

Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor α antibody, in a phase I study of subjects with mild asthma

William W. Busse, MD,^a Rohit Katial, MD,^b David Gossage, MD, MBA,^c Suha Sari, PhD,^{c*} Bing Wang, PhD,^c Roland Kolbeck, PhD,^c Anthony J. Coyle, PhD,^c Masamichi Koike, PhD,^d George L. Spitalny, PhD,^d Peter A. Kiener, PhD,^{c**} Gregory P. Geba, MD, MPH,^c and Nestor A. Molfinio, MD^c Madison, Wis, Denver, Colo, Gaithersburg, Md, Princeton, NJ

Background: Increased eosinophil levels have been linked to airway inflammation and asthma exacerbations. IL-5 is responsible for eosinophil differentiation, proliferation, and activation; IL-5 receptors are expressed on eosinophils and their progenitors, and targeting such receptors induces eosinophil apoptosis.

Objective: To evaluate the safety profile, pharmacokinetics, and pharmacodynamics of MEDI-563, a humanized mAb targeting the IL-5 receptor α chain.

Methods: Single, escalating, intravenous doses (0.0003–3 mg/kg) of MEDI-563 were administered to subjects with mild atopic asthma ($n = 44$) over ~ 3 to 30 minutes in this open-label study. Pulmonary function, symptom scores, adverse events, MEDI-563 pharmacokinetics, and levels of C-reactive protein (CRP), IL-6, eosinophil cationic protein (ECP), and eosinophils were evaluated.

Results: Mean peripheral blood (PB) eosinophil levels decreased in a dose-dependent fashion (baseline $\pm$ SD, $0.27 \pm 0.2 \times 10^3/\mu\text{L}$; 24 hours postdose, $0.01 \pm 0.0 \times 10^3/\mu\text{L}$); 94.0% of subjects receiving ≥ 0.03 mg/kg exhibited levels between $0.00 \times 10^3/\mu\text{L}$ and $0.01 \times 10^3/\mu\text{L}$. Eosinopenia lasted at least 8 or 12 weeks with doses of 0.03 to 0.1 and 0.3 to 3 mg/kg, respectively. ECP levels were reduced from $21.4 \pm 17.2 \mu\text{g/L}$ (baseline) to $10.3 \pm 7.0 \mu\text{g/L}$ (24 hours postdose). The most frequently reported adverse events were reduced white blood cell counts (34.1%), nasopharyngitis (27.3%), and increased blood creatine phosphokinase (25.0%). Mean C-reactive protein levels increased ~ 5.5 -fold at 24 hours postdose but returned to baseline by study end; mean IL-6 levels increased ~ 3.9 -fold to

4.7-fold at 6 to 12 hours postdose, respectively. Pharmacokinetic activity was dose proportional at doses of 0.03 to 3 mg/kg.

Conclusion: Single escalating doses of MEDI-563 had an acceptable safety profile and resulted in marked reduction of PB eosinophil counts within 24 hours after dosing. (J Allergy Clin Immunol 2010;125:1237–44.)

Key words: Asthma, eosinophils, IL-5, IL-5 receptor, monoclonal antibody, antibody-dependent cell-mediated cytotoxicity

Eosinophils have been implicated in the pathogenesis of asthma,¹ although their exact biological role is not entirely understood.² In a recent review, 40% to 60% of subjects with asthma were estimated to have eosinophilic airway inflammation.³ Eosinophils produce mediators of inflammation, such as eosinophil cationic protein (ECP) and leukotrienes,⁴ and a rise in sputum eosinophils has been associated with asthma exacerbations.^{5,6} Secreted by T_H2 and mast cells, IL-5 is a key cytokine responsible for the differentiation, proliferation, activation, and pulmonary trafficking of eosinophils,^{7,8} and it may contribute to the onset of asthma symptoms. IL-5 works synergistically with granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, IL-4, IL-13, eotaxins, and other signaling proteins to induce eosinophil-mediated inflammatory responses.²

