. A total of 50 (96.2 %) subjects in Part A completed the study. In Part B, of the 100 subjects screened, 80 were deemed ineligible based on inclusion/exclusion criteria. Of these, 71 subjects did not have the requisite serum total IgE level > 700 IU/mL at the time of screening and were excluded. Another 4 subjects withdrew consent and the remaining 16 subjects were enrolled and randomized. One subject withdrew consent and dropped out, and the remaining 15 (93.8 %) subjects completed the study. All subjects in both Part A and Part B who received the IP were followed up for safety assessments.

3.1. Subject demographics and clinical characteristics

Table 1 shows the demographic and baseline characteristics of the subjects. In Part A, the mean (SD) age of YH35324-treated subjects was 32.6 (9.9) years with similar proportions of males and females. These subjects had median serum-free and serum total IgE levels of 316 ng/mL and 142 IU/mL, respectively. In Part B, the mean (SD) age of YH35324-treated subjects was 25.8 (5.2) years, and 75.0 % were males. In YH35324-treated subjects, the median serum-free and serum total IgE levels were 1,955 ng/mL and 1,386 IU/mL, respectively.

Table 1
Summary of subject demographics and baseline characteristics.

Variables	YH35324	Omalizumab	Placebo	All subjects
Part A	n = 35	n = 8	n = 9	n = 52
Age, mean (SD), years	32.6 (9.9)	29.0 (9.6)	31.2 (9.2)	31.8 (9.7)
Sex, n (%)				
Male	17 (48.6)	6 (75.0)	3 (33.3)	26 (50.0)
Female	18 (51.4)	2 (25.0)	6 (66.7)	26 (50.0)
BMI, mean (SD), kg/m^2	23.5 (3.3)	23.7 (2.5)	24.6 (3.8)	23.7 (3.3)
Disease duration, mean (SD), months	155.5 (122.3)	170.4 (98.8)	109.7 (75.7)	149.9 (112.1)
Serum-free IgE,median (min–max), ng/mL	316 (38–1,648)	276 (129–760)	234 (135–720)	282 (38–1,648)
Serum total IgE,median (min–max), IU/mL	142 (33–728)	127 (42–263)	119 (51–327)	135 (33–728)
Allergic diseases, n (%)	35 (100.0)	8 (100.0)	9 (100.0)	52 (100.0)
Allergic rhinitis, n (%)	31 (88.6)	7 (87.5)	9 (100.0)	47 (90.4)
Atopic dermatitis, n (%)	3 (8.6)	1 (12.5)	1 (11.1)	5 (9.6)
Food allergy, n (%)	9 (25.7)	1 (12.5)	3 (33.3)	13 (25.0)
Urticaria, n (%)	4 (11.4)	1 (12.5)	2 (22.2)	7 (13.5)
Part B	n = 8	n = 8	–	n = 16
Age, mean (SD), years	25.8 (5.2)	32.8 (11.2)		29.3 (9.2)
Sex, n (%)				
Male	6 (75.0)	2 (25.0)		8 (50.0)
Female	2 (25.0)	6 (75.0)		8 (50.0)
BMI, mean (SD), kg/m^2	24.0 (6.1)	26.3 (3.7)		25.2 (5.0)
Disease duration, mean (SD), months	270.9 (98.6)	229.0 (86.5)		250.0 (92.2)
Serum-free IgE,median (min–max), ng/mL	1,955 (1,243–3,639)	2,547 (1,156–3,634)		2,225 (1,156–3,639)
Serum total IgE,median (min–max), IU/mL	1,386 (718–5,000)	1,499 (989–5,000)		1,386 (718–5,000)
Allergic diseases, n (%)	8 (100)	8 (100)		16 (100)
Allergic rhinitis, n (%)	4 (50.0)	7 (87.5)		11 (68.8)
Atopic dermatitis, n (%)	6 (75.0)	7 (87.5)		13 (81.3)
Food allergy, n (%)	2 (25.0)	2 (25.0)		4 (25.0)
Urticaria, n (%)	2 (25.0)	1 (12.5)		3 (18.8)

Notes: n = number of subjects in that category, %=percentages (%) are based on the subjects within each treatment group.

