

Tolerability, pharmacokinetics and pharmacodynamics of CMAB007, a humanized anti-immunoglobulin E monoclonal antibody, in healthy Chinese subjects

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Key words: pharmacokinetics, pharmacodynamics, safety, IgE, humanized antibody

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AUC, area under the concentration-time curve; BSA, bovine serum albumin; Cl/F , clearance; C_{max} , maximum drug concentration; C_{ss-max} , maximum steady-state drug concentration; CV, coefficient of variation; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; IgE, immunoglobulin E; k_e , elimination rate constant; LLOQ, lower limit of quantification; mAb, monoclonal antibody; NERCAM, national engineering research center of antibody medicine of China; OD, optical density; PD, pharmacodynamics; PK, pharmacokinetics; QC, quality control; SC, subcutaneous; SD, standard deviation; $T_{1/2}$, terminal half-life; TBS, tris-buffered saline; T_{max} , time to C_{max} ; TMB, tetramethylbenzidine; T_{ss-max} , time to C_{ss-max} ; V_z/F , apparent volume of distribution during the terminal phase

The goal of the studies presented here was to determine the tolerability, pharmacokinetic and pharmacodynamic profiles of CMAB007, a biosimilar of omalizumab (Xolair; a humanized anti-immunoglobulin E monoclonal antibody), in healthy, male Chinese subjects. Thirty-six healthy Chinese men participated in two open-label, dose-escalation studies: 27 in a single-dose study (150, 300 or 600 mg) and 9 in a multiple-dose study (150 or 300 mg every 4 weeks for 20 weeks). The safety profiles of both studies were generally unremarkable. No drug-related adverse event was observed. CMAB007 exhibited a linear PK profile over the dose range of 150–600 mg. In the single-dose study, maximum concentration (C_{max}) was reached within 6–8 d, and C_{max} and area under concentration-time curve (AUC) increased linearly with the dose. In the multiple-dose study, steady-state appeared to have been achieved after the third dose. C_{ss-max} and AUC_{0–∞} also showed dose-linearity. A dose-dependent suppression of free IgE was observed during treatment, as a median percentage change from baseline, 91.9–98.8%, in the three single-dose groups. No anti-CMAB007 antibodies were detected after dosing in any subject. Subcutaneous administration of CMAB007 was well-tolerated and seemed to be effective in reducing free IgE in healthy Chinese volunteers, which provides important information for further clinical studies.

Introduction

Omalizumab (Xolair®) is a recombinant humanized anti-immunoglobulin E (IgE) monoclonal antibody (mAb). It binds to the serum free IgE molecule and forms small biologically inert complexes, thereby blocking the interaction between IgE and effector cells, which trigger the allergic response irrespective of allergen type.^{1,2} Previous studies showed that the administration of omalizumab resulted in a rapid dose- and baseline IgE-dependent reduction of free IgE level in serum.³ Meanwhile, a simultaneous increase in total IgE (free IgE plus IgE-omalizumab complex)

in the omalizumab-treated subjects was observed because IgE-omalizumab complexes are cleared slower than free IgE.³ Clinical benefit with omalizumab was observed when serum free IgE levels were reduced to 50 ng/mL,⁴ but little additional benefit was seen at serum free IgE levels lower than the average target of 25 ng/mL.⁵ To achieve this free IgE suppression level, an individualized dosing table was developed, from which patients with asthma, depending on weight and starting IgE level, received omalizumab 150–375 mg by subcutaneous (SC) injection every 2 or 4 weeks. Omalizumab has been available in more than 30 countries worldwide, including United States and European Union, since

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2003. In China, however, omalizumab has not been approved for use and the clinical trials of omalizumab are ongoing.

CMAB007, a biosimilar of omalizumab, was developed by National Engineering Research Center of Antibody Medicine of China (NERCAM). CMAB007 has the same amino acid sequence as omalizumab and both mAbs are expressed in Chinese hamster ovary (CHO) cells. Our *in vitro* assays indicated that CMAB007 displayed similar ability as omalizumab to inhibit the binding of IgE to FcεRI-Ig fusion protein and membrane FcεRI (Fig. S1A and B). CMAB007 did not bind to IgE already bound by membrane FcεRI, suggesting that it did not crosslink IgE-receptor complexes on cells to release histamine (Fig. S1C and D). The *in vivo* studies in cynomolgus monkeys demonstrated that CMAB007 was absorbed with an average absolute bioavailability of 60.5% after SC administration (Fig. S2 and Table S1), very similar to that of omalizumab.⁶

Based on our preclinical data, we performed the current Phase 1 single- and multiple-dose studies in healthy male volunteers to characterize the initial safety profile of CMAB007 and assess its pharmacokinetics (PK) and pharmacodynamics (PD), which would provide some theoretical guidance for future clinical trials. It is also the first report about the safety, tolerability, PK and PD of anti-IgE mAb in a Chinese population.

Results

Demographics. A total of 36 healthy Chinese men were enrolled in the two studies. Twenty-seven subjects received CMAB007 at one of three levels in a single-dose study (150, 300 or 600 mg) and 9 received CMAB007 at either of the levels in a multiple-dose study (150 or 300 mg every 4 weeks for 20 weeks). None of the subjects participated in both studies. Thirty-five subjects completed the study and were included in safety, PK and PD analyses. One subject voluntarily withdrew from the multiple-dose study on day 2 after the 6th dose drug administration for personal reasons. Demographic characteristics for all study groups were summarized in Table 1. Overall, the dose cohorts in each study were similar in terms of demographic and other baseline characteristics. The body weights ranged from 51.0–73.0 kg, since all subjects were Chinese medical school students.