Human IL-5 interacts with eosinophils via the IL-5 receptor (IL-5R), which is expressed on eosinophils, basophils,^{7,9} and the surface of some cultured human mast cells.¹⁰ IL-5R is a heterodimer consisting of an α and a β chain. Monomeric IL-5R α specifically binds IL-5 but with low affinity, whereas dimerization with the β chain produces a high-affinity phenotype.¹¹ The intracellular portion of IL-5R α is associated with Janus Activating Kinase 2 (JAK2), a protein tyrosine kinase essential in IL-5 signal transduction.^{12,13}

Previous asthma clinical studies with humanized anti-IL-5 mAb therapies, such as mepolizumab and SCH55700, have shown improvement in some efficacy parameters, although the extent of their clinical benefit remains unclear.^{14,15} Recently, positive results were reported from 2 studies with mepolizumab.^{16,17} In 1 study with a 50-week observational period, subjects with refractory eosinophilic asthma ($n = 61$) received 12 monthly intravenous infusions of mepolizumab (750 mg). Active treatment led to significant reductions of severe asthma exacerbations (mean, 2.0/subject) compared with placebo (mean, 3.4/subject; $P = .02$).¹⁶ Statistically significant improvements were also found in quality of life ($P = .02$) and eosinophil levels in the blood ($P < .001$) and sputum ($P = .002$). In contrast, parameters such as forced expiratory volume in 1 second (FEV₁), symptom scores, and airway hyperresponsiveness (AHR) failed to improve. In another smaller study of subjects with prednisone-dependent eosinophilic airway

From ^athe University of Wisconsin School of Medicine and Public Health; ^bNational Jewish Health, Denver; ^cMedImmune, LLC, Gaithersburg; and ^dBiowa, Inc, Princeton.

*S. Sari is currently employed by Emergent BioSolutions, Rockville, Md.

**P. A. Kiener is currently employed by Zyngenia, Inc, Gaithersburg, Md.

Supported by MedImmune, LLC, Gaithersburg, Md, and Biowa, Inc, Princeton, NJ.

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Reprint requests: Nestor A. Molfinio, MD, MedImmune, LLC, One MedImmune Way, Gaithersburg, MD 20878. E-mail: Molfinon@MedImmune.com.

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Abbreviations used

ADCC:	Antibody-dependent cell-mediated cytotoxicity
AE:	Adverse event
AHR:	Airway hyperresponsiveness
CPK:	Creatine phosphokinase
CPKMB:	Creatine phosphokinase, muscle and brain
CRP:	C-reactive protein
ECP:	Eosinophil cationic protein
FE _{NO} :	Fractional exhaled nitric oxide
IL-5R:	IL-5 receptor
JAK2:	Janus Activating Kinase 2
PB:	Peripheral blood
WBC:	White blood cell

inflammation ($n = 20$), 5 monthly intravenous infusions with mepolizumab (also 750 mg) resulted in fewer asthma exacerbations compared with placebo over a 24-week period.¹⁷ On average, the mepolizumab-treated subjects reduced their prednisone dose by nearly 84% versus approximately 48% observed in those in the placebo arm ($P = .04$).

MEDI-563, a humanized, recombinant immunoglobulin IgG_{1κ} isotype mAb, binds with high affinity to a conformationally distinct epitope within domain 1 of the α -chain of IL-5R,¹⁸ and it has the capacity to inhibit proliferation of IL-5-dependent cell lines (half-maximal inhibitory concentration, 0.3 nmol/L).¹⁹ In addition, MEDI-563 has been engineered without a fucose sugar residue in the CH2 region, a modification that results in augmentation of eosinophil apoptosis *in vitro* via antibody-dependent cell-mediated cytotoxicity (ADCC).¹⁹

This first in-human, multicenter, open-label study evaluated the safety profile, biological effects, and pharmacokinetic activity of single ascending intravenous doses of MEDI-563 in adults with mild atopic asthma.