3.2. YH35324 demonstrated a favorable safety profile

In Part A, 20 subjects (38.5 %) experienced ≥ 1 treatment-emergent adverse event (TEAE) (YH35324: 37.1 %; omalizumab: 50.0 %; placebo: 33.3 %) (Table 2, see Supplementary Tables S1 and S2 for TEAEs classified by MedDRA preferred terms). All TEAEs were grade 1 or 2 except for 1 subject in the omalizumab group who had elevated creatine phosphokinase (grade 3), which occurred 28 days after IP administration. Drug-related TEAEs were reported in 3 subjects (8.6 %) in the YH35324 group and 3 subjects (37.5 %) in the omalizumab group. The most common TEAE in the YH35324 group was headache (4 subjects, 11.4 %). All of them were considered not related to YH35324. In Part B, 10 subjects (62.5 %) reported TEAEs, all of which were grade 1 or 2. Drug-related TEAEs were reported by 1 subject (12.5 %) in the YH35324 group and 2 subjects (25.0 %) in the omalizumab group. No serious AEs, treatment discontinuation due to AEs, or anaphylaxis were reported in either Part A or Part B of the study.

3.3. YH35324 demonstrated a dose-proportional increase in C_{max} and AUC_{last} over the dose range

In Part A, following a single subcutaneous injection, the peak serum level for YH35324 was reached for all doses at similar times, and the peak serum level increased with the dose level (Fig. 2). In Part B, the peak serum level for YH35324 was reached at a similar time (Fig. 2).

The median T_{max} of YH35324 ranged from 48 to 51 h and the arithmetic mean of $t_{1/2}$ ranged from 226 to 274 h across all doses (Table 3). AUC_{last} and C_{max} of YH35324 increased in a dose-proportional manner over the dose range of 0.3 to 9 mg/kg (Table 3) (Supplementary

Table 2
Summary of treatment-emergent adverse events.

Number of subjects, n* (%)	YH35324	Omalizumab	Placebo	All subjects
Part A	n = 35	n = 8	n = 9	n = 52
TEAEs‡	13 (37.1)	4 (50.0)	3 (33.3)	20 (38.5)
Drug-related TEAEs†	3 (8.6)	3 (37.5)	1 (11.1)	7 (13.5)
Serious TEAEs or anaphylaxis, or discontinuation or death due to TEAEs	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Injection site related TEAEs	2 (5.7)	0 (0.0)	1 (11.1)	3 (5.8)
	YH35324 (6 mg/kg)	Omalizumab		All subjects
Part B	n = 8	n = 8		n = 16
TEAEs‡	8 (100)	2 (25.0)	–	10 (62.5)
Drug-related TEAEs†	1 (12.5)	2 (25.0)	–	3 (18.8)
Serious TEAEs or anaphylaxis, or discontinuation or death due toTEAEs	0 (0.0)	0 (0.0)	–	0 (0.0)
Injection site related TEAEs	0 (0.0)	1 (12.5)	–	1 (6.3)

Abbreviations: TEAE = Treatment-emergent adverse events.
*n = number of subjects in that category, percentage; (%) = percentages are based on the subjects within each treatment group.
†Drug related TEAE = TEAE which had 'Certain', 'Probable/Likely', 'Possible' or 'Unassessable/Unclassifiable' relationship with the investigational product (IP).
‡Only TEAEs that occurred within 28 days from IP administration were analyzed.