Safety and tolerability. CMAB007 SC injection was well-tolerated in healthy, male Chinese subjects. No serious clinical or laboratory adverse events were observed in either study and none of the subjects discontinued the study due to an adverse event. No subjects were found to have anti-CMAB007 antibodies at the end of the study.

A total of nine treatment-emergent adverse events were reported by 7 (19.4%) of the 36 subjects during the study, including one clinically significant signs/symptoms and eight abnormal test findings, i.e., laboratory values, chest X-ray and electrocardiogram (ECG) (Table 2). One clinical adverse event, upper respiratory tract infection, was observed after the third administration of CMAB007 to one subject in the 300 mg multi-dose group; the adverse event was minor and the subject recovered after 24 h. Two laboratory adverse events of alanine aminotransferase (ALT) increase were observed, which occurred in the 300

mg single- and multi-dose group, respectively. Increases in ALT were low in magnitude (within 2× the upper limit of the normal range) and did not appear to be dose-related. Abnormal chest X-ray results were observed in five subjects after a single dose administration, including 3 in 150 mg group, 1 in 300 mg group and 1 in 600 mg group. All of them showed an increase in double-lung markings, which was defined as not clinically meaningful by investigators. Also in the single-dose study, one subject in the 150 mg group had abnormal ECG (benign sinus tachycardia), which had returned to normal when rechecked after one day. Overall, the severity of all adverse events that occurred in the studies was evaluated as “mild,” in accordance with the study protocol. There were no drug-related changes from baseline in clinical adverse experience, vital sign assessments or physical examination results after dosing.

Pharmacokinetics. Following the initial single dose of 150, 300 or 600 mg, CMAB007 was slowly absorbed with the time to maximum drug concentration (T_{max}) of 6–8 d and gradually decreased throughout the sampling intervals (Fig. 1). Serum concentrations of CMAB007 were detected in all subjects after dosing (2 h, first post-dose collection) and were detectable throughout the study period until 84, 112 and 112 d for the 150, 300 and 600 mg group, respectively.

Noncompartmental pharmacokinetic parameters analysis showed that the maximum drug concentration (C_{max}) and the area under the concentration-time curve (AUC) increased proportionally within CMAB007 doses range of 150, 300 and 600 mg, while mean estimates of the clearance (Cl/F) was similar across the three dose levels (Table 3). The dose proportionality of C_{max} and AUC was further investigated by normalizing C_{max} and the area under the serum concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) with the amount of CMAB007 actually administered (Table 3). Statistical analysis of dose-normalized parameters and regression analysis (Fig. 2) of non-dose-normalized parameters confirmed the assumption of linear PK over the dose range. The mean terminal half-life ($T_{1/2}$) was 20.4, 24.0 and 24.6 d for the 150, 300 and 600 mg single dose, respectively. This roughly coincides with the reported half-life of 21 d for IgG mAbs.⁷ Mean estimates of the apparent volume of distribution during the terminal phase (V_z/F) ranged from 82.8–113.7 mL/kg, indicating that CMAB007 was confined primarily to the vascular system, which was consistent with results for endogenous IgG.

After 150 mg and 300 mg multiple dose administration of CMAB007, steady-state appears to have been achieved after the third dose because the trough concentration (the serum concentration of CMAB007 before injection) on week 12, 16 and 20, remained constant. Serum concentrations vs. time profiles and the principal pharmacokinetic parameters for steady-state condition were shown in Figure 3 and Table 4. Although PK parameters variability was observed due to the small sample size ($n = 3$ or 5) for both groups, it appeared that increases in the maximum values of the steady-state serum drug concentration (C_{ss-max}) and the area under the concentration-time curve during dose interval τ (AUC_{τ}) were generally proportional to CMAB007 dose as the dose increased from 150 to 300 mg every 4 weeks. The mean

Table 1. Demographic characteristics of subjects enrolled in single- and multiple-dose studies

Characteristic	Single-dose groups			Multiple-dose groups	
	150 mg (n = 9)	300 mg (n = 9)	600 mg (n = 9)	150 mg q4wk × 6 (n = 4)	300 mg q4wk × 6 (n = 5)
Age (range; years)	23.7 (21–28)	22.7 (20–26)	21.9 (19–25)	22.8 (22–24)	21.2 (21–22)
Weight (kg)	61.6 ± 5.7	59.3 ± 6.0	59.8 ± 4.5	62.0 ± 8.0	58.3 ± 4.2
Height (cm)	170.0 ± 3.6	169.9 ± 6.1	170.2 ± 5.3	172.0 ± 5.0	166.6 ± 3.0
Body mass index (kg/m ²)	21.3 ± 1.6	20.5 ± 1.3	20.6 ± 0.7	20.9 ± 1.8	21.0 ± 0.8
Baseline IgE value (range; ng/mL)	210.1 (87.9–346.3)	195.7 (75.61–323.54)	184.4 (85.38–311.34)	162.2 (44.2–290.6)	174.2 (62.3–350.2)

Values are shown as mean ± SD, except for age and baseline IgE value, which are shown as mean (range).