METHODS

Subjects

Adults (age 18–45 years) with mild atopic asthma (nighttime symptoms $\leq 1/\text{wk}$ and daytime symptoms $< 1/\text{d}$, FEV₁ or peak expiratory flow $\geq 80\%$ of predicted, and peak expiratory flow variability $\leq 30\%$) were eligible. A methacholine provocation dose causing 20% decrease in FEV₁ (PC₂₀) of $\leq 8 \text{ mg/mL}$ was required during the first visit (day -14 to -7) or documented within 6 months previously. Women were eligible if postmenopausal, surgically sterile, or willing to use birth control for the duration of the study. Subjects who received topical corticosteroids other than nonprescription topical steroids for dermatologic conditions within 6 months before the first visit or those requiring a prescription medication other than contraceptives, β_2 -agonists, or anticholinergic agents for the treatment of mild asthma were excluded. Individuals were excluded if they had smoked tobacco within 1 year before the second visit on day 0 or had a smoking history of > 5 pack-years; documented evidence of hepatitis B virus, HIV, or hepatitis C virus infection; any condition resulting in an increased eosinophil count (except for asthma); or a body mass index $< 15 \text{ kg/m}^2$ or $> 30 \text{ kg/m}^2$ at the first visit. The study was conducted in accordance with the Declaration of Helsinki and was approved by an institutional review board/independent ethics committee. Written informed consent was obtained from each subject before study entry.

Study design

This was a phase I, multicenter, open-label, sequential dose-escalation study with unique subjects for each cohort. A single intravenous dose of

MEDI-563 was administered over ~ 3 to 30 minutes in the morning, according to the following dose-escalation scheme: cohort 1 ($n = 6$), 0.03 mg/kg; cohort 2 ($n = 6$), 0.1 mg/kg; cohort 3 ($n = 6$), 0.3 mg/kg; cohort 4 ($n = 9$), 1 mg/kg; and cohort 5 ($n = 6$), 3 mg/kg. Two additional cohorts (cohort 6 [$n = 6$], 0.003 mg/kg; cohort 7 [$n = 5$], 0.0003 mg/kg) were added to aid in identifying the minimum effective dose (see Results). MEDI-563 was administered over 3 to 6 minutes in 84% of the subjects. The drug was initially manually filtered by using a protein-sparing/low-protein binding filter (0.22 μm) before infusion. However, because of some mild infusion-related reactions observed in cohort 5 (3 mg/kg; see Results), an in-line filter was subsequently used, and the infusion rate was extended to 30 minutes.

Once all subjects within each cohort were administered the study drug at a particular dose level, dose escalation continued to the next cohort if the safety profile was deemed acceptable. Day 58 postdose was the end of study for cohorts 1 and 2; however, as per a protocol amendment to lengthen the observation time, day 84 postdose was the end of study for cohorts 3 to 7. Subjects in cohorts 3 to 7 whose peripheral blood (PB) eosinophil counts persisted at a level less than 70% of baseline were followed monthly beyond day 84 postdose for 6 months and every 3 months thereafter for a maximum of 1 year, or until the eosinophil counts reached a level of ≥ 100 eosinophils/ mm^3 or $\geq 70\%$ of the baseline value.

Pharmacologic activity

The pharmacologic activity of MEDI-563 was evaluated by measuring PB eosinophil levels throughout the study and ECP levels on day 0 before dosing and on days 1, 2, 7, 58, and 84.

Skin testing and assessment of pulmonary function, airway inflammation, AHR, symptom scoring, and β_2 -agonist use were also performed (see this article's **Methods and Results** in the Online Repository at www.jacionline.org for details).