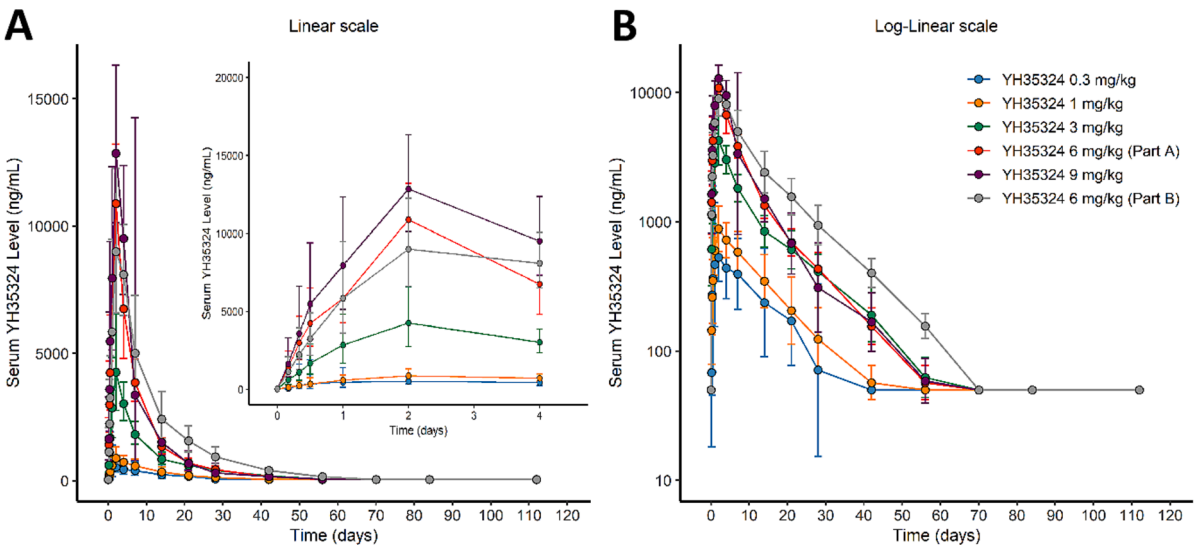

Fig. 2. Geometric mean serum levels of YH35324 following a single subcutaneous injection. The peak serum level for YH35324 was reached for all doses at similar times, and the peak serum level increased with the dose level, shown in (A) linear scale, including an expanded linear scale of up to 4 days after YH35324 administration, and (B) log-linear scale. Actual sampling times were used and if the level measured was below the lower limit of quantification (LLOQ), it was recorded as "ND" (not detected) or "BLQ" (below the limit of quantitation). Serum levels recorded as ND or BLQ were considered as LLOQ/2 for calculation of geometric means and 0 for calculation of arithmetic means. For individual curves, serum levels recorded as ND or BLQ were considered as 0 for the linear curves and as LLOQ/2 for the log-linear curves. Data shown represent geometric means ± 95 % confidence intervals.

Table 3
Summary of YH35324 pharmacokinetic parameters.

Pharmacokinetic parameters	Part A					Part B
	0.3 mg/kg (n = 3)	1 mg/kg (n = 8)	3 mg/kg (n = 8)	6 mg/kg (n = 8)	9 mg/kg (n = 8)	6 mg/kg (n = 7)
AUC_{last}, hour·ng/mL	181,880	304,421	1,115,032	1,998,194	2,720,413	2,886,371
	(34)	(53)	(23)	(18)	(34)	(27)
AUC_{inf}, hour·ng/mL	236,448	350,608	1,189,279	2,045,456	2,782,216	2,943,170
	(27)	(47)	(21)	(17)	(33)	(26)
C_{max}, ng/mL	556	1023	4,780	10,118	13,345	10,580
	(21)	(37)	(37)	(34)	(31)	(22)
T_{max}, hour	50	51	48	48	48	49
	(24–51)	(24–119)	(24–95)	(46–117)	(47–50)	(24–121)
$t_{1/2}$, hour	263	226	274	233	234	248
	(7)	(18)	(15)	(9)	(16)	(6)

Data: Arithmetic mean (CV%), T_{max}=median (min-max)
Abbreviations: AUC_{last} = area under the serum concentration-time curve from zero to the time of the last quantitative concentration; AUC_{inf} = area under the serum concentration-time curve from zero to infinity; CV = Coefficient of Variation; C_{max} = peak serum concentration; mean = arithmetic mean; T_{max} = time to peak serum concentration; $t_{1/2}$ = apparent terminal elimination half-life.
Notes: The actual sampling times were used and if the concentration measured was below the lower limit of quantification (LLOQ), it was recorded as "ND" (not detected) or "BLQ" (below the limit of quantitation). Serum concentrations recorded as ND or BLQ were considered as LLOQ/2 for calculation of geometric means and 0 for calculation of arithmetic means. For individual time-concentration curves, serum concentrations recorded as ND or BLQ were considered as 0 for the linear curves and as LLOQ/2 for the log-linear curves.

Fig. 1). In regression plots using the power model, the 90 % confidence intervals (CIs) of the C_{max} and AUC_{last} versus dose slopes included 1.0 (90 % CI: C_{max} 0.9543–1.2159; AUC_{last} 0.8286–1.0522), demonstrating dose-proportionality (Supplementary Fig. 1).

In Part B, in subjects with baseline serum total IgE level of > 700 IU/mL, the arithmetic mean (coefficient of variation, CV%) for AUC_{last} and C_{max} of YH35324 at the 6 mg/kg dose were 2,886,371 (27 %) hour·ng/mL and 10,580 (22 %) ng/mL, respectively (Table 3). The median T_{max} of YH35324 was 49 h and arithmetic mean $t_{1/2}$ was 248 h.