Table 2. Treatment-emergent adverse events reported during single- and multiple-dose administration of CMAB007

Adverse event	Single-dose groups			Multiple-dose groups		Any grade total	Grade 3/4 total
	150 mg (n = 9)	300 mg (n = 9)	600 mg (n = 9)	150 mg q4wk × 6 (n = 4)	300 mg q4wk × 6 (n = 5)		
Patients with any event	3 (33)	2 (22)	1 (11)	0	1 (20)	7 (19)	0
Any adverse event	4 (44)	2 (22)	1 (11)	0	2 (40)	9 (25)	0
Respiratory system disorder							
Upper respiratory tract infection	0	0	0	0	1 (20)	1 (3)	0
Chest X-ray abnormal	3 (33)	1 (11)	1 (11)	0	0	5 (14)	0
Liver and biliary system disorder							
Increased ALT	0	1 (11)	1 (11)	0	1 (20)	3 (8)	0
Cardiovascular general disorder							
ECG abnormal	1 (11)	0	0	0	0	1 (3)	0
Serious adverse event	0	0	0	0	0	0	0
Withdrawal due to adverse event	0	0	0	0	0	0	0

Values are shown as n (%).

serum CMAB007 concentration-time curves after single dose and the last dose of steady-state with the same dose were similar in shape (Fig. 4). In general, pharmacokinetic parameters after multiple dosing were similar to those obtained after a single dose, indicating that no accumulation was found after multiple doses of CMAB007.

The compartment model was also used in the study and a one-compartmental model with first-order absorption and first-order elimination was shown to be the best to describe the pharmacokinetics of CMAB007. The PK parameters obtained from the compartmental analysis showed excellent agreement with those from the above noncompartmental analysis (Tables 3 and 4).

Pharmacodynamics. Figure 5A and B showed the time-course of total IgE and free IgE levels in three groups after single dose administration of CMAB007, respectively. It was observed that an immediate fall to nearly undetectable levels in the concentrations of free IgE occurred within 7 days and a gradual increase of total IgE levels occurred after the single administration.

Decreases in free serum IgE concentrations were dose-dependent with CMAB007. The mean maximum percentage decline in the serum concentrations of free IgE was 91.9%, 97.3% and 98.8% from baseline levels in 150, 300 and 600 mg dose levels, respectively. Free IgE levels in serum at 28 d were below the

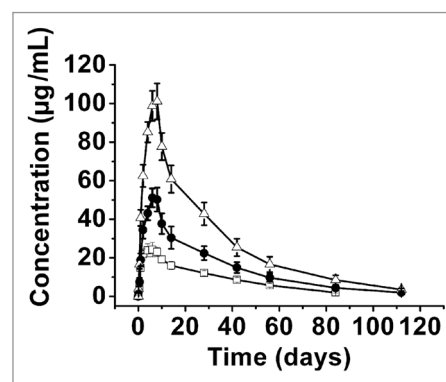


Figure 1. Serum CMAB007 concentration-time curve in healthy, male Chinese subjects after single SC administration of different doses. CMAB007 150 mg (□); CMAB007 300 mg (●); CMAB007 600 mg (△) (n = 9 for each dose group). Data were expressed as mean ± SD.

crucial level 50 ng/mL in four (44.4%) subjects receiving 150 mg CMAB007, six (66.7%) receiving 300 mg and all (100%) of those receiving 600 mg CMAB007. Additionally, there were still five (55.6%) subjects with free IgE levels of below 50 ng/mL at

Table 3. Noncompartmental and one-compartmental pharmacokinetic parameters of CMAB007 after administration of single doses in healthy Chinese subjects

Parameters (units)	CMAB007 dose		
	150 mg (n = 9)	300 mg (n = 9)	600 mg (n = 9)
Noncompartmental analysis			
AUC _{0-t} (mg·d/mL)*			
Mean (SD)	0.8 (0.1)	1.6 (0.2)	3.0 (0.3)
Median (range)	0.8 (0.6–1.0)	1.5 (1.3–2.0)	2.9 (2.6–3.5)
AUC _{0-∞} (mg·d/mL)*			
Mean (SD)	0.9 (0.1)	1.7 (0.3)	3.2 (0.3)
Median (range)	0.9 (0.7–1.0)	1.6 (1.3–2.1)	3.1 (2.6–3.7)
T _{1/2} (d)			
Mean (SD)	20.4 (2.2)	24.0 (2.0)	24.6 (2.8)
Median (range)	20.0 (18.0–24.9)	23.9 (20.8–26.9)	23.7 (20.6–30.2)
Cl/F (mL/kg·d)			
Mean (SD)	2.8 (0.4)	3.1 (0.5)	3.2 (0.3)
Median (range)	2.7 (2.4–3.8)	3.2 (2.4–3.8)	3.3 (2.7–3.8)
V _z /F (mL/kg)			
Mean (SD)	82.8 (13.0)	106.2 (15.2)	113.7 (16.7)
Median (range)	79.5 (68.2–107.1)	109.6 (84.3–128.2)	112.0 (92.0–148.8)
C _{max} (μg/mL)*			
Mean (SD)	24.9 (3.3)	53.2 (4.9)	103.1 (8.7)
Median (range)	26.1 (18.8–28.8)	53.3 (44.1–58.7)	102.3 (89.6–118.7)
T _{max} (d)			
Mean (SD)	6.0 (1.7)	6.9(1.1)	7.6(0.9)
Median (range)	6.0(4.0–8.0)	6.0(6.0–8.0)	8.0(6.0–8.0)
AUC _{0-∞} (norm)**			
Mean (SD)	5.8 (0.7)	5.6(0.9)	5.3(0.6)
Median (range)	5.9 (4.3–6.8)	5.3(4.5–7.1)	5.1 (4.4–6.1)
C _{max(norm)} **			
Mean (SD)	0.17 (0.02)	0.18 (0.02)	0.17 (0.01)
Median (range)	0.17 (0.13–0.19)	0.18 (0.15–0.20)	0.17 (0.15–0.21)
One-compartmental analysis			
AUC (mg·d/mL)*			
Mean (SD)	0.9 (0.1)	1.7 (0.3)	3.1 (0.4)
Median (range)	0.9 (0.7–1.0)	1.6 (1.3–2.1)	3.0 (2.6–3.7)
Cl/F (mL/kg·d)			
Mean (SD)	2.8 (0.4)	3.1(0.5)	3.2 (0.4)
Median (range)	2.8 (2.4–3.7)	3.1 (2.4–3.8)	3.3 (2.7–3.8)
T _{1/2} (d)			
Mean (SD)	22.1 (1.4)	22.9(1.8)	21.9 (2.3)
Median (range)	21.7 (20.2–24.7)	23.5 (20.0–25.3)	22.4 (17.9–25.1)
V _z /F (mL/kg)			
Mean (SD)	89.3 (11.4)	101.2(11.8)	102.0 (10.0)
Median (range)	87.7 (79.5–113.6)	102.9 (80.9–114.7)	100.3 (85.3–117.1)