Safety profile

The safety profile was primarily assessed by monitoring treatment-emergent adverse events (AEs) throughout the study following administration of MEDI-563. Other safety variables included assessment of vital signs, hematology, C-reactive protein (CRP) levels, IL-6 levels, urinalysis, and biochemistry parameters. Hematology, urinalysis, and biochemistry parameters were assessed via standard techniques at local laboratories.

Pharmacokinetic sampling

Blood samples were collected on day 0 (2, 30, and 60 minutes; 2, 4, 8, and 12 hours postdose), day 1 (24 and 36 hours postdose), days 2 to 14 (same time as dosing on day 0 ± 1 hour), and days 28, 58, and 84. Plasma MEDI-563 concentrations were determined using a validated ELISA with a lower limit of quantitation of $\sim 60 \text{ ng/mL}$.

Statistical analyses

Descriptive statistics were used for continuous variables, frequency distributions, and percentages for discrete variables. A sample size of 6 subjects/dose level was deemed clinically adequate to determine whether subjects could be treated with the next dose level in this phase I study. The intent-to-treat population consisted of all subjects assigned a study number, whereas the safety population included all subjects who received the study drug. These 2 populations were identical in this study, and the results reported apply to both intent-to-treat and safety populations.

The primary safety endpoint evaluated the overall incidence of AEs reported in each dose group. Secondary safety endpoints included changes in laboratory assessments (hematology, biochemistry, and urinalysis), vital signs, physical examinations, and electrocardiogram tests. Secondary pharmacologic endpoints included assessment of eosinophil counts in the PB and levels of fractional exhaled nitric oxide (FE_{NO}) and ECP. AHR was evaluated as an exploratory endpoint in the new subjects enrolled in cohort 4 and subjects in cohorts 5 to 7.

TABLE I. Demographics and baseline characteristics

Parameter	0.0003 mg/kg (n = 5)	0.003 mg/kg (n = 6)	0.03 mg/kg (n = 6)	0.1 mg/kg (n = 6)	0.3 mg/kg (n = 6)	1 mg/kg (n = 9)	3 mg/kg (n = 6)
Age (y)							
Mean	26.8	23.5	21.3	21.8	24.8	28.1	25.0
Range	19-38	18-30	19-25	18-29	20-41	20-40	20-34
Sex, n (%)							
Male	0 (0.0)	1 (16.7)	5 (83.3)	2 (33.3)	3 (50.0)	4 (44.4)	2 (33.3)
Female	5 (100)	5 (83.3)	1 (16.7)	4 (66.7)	3 (50.0)	5 (55.6)	4 (66.7)
Race, n (%)							
White	4 (80)	5 (83.3)	6 (100)	6 (100)	6 (100)	8 (88.9)	6 (100)
Black	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)
BMI (kg/m ²)							
Mean	24.5	22.8	23.9	24.2	24.7	25.9	25.0
Range	19.3-30.8	17.8-25.5	19.1-32.6	21.7-30.2	21.7-28.7	20.0-32.0	20.9-29.4
FEV ₁ (L),*							
Mean (SD)	3.1 (0.3)	3.2 (0.4)	3.7 (0.5)	3.4 (0.4)	3.6 (0.6)	3.7 (0.8)	3.4 (0.6)
FE _{NO} (ppb),*							
Mean (SD)	22.6 (18.2)	64.4 (41.4)	49.7 (50.2)	39.6 (29.7)	27.6 (12.7)	52.7 (41.4)	30.7 (26.6)

BMI, Body mass index; ppb, parts per billion.

*Measurements were within the normal predicted values.

Noncompartmental pharmacokinetic data analysis was performed on individual MEDI-563 plasma concentration data by using WinNonlin Professional (version 5.2; Pharsight Corp, Mountain View, Calif). Nominal collection times as prespecified in the study protocol were used for the data analysis.

RESULTS

Demographics and baseline characteristics

Of the 44 subjects enrolled, 39 (88.6%) completed the study. Three subjects withdrew consent (0.1 mg/kg, n = 1; 1 mg/kg, n = 1; 3 mg/kg, n = 1), and 2 subjects (0.0003 mg/kg) were lost to follow-up. Baseline characteristics were generally comparable among all treatment groups (Table I).