3.4. Serum-free IgE level was suppressed below 25 ng/mL and 82.8 ng/mL for longer durations with YH35324 compared with omalizumab

In Part A, serum-free IgE level was rapidly suppressed at all doses of YH35324 and to a greater extent compared with both omalizumab and placebo (Fig. 3A, Supplementary Fig. 2A [individual subjects]). This suppression was more pronounced within the first 15 days following YH35324 administration (Fig. 3B). The mean maximum percentage decrease in serum-free IgE levels in the YH35324-treated groups ranged from 99.9 % to 100 %, all of which were higher than the 64.7 % decrease in the omalizumab group (Supplementary Table S3). At Day 15, the median serum-free IgE level was 0 ng/mL in subjects who received YH35324 doses of 6 and 9 mg/kg, whereas the median level was 205.0 ng/mL in the omalizumab group. The median duration of serum-free IgE suppression below 25 ng/mL was longer in the groups treated with higher YH35324 doses (29.0 days in the 6 mg/kg group, and 25.5 days in the 9 mg/kg group) than the groups treated with lower doses (8.0–18.5 days in the 0.3–3 mg/kg groups) (Table 4). In all YH35324-treated groups, the duration of serum-free IgE suppression was significantly longer than in the omalizumab group (Table 4). All YH35324-treated subjects (n = 35, 100 %) achieved serum-free IgE levels of < 25 ng/mL at least once during the study period (Supplementary Table S4). This

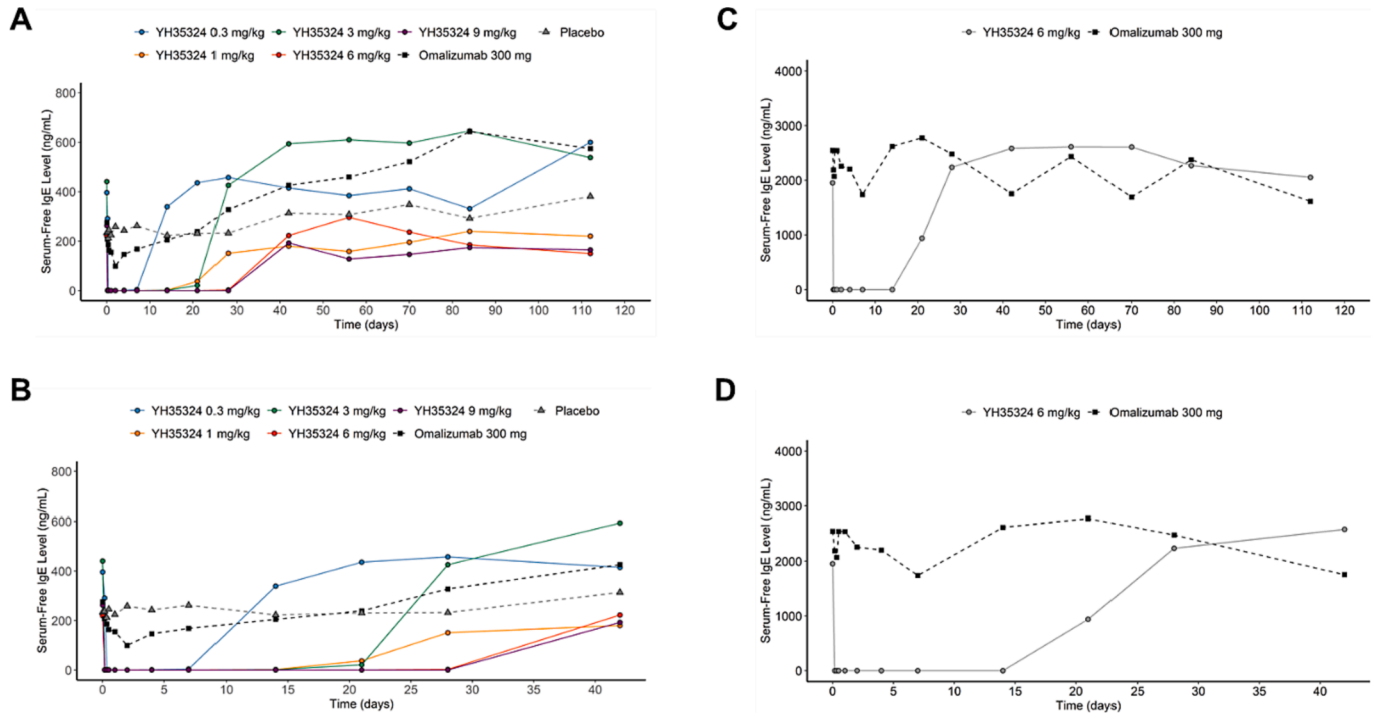

Fig. 3. Serum-free IgE levels (median values) at each time point. In subjects with total IgE levels of 30–700 IU/mL at baseline (A and B), serum-free IgE level was rapidly suppressed with all doses of YH35324 and to a greater extent compared with both omalizumab and placebo. In subjects with total IgE levels > 700 IU/mL at baseline (C and D), treatment with 6 mg/kg of YH35324 rapidly suppressed serum-free IgE to a greater extent than omalizumab.