*Regression analysis of non-dose-normalized parameters revealed no statistically significant difference of the intercept from zero. **Analysis of variance statistical analysis did not identify any difference between dose-normalized mean values.

56 d in 600 mg dose group. Higher doses of CMAB007 appeared to result in more complete suppression of free IgE concentrations.

In contrast to free IgE concentrations, total IgE concentrations increased in all treated subjects after administration of CMAB007. Mean maximum levels were up to 4.2-fold of baseline IgE concentrations in those receiving 150 mg of CMAB007, 5.4-fold in those receiving 300 mg and 6.1-fold in those receiving 600 mg of CMAB007. This dose-dependent increase in total IgE concentration appeared to be due to the formation of CMAB007: IgE complexes with a slower elimination rate compared with free IgE, which was consistent with previous studies of omalizumab.⁶

Discussion

In recent years, the question of which changes are acceptable for biosimilar products has been debated extensively. Due to the complexity of biologics, even different lots of the same mAb manufactured by the same company showed changes of N- and C-terminal heterogeneity and also showed variation in ADCC activity among batches.⁸ This issue makes it difficult to show the quality of the biosimilars to be comparable to the originals. Although several analytical assays to compare physicochemical and biological properties between the biosimilars and the originals are available, reliable tests for safety and efficacy still require development. Currently, well-controlled clinical trials are considered valuable to support the efficacy and safety of the biosimilars.⁹ Phase 1 studies play a more critical role in this issue because these are considered “first in human” studies that can give preliminary safety and pharmacokinetic profiles needed to support larger investigation that may lead to eventual regulatory approval and widespread usage of the biosimilars. However, few studies describing Phase 1 studies of biosimilars can be found in the published literature.

In this paper, we reported the first clinical evaluation of a humanized anti-IgE mAb CMAB007, a biosimilar version of omalizumab. As regards clinical development of the biosimilar, a parallel group design with CMAB007 and omalizumab was not conducted because omalizumab is unavailable in China. Accordingly, this single-arm, open-label study was conducted to evaluate the PK/PD of CMAB007 after single doses ranging from 150–600 mg and after repeated dosing at 150 or 300 mg every 4 weeks, as well as the safety profile of CMAB007 in healthy, male Chinese volunteers.

CMAB007 appeared to be generally safe and well-tolerated at the dose regimens studied. The pharmacokinetic profiles of CMAB007 after single- and multiple-dose administration were linear and dose proportional within the dose range evaluated. Negligible accumulation of CMAB007 was observed after multiple dosing. Since *Cl/F* of CMAB007 did not depend on the dose or the serum concentration, the observed elimination profiles of the mAb could be described by linear clearance, which was also showed in the PK of other mAbs (e.g., rituximab,¹⁰ pertuzumab,¹¹ bevacizumab,¹² adalimumab¹³). Target-mediated elimination, which is nonlinear and saturable and is attributed to the antigen,

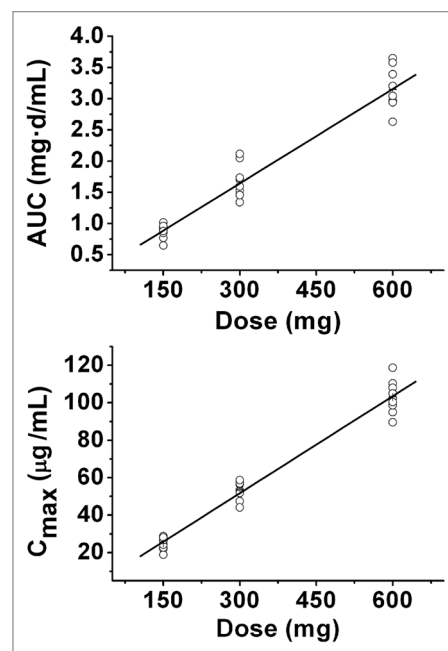


Figure 2. Linear regression analyses of (A) $AUC_{0-\infty}$ (R^2 , 0.9997) and (B) C_{max} (R^2 , 0.9979) of CMAB007 after single SC administration. Individual value (○).