Table 4
Duration (days) during which the serum-free IgE level was maintained at < 25.0 ng/mL and < 82.8 ng/mL.

Duration, days	Part A YH35324 0.3 mg/kg (n = 3)	1 mg/kg (n = 8)	3 mg/kg (n = 8)	6 mg/kg (n = 8)	9 mg/kg (n = 8)	Omalizumab (n = 8)	Placebo (n = 9)	Part B YH35324 6 mg/kg (n = 7)	Omalizumab (n = 8)
Serum-free IgE level maintained at < 25.0 ng/mL									
Median	8.0	15.0	18.5	29.0	25.5	0.0	0.0	15.0	0.0
(min–max)	(8.0–8.0)	(8.0–29.0)	(15.0–29.0)	(13.0–29.0)	(15.0–43.0)	(0.0–1.0)	(0.0–8.0)	(5.0–15.0)	(0.0–0.0)
P-value‡	0.010	<0.001	<0.001	<0.001	<0.001	>0.999	–	–	–
P-value§	0.006	<0.001	<0.001	<0.001	<0.001	–	–	<0.001	–
Serum-free IgE level maintained at < 82.8 ng/mL									
Median	8.0	22.0	22.0	29.0	29.0	0.0	0.0	15.0	0.0
(min–max)	(8.0–8.0)	(8.0–113.0)	(15.0–57.0)	(13.0–29.0)	(15.0–113.0)	(0.0–8.0)	(0.0–8.0)	(5.0–22.0)	(0.0–0.0)
P-value‡	0.015	<0.001	<0.001	<0.001	<0.001	0.632	–	–	–
P-value§	0.028	0.001	<0.001	<0.001	<0.001	–	–	<0.001	–

‡Wilcoxon rank sum test versus placebo group.
§ Wilcoxon rank sum test versus omalizumab group.

was significantly higher than subjects treated with omalizumab (n = 1, 12.5 %) (P = 0.001 for YH35324 1–9 mg/kg groups; P = 0.024 for YH35324 0.3 mg/kg group) or placebo (n = 1, 11.1 %) (P < 0.001 for YH35324 1–9 mg/kg group; P = 0.018 for YH35324 0.3 mg/kg group). Similarly, all YH35324-treated subjects (n = 35, 100 %) achieved a serum-free IgE level of < 82.8 ng/mL at least once during the study period, a significantly higher proportion than either the omalizumab group (n = 3, 37.5 %) (P = 0.026 for YH35324 1–9 mg/kg dose groups; P = 0.182 for YH35324 0.3 mg/kg group) or the placebo group (n = 2, 22.2 %) (P = 0.002 for YH35324 1–9 mg/kg groups; P = 0.046 for YH35324 0.3 mg/kg dose group).

In Part B, in subjects with higher baseline levels of serum total IgE (>700 IU/mL), treatment with 6 mg/kg of YH35324 rapidly suppressed serum-free IgE to a greater extent than omalizumab (Fig. 3C, Supplementary Fig. 2B [individual subjects]). The mean maximum percentage decrease in serum-free IgE level was 100 % for YH35324, which was higher than the 33.73 % decrease for omalizumab (Supplementary Table S3). At Day 15, median serum-free IgE level was 0 ng/mL for YH35324 and 2,619.3 ng/mL for omalizumab (Fig. 3D). Serum-free IgE levels were maintained at < 25 ng/mL and < 82.8 ng/mL for a median duration of 15.0 days for YH35324 and 0 days for omalizumab (Table 4). All subjects (100 %) in the YH35324 6 mg/kg dose group achieved IgE levels of 25 ng/mL or 82.8 ng/mL or below at least once during the study period. In the omalizumab group, no subjects (0 %) achieved these levels of IgE suppression (Supplementary Table S4). The difference between the two groups was statistically significant (P < 0.001).

3.5. *Serum total IgE level increased after treatment with YH35324 or omalizumab but not placebo*

In Part A, median serum total IgE level increased over time in subjects after they received YH35324 (all doses) or omalizumab, but not in

the placebo group (Supplementary Fig. 3A). No dose-dependent increase in serum total IgE level was observed for YH35324. In Part B, in subjects with higher baseline total IgE levels, serum total IgE level also increased over time in both the YH35324 and omalizumab groups (Supplementary Fig. 3B). For YH35324-treated cohorts in Part A, the mean ratio of the IgE levels at week 4 and baseline (w4IgE:bIgE) ranged from 1.90 to 8.31, compared with 5.82 for omalizumab and 1.11 for placebo, respectively. In Part B, the w4IgE:bIgE mean ratio was 3.12 for YH35324 and 1.97 for omalizumab.