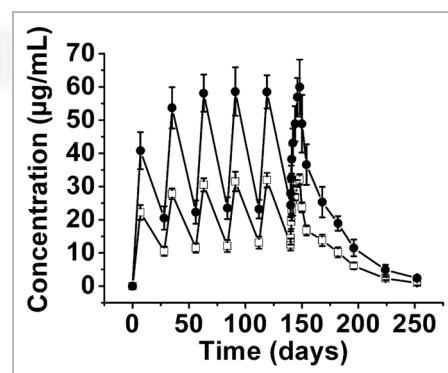


Figure 3. Serum CMAB007 concentration-time curve in healthy, male Chinese subjects following multiple SC doses of 150 mg q4wk x 6 (□, n = 3) and 300 mg q4wk x 6 (●, n = 5). Data were expressed as mean ± SD.

might be of less importance for these agents compared with other linear elimination pathways.

Pharmacodynamic responses were also achieved, although only healthy volunteers with normal IgE levels were selected in this study. Free serum IgE, as direct indicator of pharmacodynamic efficacy, decreased in a dose-dependent fashion to undetectable levels. At the same time, total IgE concentrations increased with CMAB007 therapy. Based on clinical findings with omalizumab, 50 ng/mL was the average serum free IgE level associated with potential clinical benefit. In this study, across all CMAB007 dose ranges, a reduction in mean free IgE concentrations below 50 ng/mL were all achieved and exhibited in a dose-dependent

Table 4. Noncompartmental and one-compartmental of individual pharmacokinetic parameters of CMAB007 at steady-state in healthy Chinese subjects

Dose and subject no.	Noncompartmental model					Compartmental model		
	AUC _t (mg·d/mL)	C _{ss-max} (µg/mL)	T _{ss-max} (d)	T _{1/2} (d)	AUC _{t(norm)}	C _{ss-max (norm)}	AUC _t (mg·d/mL), %CV	T _{1/2} (d), %CV
150 mg q4wk x 6*								
1	1.0	33.0	8.0	18.3	7.0	0.22	1.0, 14	23.0, 16
2	1.1	33.0	8.0	21.0	7.2	0.22	1.1, 13	27.9, 16
3	0.93	32.0	6.0	19.4	6.2	0.21	0.91, 11	21.9, 12
300 mg q4wk x 6								
1	1.6	52.7	6.0	24.7	5.4	0.18	1.5, 12	23.9, 11
2	2.5	69.3	8.0	26.5	8.2	0.23	2.4, 12	26.2, 14
3	1.9	61.2	8.0	23.1	6.4	0.20	1.8, 12	23.7, 11
4	2.2	64.3	8.0	21.7	7.3	0.21	2.0, 11	26.4, 11
5	1.8	57.5	8.0	23.0	6.1	0.19	1.7, 10	23.6, 10
Median values								
150 mg q4wk x 6	1.0	33	8.0	19.4	7.0	0.22	1.0, 14	23.0, 16
300 mg q4wk x 6	1.9	61.2	8.0	23.1	6.4	0.20	1.8, 12	23.9, 11

*One subject discontinued was excluded.

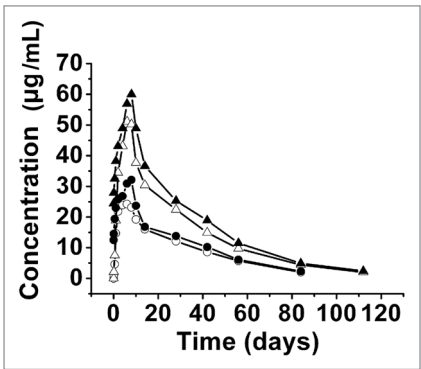


Figure 4. Serum CMAB007 concentration-time curve in healthy, male Chinese subjects following single and multiple doses of 150 mg and 300 mg. Single-dose of 150 mg (○, n = 9); multiple-dose of 150 mg (●, n = 3); single-dose of 300 mg (△, n = 9); multiple-dose of 300 mg (▲, n = 5). Data were expressed as mean values.

manner. A randomized, placebo-controlled trial in patients with severe persistent allergic asthma showed that, after omalizumab treatment cessation, asthma symptoms re-emerged followed by the return to baseline of free IgE and omalizumab.⁴ It was demonstrated that omalizumab and free IgE closely correlated with clinical symptoms, which strengthens our confidence to carry out further trials in patients with higher baseline IgE levels.

The present study demonstrates that PK/PD results of CMAB007 are comparable to those of omalizumab. For comparison purpose, key PK parameters and PD response between historical data of omalizumab and results of CMAB007 are summarized in Table 5. Due to their similarities in molecular mass, manufacturing processes and in vitro activity, it is not surprising that both anti-IgE antibodies are similar in PK/PD profiles.

Many factors can influence PK parameters of antibodies, including charge, glycosylation and aggregation.¹⁵ In general,

different expression systems may yield mAbs with different glycosylation patterns, which might result in different PK profiles for the biosimilar products.¹⁶ However, our previous studies suggest that this is not always the case. We conducted a comparative study with daclizumab (Zenapax®) and its biosimilar HuCD25mAb (also called CMAB002).¹⁷ CMAB002 and the innovator product were produced from different expression systems; however, the two mAbs showed similar PK parameters. A similar case is seen with the approved product Valtropin®, a biosimilar version of Humatrope®. Although they have exhibited similar PK profiles, efficacy and safety, Humatrope® is synthesized in *E. coli* and Valtropin® is synthesized in the yeast *Saccharomyces cerevisiae*.¹⁸ Future studies are needed to provide more evidence on comparable efficacy and safety of CMAB007 to its reference product.