3.6. Immunogenicity

Anti-YH35324 antibodies were detected in 4 subjects treated with YH35324 6 mg/kg (2 subjects each in Part A and Part B). In Part A, none of these were neutralizing antibodies. One of the subjects tested positive for anti-YH35324 antibodies even at baseline. The other subject tested negative for anti-YH35324 antibodies approximately 10 months after IP administration. In Part B, both subjects tested positive for neutralizing antibodies. One of them tested positive for anti-YH35324 antibodies even at baseline. The other subject tested negative for anti-YH35324 antibodies on Day 113 after IP administration.

4. Discussion

In this FIH study which evaluated the safety, PK, PD, and tolerability of 0.3 mg/kg–9 mg/kg doses of YH35324 in subjects with atopy, YH35324 showed a favorable safety profile. In addition, YH35324 across the dose groups exhibited a more substantial decrease and sustained suppression of serum-free IgE levels than 300 mg of omalizumab, which is the highest approved dose for CSU and covers most of the dosing table for asthma. However, given that some asthmatic patients with higher serum total IgE levels or body weight are treated with > 300 mg of omalizumab [12], it remains to be further investigated whether YH35324 can effectively outcompete omalizumab at these higher doses in reducing serum-free IgE levels in such patients.

The results suggest that subcutaneous administration of YH35324 was both safe and well-tolerated in subjects with baseline serum total IgE of 30–700 IU/mL (Part A). No clinically significant safety concerns emerged during the study, and there were no serious AEs, AEs leading to study discontinuation, or anaphylaxis, and only 3 (8.6 %) YH35324-treated subjects and 3 (37.5 %) omalizumab-treated subjects experienced drug-related AEs in Part A. Headache was the most common TEAE reported in the YH35324 group (4 subjects, 11.4 %), but was not considered related to the study drug. In addition, there were no clinically significant safety concerns observed in subjects with higher serum total IgE level (>700 IU/mL, Part B). No serious AEs or anaphylaxis events were reported during the study. Only 1 YH35324-treated subject (12.5 %) and 2 omalizumab-treated subjects (25.0 %) reported drug-related TEAEs. Use of omalizumab has been previously reported to be associated with AEs ranging from pruritus to anaphylaxis [12]. These adverse effects have been attributed to the formation of immune complexes between omalizumab and IgE, which can trigger these effects through FcγR binding [21,22]. The IgD/IgG4 Fc region of the YH35324 molecule has been engineered so as not to elicit FcγR-mediated or C1q-mediated immune effector functions, thereby avoiding unwanted side effects such as anaphylaxis [18].

Systemic exposure of YH35324 increased in a dose-proportional manner over the dose range of 0.3 to 9 mg/kg in subjects with baseline serum total IgE levels of 30–700 IU/mL. At the 6 mg/kg dose, YH35324 maximal levels (arithmetic mean C_{max}) were similar in subjects with baseline serum total IgE levels of 30–700 IU/mL and those with > 700 IU/mL, whereas subjects with > 700 IU/mL showed a higher arithmetic mean AUC_{last} than those with 30–700 IU/mL, since YH35324-IgE complexes are eliminated more slowly by intracellular proteolysis than unbound YH35324. The $t_{1/2}$ and T_{max} of YH35324 were found to be similar across all doses.

Previous studies suggest that serum-free IgE could be a suitable biomarker for predicting treatment response as it was shown to decrease substantially in asthmatic patients with a clinical response to omalizumab treatment [23–26]. In practice, the usefulness of serum total IgE (bound and unbound IgE) for monitoring treatment response is limited [20]. Serum total IgE levels have been reported to increase after omalizumab treatment due to the detection of IgE-omalizumab complexes [20]. The present study demonstrated that all doses of YH35324 could rapidly suppress serum-free IgE levels to a greater extent than omalizumab in subjects with serum total IgE of 30–700 IU/mL. Moreover, the greater extent of serum-free IgE suppression by YH35324 (compared with omalizumab) was noted in subjects with higher total IgE (>700 IU/mL). *In vitro* studies also showed that YH35324 has superior binding affinity for human IgE compared with omalizumab [19], suggesting the therapeutic potential of YH35324 for subjects with IgE-mediated allergic disease, via suppression of serum-free IgE levels.

Durable binding of YH35324 to IgE translated into a longer duration of action than omalizumab. In our study, higher YH35324 doses resulted in a longer duration of suppression of serum-free IgE levels (< 82.8 ng/mL), which was longer for all doses of YH35324 than for omalizumab. This longer duration of IgE suppression relative to omalizumab was also observed in subjects with higher baseline total IgE levels (>700 IU/mL; Part B). Serum-free IgE level at Day 15 was negligible (< 25 ng/mL) and below the cut-off level (82.8 ng/mL) for atopy [20], supporting the exploration of YH35324 as a therapeutic option, even for subjects with high serum total IgE levels. Although 4 subjects who received the 6 mg/kg dose were found to have anti-YH35324 antibodies, none of these subjects reported serious AEs or anaphylaxis. YH35324 exhibits sustained IgE-suppressive effects with low immunogenicity that does not affect its safety or PD profile.

An earlier omalizumab study identified the w4IgE:bIgE ratio as the best predictor of response to treatment as it was significantly correlated with reduction in disease activity [27]. It was suggested that a cut-off value of 1.9 or higher for this ratio indicates a treatment response. In our study, the mean w4IgE:bIgE ratios for all YH35324 dose groups ranged from 1.90 to 8.31, reaching or exceeding this cutoff value, which indicates potential reduction in disease activity [27].

As this was a first-in-human study with a single-dose design, limited numbers of subjects were recruited for the YH35324, omalizumab, and placebo treatment groups. The small sample size may limit the generalization of the results and preclude meaningful statistical comparisons between different groups. Given that this study is a Phase 1 study in subjects with healthy or mild allergic disease, clinical response assessment was limited; therefore, further investigation is required to ascertain whether the effect of YH35324 on IgE translates into favorable clinical responses in patients with allergic diseases. Currently, two Phase 1 studies are still in progress (NCT05564221 and NCT05960708).

5. Conclusion

This study showed that YH35324 has a favorable safety profile and is effective in reducing serum-free IgE levels in atopic subjects with major allergic diseases such as allergic rhinitis, atopic dermatitis, food allergy, and urticaria. The results support the continued development of YH35324 as a therapeutic option for IgE-mediated allergic diseases.

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CRediT authorship contribution statement

Young-Min Ye: Writing - review&editing, Resources, Methodology, Investigation, Conceptualization. **Jung-Won Park:** Writing – review & editing, Resources, Investigation. **Sae-Hoon Kim:** Writing – review & editing, Resources, Investigation. **You Sook Cho:** Writing -

review&editing, Resources, Investigation. **Sook Young Lee:** Writing - review&editing, Resources, Investigation. **Sae Young Lee:** Conceptualization, Writing-review & editing. **Sujin Sim:** Writing – review & editing, Validation, Software, Formal analysis, Data curation. **Eunji Song:** Writing-review & editing, Resource. **Bomin Kim:** Writing – review & editing, Writing – original draft, Visualization. **Jieon Lee:** Writing – original draft, Writing – review & editing, Visualization. **Su Kyung Kim:** Writing – review & editing. **Myoung Ho Jang:** Writing – review & editing. **Hae-Sim Park:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