A mechanism-based population PK/PD model of omalizumab revealed that the PD response is mainly a function of body weight, baseline IgE and dosage.¹⁹ Given the similarities in structure and behavior between CMAB007 and omalizumab in the in vitro and in vivo studies, it was expected that individual covariates of CMAB007 would be similar to those of omalizumab. Nonetheless, the healthy subjects in this study had similar body weight and baseline IgE levels and, thus, our results should be confirmed in future studies involving larger numbers of subjects.

These studies have demonstrated that CMAB007 was well-tolerated with predictable, dose-proportional PK and dose-dependent suppression of free IgE in healthy Chinese subjects. Furthermore, the PK and PD of CMAB007 were similar to reported values for its reference product omalizumab, suggesting that the same dosing schedule could be feasible for future clinical studies of CMAB007.

Materials and Methods

Study subjects. Healthy, male Chinese volunteers aged 18–45 y were enrolled in the two studies. Volunteers were selected for

enrollment in the study on the basis of the results of a physical examination, medical history and clinical laboratory tests performed prior to drug administration. Subjects with any known immunology disorder or allergic history were excluded.

Study design. In the single-dose study, 27 subjects were sequentially assigned at a 1:1:1 ratio into one of three escalating-dose cohorts (150, 300 or 600 mg). For each cohort, each subject was arranged to receive single SC administration of CMAB007. The first cohort received CMAB007 at a dose level of 150 mg. Enrollment of sequential, dose-escalating cohorts proceeded following a review of the safety data from at least eight of nine patients from the prior cohort.

In the subsequent multiple-dose study, nine subjects were recruited and divided into two groups ($n = 4$ for 150 mg dose level; $n = 5$ for 300 mg dose level). At each dose level, subjects were administered every 4 weeks for 20 weeks for a total of six doses.

Ethical considerations. The two studies were conducted at Southwest Hospital of the Third Military Medical University in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice. Before implementation of the study, the research protocol and informed consent form were approved by the ethics committee of the medical center. All subjects provided written informed consents prior to study participation.

Study drug. CMAB007 is a recombinant, DNA-derived, humanized mAb that competes for the same region of the IgE molecule that interacts with IgE receptors on effector cells, i.e., the Cε3 domain. The reagent is 100 mg/vial, supplied as a sterile, white-to-off-white, preservative-free, lyophilized powder.

Safety and tolerability. Safety and tolerability were assessed frequently in each study by physical examinations, vital signs, laboratory measurements (including hematology, serum chemistry tests, routine urinalysis and scotscopy), ECG and chest X-ray. Clinical adverse events, including systemic adverse events and those occurring locally at the SC injection site, were monitored throughout the study. Investigators evaluated all adverse events in terms of intensity (mild, moderate or severe), duration, outcome and relationship to study drug (none, remote, possible, probable or definitely related).

Pharmacokinetic sampling and analysis. In the single-dose study, blood samples for drug concentration detection were drawn from the radial vein before administration and 2, 12 and 24 h after administration. Additional samples were collected on day 2, 4, 6, 8, 10, 14, 28, 42, 56, 84, 112 and 140 after the administration ended. In the multiple-dose study, blood samples for the analysis were obtained before each administration and 7 d after every administration. Other blood samples were collected at 2, 12, 24 h and on day 2, 4, 8, 10, 14, 28, 42, 56, 84, 112 and 140 after the last administration. For each sample, serum was harvested by means of centrifugation from 3 mL whole blood and stored for analysis at -20°C .

Serum concentrations of CMAB007 were determined by a competitive enzyme-linked immunosorbent assay (ELISA) using CMAB007 in tested samples and a horseradish peroxidase (HRP)-labeled IgE binding competitively with human FcεRI-Ig fusion protein coated on the microplates. Human IgE-secreting

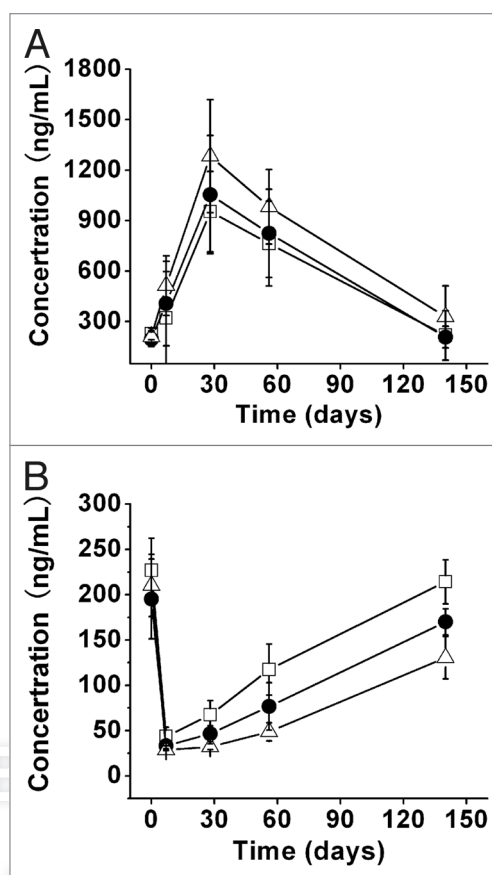


Figure 5. Serum concentration-time curve of (A) total IgE and (B) free IgE after single SC administration of different doses. CMAB007 150 mg (□); CMAB007 300 mg (●); CMAB007 600 mg (Δ) ($n = 9$ for each dose group). Data were expressed as mean $\pm$ SD.