[1] T. Zuberbier, W. Aberer, R. Asero, A.H. Abdul Latiff, D. Baker, et al., The EAACI/GA(2)LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria, Allergy 73 (7) (2018) 1393–1414.
[2] A.N. Fasseeh, B. Elezbawy, N. Korra, M. Tannira, H. Dalle, et al., Burden of atopic dermatitis in adults and adolescents: a systematic literature review, Dermatol Ther (heidelb) 12 (12) (2022) 2653–2668.
[3] S.W. Son, J.H. Lee, J. Ahn, S.E. Chang, E.H. Choi, et al., Assessment of disease severity and quality of life in patients with atopic dermatitis from south korea, Ann Dermatol. 34 (6) (2022) 419–430.
[4] J. Gutowska-Slesik, B. Samolinski, E. Krzych-Falta, The increase in allergic conditions based on a review of literature, Postepy Dermatol Alergol. 40 (1) (2023) 1–7.
[5] R.G. Hamilton, D.W. MacGlashan Jr., S.S. Saini, IgE antibody-specific activity in human allergic disease, Immunol Res. 47 (1–3) (2010) 273–284.
[6] S.C. Garman, B.A. Wurzburg, S.S. Tarchevskaya, J.P. Kinet, T.S. Jardetzky, Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha, Nature. 406 (6793) (2000) 259–266.
[7] B.A. Wurzburg, S.S. Tarchevskaya, T.S. Jardetzky, Structural changes in the lectin domain of CD23, the low-affinity IgE receptor, upon calcium binding, Structure. 14 (6) (2006) 1049–1058.
[8] S. Kraft, J.P. Kinet, New developments in FcepsilonRI regulation, function and inhibition, Nat Rev Immunol. 7 (5) (2007) 365–378.
[9] J. Eckl-Dorna, S. Villazala-Merino, N.J. Campion, M. Byazrova, A. Filatov, et al., Tracing IgE-Producing Cells in Allergic Patients, Cells. 8 (9) (2019) 994.
[10] E. Jensen-Jarolim, H.J. Bax, R. Bianchini, M. Capron, C. Corrigan, et al., AllergoOncology - the impact of allergy in oncology: EAACI position paper, Allergy. 72 (6) (2017) 866–887.
[11] B. Balbino, E. Conde, T. Marichal, P. Starkl, L.L. Reber, Approaches to target IgE antibodies in allergic diseases, Pharmacol Ther 191 (2018) 50–64.
[12] US Food and Drug Administration, XOLAIR: Full prescribing information, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/103976Orig1s5243lbl.pdf. (Accessed 16 October 2023).
[13] P. Gasser, S.S. Tarchevskaya, P. Guntern, D. Brigger, R. Ruppli, et al., The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab, Nat Commun. 11 (1) (2020) 165.
[14] M. Maurer, A.M. Gimenez-Arnau, G. Sussman, M. Metz, D.R. Baker, et al., Ligelizumab for chronic spontaneous urticaria, N Engl J Med 381 (14) (2019) 1321–1332.
[15] L.F. Ensina, J. Bernstein, G. Sussman, M. Maurer, A.M. Giménez-Arnau, et al., Efficacy and Safety of Ligelizumab in Chronic Spontaneous Urticaria: results from the Phase-3 Pivotal Trials, J. Allergy Clin. Immunol. 151 (2) (2023) AB130.
[16] M. Maurer, L.F. Ensina, A.M. Gimenez-Arnau, G. Sussman, M. Hide, et al., Efficacy and safety of ligelizumab in adults and adolescents with chronic spontaneous urticaria: results of two phase 3 randomised controlled trials, Lancet 403 (10422) (2023).
[17] J. Trischler, I. Bottoli, R. Janocha, C. Heusser, X. Jaumont, et al., Ligelizumab treatment for severe asthma: learnings from the clinical development programme, Clin & Trans Immunol 10 (3) (2021) e1225.
[18] S.B. An, B.G. Yang, G. Jang, D.Y. Kim, J. Kim, et al., Combined IgE neutralization and Bifidobacterium longum supplementation reduces the allergic response in models of food allergy, Nat Commun 13 (1) (2022) 5669.
[19] Lee, K.W., Yang, B-G., Oh, E.Y., Park, K.H., Ryu, M.S., et al., GI-301, a novel long-acting IgE_{Trap}-Fc fusion protein with durable and high binding to IgE translates therapeutic efficacy in various animal models (Poster), (Urticaria Conference 2019).
[20] S.D. Woo, E.M. Yang, J. Jang, Y. Lee, Y.S. Shin, et al., Serum-free immunoglobulin E: a useful biomarker of atopy and type 2 asthma in adults with asthma, Ann Allergy Asthma Immunol 127 (1) (2021) 109–115 e1.
[21] B. Balbino, P. Herviou, O. Godon, J. Stackowicz, O.R. Goff, et al., The anti-IgE mAb omalizumab induces adverse reactions by engaging Fcgamma receptors, J Clin Invest 130 (3) (2020) 1330–1335.
[22] F. Jonsson, L. de Chaisemartin, V. Granger, A. Gouel-Cheron, C.M. Gillis, et al., An IgG-induced neutrophil activation pathway contributes to human drug-induced anaphylaxis, Sci Transl Med. 11 (500) (2019) eaat1479.
[23] Y. Gon, R. Ito, S. Maruoka, K. Mizumura, Y. Kozu, et al., Long-term course of serum total and free IgE levels in severe asthma patients treated with omalizumab, Allergol Int 67 (2) (2018) 283–285.
[24] R.G. Hamilton, G.V. Marcotte, S.S. Saini, Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy, J Immunol Methods. 303 (1–2) (2005) 81–91.
[25] R. Ito, Y. Gon, S. Nunomura, R. Atsuta, N. Harada, et al., Development of assay for determining free IgE levels in serum from patients treated with omalizumab, Allergol Int. 63 (Suppl 1) (2014) 37–47.
[26] S. Korn, I. Haasler, F. Fliedner, G. Becher, P. Strohner, et al., Monitoring free serum IgE in severe asthma patients treated with omalizumab, Respir Med 106 (11) (2012) 1494–1500.
[27] R. Ertas, K. Ozyurt, M. Atasoy, T. Hawro, M. Maurer, The clinical response to omalizumab in chronic spontaneous urticaria patients is linked to and predicted by IgE levels and their change, Allergy 73 (3) (2018) 705–712.