cell line (SKO-007) was obtained from the American Type Culture Collection (ATCC). The IgE was purified from the cell culture supernatant of human IgE-secreting cell line, SKO-007, by affinity chromatography on a Sepharose 4B column conjugated with omalizumab. HRP-labeled human IgE was obtained by using a HRP labeling kit (Pierce; Rockford, IL). Human FcεRI-Ig fusion proteins were provided by NERCAM. In detail, Nunc Easywash 96-well microplates (Thermo Fisher Scientific; Waltham, MA) were coated with human FcεRI-Ig at a concentration of $5 \mu\text{g/mL}$ ($100 \mu\text{L/well}$) or buffer alone at 37°C for 2 h. The solution was aspirated and the microplates were blocked with $200 \mu\text{L/well}$ of 3% bovine serum albumin (BSA) in tris-buffered saline (TBS). Then the microplates were washed three times with tris-buffered saline tween-20 (TBST). CMAB007 standards, quality control (QC) samples and tested samples were added at $100 \mu\text{L/well}$. The sub-saturated HRP-labeled IgE was then added, and the microplates were incubated at 37°C . After 45 min, the solution was aspirated and the microplates were washed three times with TBST. The tetramethylbenzidine (TMB) substrate solution was added for coloration. The standard curve was obtained through logistic four-parameter regression, and the serum CMAB007 concentration was calculated through standard curve. Calibration standards and QC samples were run

Table 5. PK/PD of CMAB007 vs. Xolair®

Dose and parameters		CMAB007	Xolair®
Dose		150~600 mg	Recommended doses
PK parameters (noncompartmental analysis)			
Dose proportionality		Linear	Linear
$T_{\max}$ (d)		6~8	7~8
V_z/F (mL/kg)		82.8~113.7	78 ± 32
Cl/F (mL/kg·d)		2.8~3.2	2.4 ± 1.1
$T_{1/2}$ (d)		20.4~24.6	26
PK parameters (compartmental analysis)			
Compartment model	One compartment model with first-order absorption and elimination		One compartment model with first-order absorption (elimination is dose-dependent) ¹⁴
PD response			
Dose proportionality	Dose-dependent decrease in free IgE concentration		Dose-dependent decrease in free IgE concentration
Extent of free IgE decrease	Greater than 91%		Greater than 96%
Extent of total IgE increase	4.2- to 6.1-fold		5-fold

PK/PD data of Xolair® were from reference 6, if not specified.

in quadruplicate and samples were run in duplicate. The serum samples were tested directly, except that the samples on day 4, 6, 8, 10 of 600 mg group were diluted 5-folds with TBS. The method was validated in the range of 0.1–100 µg/mL with a lower limit of quantification (LLOQ) of 0.1 µg/mL. The assays provided accurate and reproducible results, with the defined limits of accuracy [standard deviation (SD) <20%] and precision [coefficient of variation (CV) <20%] at the LLOQ and <15% at all other levels up to the upper limit of quantification.

Pharmacokinetic parameters. Compartmental and non-compartmental pharmacokinetic parameters were estimated with WinNonlin Professional (version 6.1) software (Pharsight Corp.). Individual pharmacokinetic parameters were calculated and then summarized by dose cohort for dose proportionality evaluations. $C_{\max}$ in serum and $T_{\max}$, as well as $C_{ss-\max}$ and the time to $C_{ss-\max}$ ($T_{ss-\max}$) were derived directly from the observed data.

For the noncompartmental analysis, the area under the serum concentration-time curve from time zero to the last measurable serum concentration time point (AUC_{0-t}) was calculated by linear-up/log-down trapezoidal summation; $AUC_{0-\infty}$ was calculated by linear-up/log-down trapezoidal summation and extrapolated to infinity by addition of the last detectable concentration (C_t) divided by the elimination rate constant (i.e., $AUC_{0-t} + C_t/k_e$); the elimination rate constant (k_e) was determined by linear regression of the terminal points of the log-linear serum concentration-time curve; $T_{1/2}$ was determined as $\ln 2/k_e$; Cl/F was calculated as the dose divided by $AUC_{0-\infty}$; V_z/F was calculated as Cl/F divided by k_e .

For the compartmental analysis, parameter estimates for CMAB007 were determined for each individual after fitting of concentration data to one-, two- and three-compartmental models. Final model selection was based on goodness-of-fit comparisons among the models using several criteria, including, among others, visual inspection of the data, CV of parameter estimates, condition numbers, the Aikake information criterion and the Schwartz criterion.

Pharmacodynamic sampling and analysis. In CMAB007-treated subjects, total serum IgE comprised free serum IgE and IgE complexed with CMAB007. Serum concentrations of total IgE and free IgE were measured at baseline before administration, on day 7, 28, 56 and 140 during the single-dose study.

The assay for total IgE is based on a Sandwich ELISA performed in microplates. During the first incubation step, total IgE from the sample is captured by anti-human IgE coated to the microwells. By a washing procedure surplus serum components are removed from the wells whereas IgE remains bound to the solid phase surface. Detection of bound IgE is performed with an anti-human IgE HRP labeled antibody. The wells are washed again and the TMB substrate solution is added and incubated, resulting in the development of a blue color. After stopping the enzymatic reaction with sulphuric acid (H_2SO_4) the color changes into yellow. The optical density (OD) of the colored product is measured spectrophotometrically at 450 nm. The IgE concentration of the sample is proportional to the OD. A standard curve is then obtained and unknown IgE concentrations of the test samples are calculated from this curve. This assay measures both free IgE and IgE complexed with CMAB007. CMAB007 binding to IgE does not interfere with the binding of either the capturing or the revealing antibody of the assay. The LLOQ of total IgE was 2.4 ng/mL with CV <12.5%.

Free IgE was measured by using an ELISA technique with CMAB007 as the capture antibody. This avoids detection of IgE that is already complexed with CMAB007 and thus measures both baseline IgE levels before administration of CMAB007 and remaining free IgE after administration. A biotinylated anti-IgE mAb that was specific for an epitope of the IgE molecule different from that of CMAB007 was used as the revealing antibody (provided by NERCAM). To prepare IgE standards, a serum of known IgE concentration was diluted with PBS/BSA/TW (phosphate buffered saline containing 1% bovine serum albumin, 0.5 mL/L Tween 20 and 0.2 mL/L of a 10%

NaN₃ solution) to concentrations between 1 and 350 ng/mL. Nunc Easywash 96-well microplates (Thermo Fisher Scientific; Waltham, MA) were coated overnight at room temperature with 50 µL/well of CMAB007 coating solution (1 mg/mL in coating buffer). The coating solution was removed and unoccupied sites blocked by incubating overnight at 4°C with 200 µL/well of assay buffer. After washing three times with 200 µL/well of PBST, standard point solutions, controls (50 µL) and test sera (5 µL in 45 µL of diluent) were added to the appropriate wells in triplicate. In order to prevent the dissociation of drug-IgE complexes, IgE-free serum was used as the diluent. The plates were incubated for 1 h at room temperature. The plates were then washed again and 50 µL/well of a 1:5,000 dilution of the biotinylated antibody was added. After an incubation for 1 h at room temperature, the plates were washed three times and the bound biotinylated anti-IgE mAb was detected with avidin alkaline phosphatase reagent. The reaction was stopped by adding 50 µL/well of 1 M NaOH, and the optical density was read at 405 nm. The free IgE concentrations in each sample were then calculated through the standard curve obtained from the calibration standards and the optical density at 405 nm. The assay had an upper limit of quantification of 350 ng/mL, a LLOQ of 1 ng/mL and CV <16.4%.

Immunogenicity analysis. Anti-CMAB007 antibody was assayed using an antibody-bridge method. It was a double-antibody capture ELISA based on CMAB007 as the capture antibody and the revealing antibody. Endogenous IgE was blocked by pre-incubating samples with an excess of a mouse anti-IgE mAb that binds to the same epitope as CMAB007 and has a higher affinity for IgE. Briefly, the serum samples were added to microplates pre-coated with mouse anti-IgE mAb and incubated at 37°C for 1 h. The IgE-free serum served as samples. 96-well microplates were coated with CMAB007 at a concentration of 20 µg/mL (100 µL/well) at 37°C for 2 h. The solution was aspirated and the microplates were washed 3 times with PBST. The microplates were blocked with 200 µL/well of 3% BSA. Samples and controls were diluted 1:10 in 300 mM acetic acid, and then were left at ambient temperature for 1 h to dissociate any immune complexes. The blocking solution was removed, and 100 µL of biotinylated CMAB007 at 2 µg/mL in blocking buffer was added to each well of the microplates, followed by the addition of 30 µL

of 1 M TRIS pH 9.5 and 100 µL of the acid treated sample. The solutions were then mixed gently, and the plates were incubated at ambient temperature for 2 h. After a wash procedure, HRP-avidin was added, and the plates were incubated at room temperature for 1 h. The solution was aspirated and the microplates were washed three times. The TMB substrate solution was added for coloration. Concentrations of anti-CMAB007 were expressed in ng equivalents/mL using the anti-idiotypic mAb against anti-CMAB007 as the reference. The lower limit of assay was 50 ng equivalents/mL of anti-idiotypic mAb when CMAB007 was 20 µg/mL. When the samples were positive, neutralizing anti-antibody antibody would be detected by competitive ELISA method subsequently.

Statistical analyses. Statistical analysis was performed with a standard computerized statistical program, SPSS 13.0 for Windows software (SPSS, Inc.; Chicago, IL). For data among at least three groups, analysis of variance (ANOVA) was applied to test differences of data on normal distribution, and the Kruskal-Wallis test (K-W *H* test) was used when the data were not normally distributed. For a difference between two groups, the t-test was employed when the data were of normal distribution and variance was homogenous; otherwise, the Wilcoxon test was used. Shapiro-Wilks *W* test and Levene's test were used for assessing normality and homogeneity of variance, respectively. To investigate whether C_{max} and $AUC_{0-\infty}$ increased dose proportionally, visual/graphical examination and/or linear regression analyses were performed to evaluate the relationships of these parameters and dose. The *p* value < 0.05 was considered statistically significant.

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Supplemental Material

Supplemental material can be found at:
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