Three additional subjects were added to the 1 mg/kg group (n = 9) when half of the 6 subjects in the 3 mg/kg group experienced a cluster of AEs after infusion with MEDI-563 (see Safety profile). All of these subjects completed the study.

Pharmacologic activity

MEDI-563 exhibited good pharmacologic activity, as evidenced by the reduced PB eosinophil and ECP levels. Eosinopenia developed quickly in all dose cohorts. Persistent PB eosinophil suppression was observed at doses ranging from 0.03 mg/kg to 3 mg/kg (Fig 1). Eosinopenia lasted at least 8 weeks in doses of 0.03 to 0.1 mg/kg and at least 12 weeks in doses of 0.3 to 3 mg/kg. One day after dosing, the PB absolute eosinophil counts decreased to a mean $\pm$ SD of $0.01 \pm 0.00 \times 10^3/\mu\text{L}$ 1 day after administration of doses ranging from 0.03 mg/kg to 3 mg/kg. Between day 7 and the study end, 94.0% (31/33) of subjects in those cohorts demonstrated persistent mean eosinophil depletion ($0.00 \times 10^3/\mu\text{L}$ to $0.01 \times 10^3/\mu\text{L}$). Two subjects in the 0.03 mg/kg ($0.17 \times 10^3/\mu\text{L}$) and 1 mg/kg ($0.22 \times 10^3/\mu\text{L}$) groups manifested partial recovery of PB absolute eosinophil counts on days 58 and 84, respectively. Eosinophil levels were not consistently reduced in the 0.003 mg/kg and 0.0003 mg/kg groups, establishing a threshold for consistent reduction of PB eosinophils at the 0.03 mg/kg dose.

Similarly, ECP levels were reduced 24 hours postdose in all the groups, with the exception of the 3 mg/kg group, which exhibited transiently increased mean levels ($\sim 13\%$) relative to baseline (Table II). Three of 6 subjects in the 3 mg/kg cohort had increased ECP levels at 24 hours postdose. Two of the 3 subjects had infusion-related AEs; 1 of these subjects had an ECP level of $42.3 \mu\text{g/L}$, accounting for most of the ECP increase observed in the 3 mg/kg cohort. By study end, mean ECP levels were below baseline values in all cohorts.

Pulmonary function did not deteriorate during the study, and asthma symptom scores and β_2 -agonist use remained low. Airway inflammation assessed by fractional exhaled nitric oxide was unchanged (see Results in the Online Repository at www.jacionline.org for details).

Safety profile

A total of 348 AEs were reported across all cohorts. Of those, 86.5% were mild and 13.5% were moderate in severity. None of the AEs was severe, and no relationship between dose level and incidence of AE was detected. Likewise, no serious AEs or deaths were observed. Transient reductions of white blood cell (WBC) counts (34.1%), nasopharyngitis (27.3%), and increased blood creatine phosphokinase (CPK; 25.0%) were the most frequently reported AEs (Table III).

A transient, mild decrease in WBC counts was observed in this study across all cohorts. The maximum mean WBC decrease of 25.6% occurred 1 day after MEDI-563 infusion (52.3% of subjects <4000 cells/ μL). However, these decreases were transient, and the proportion of subjects with WBC counts ≥ 4000 cells/ μL increased to 86.4%, 86.4%, and 92.9% by days 14, 28, and 58, respectively. Mean absolute blood eosinophils were reduced by 96.8% at day 1. Other mean absolute blood cell counts were also decreased, but to a lesser degree (lymphocytes, 35.2%; monocytes, 20.5%; and neutrophils, 13.3%). Accounting for the prevalence of each blood cell lineage and the absolute mean reductions 1 day after MEDI-563 infusion, the rank order of each cell lineage's contribution to the overall decrease in

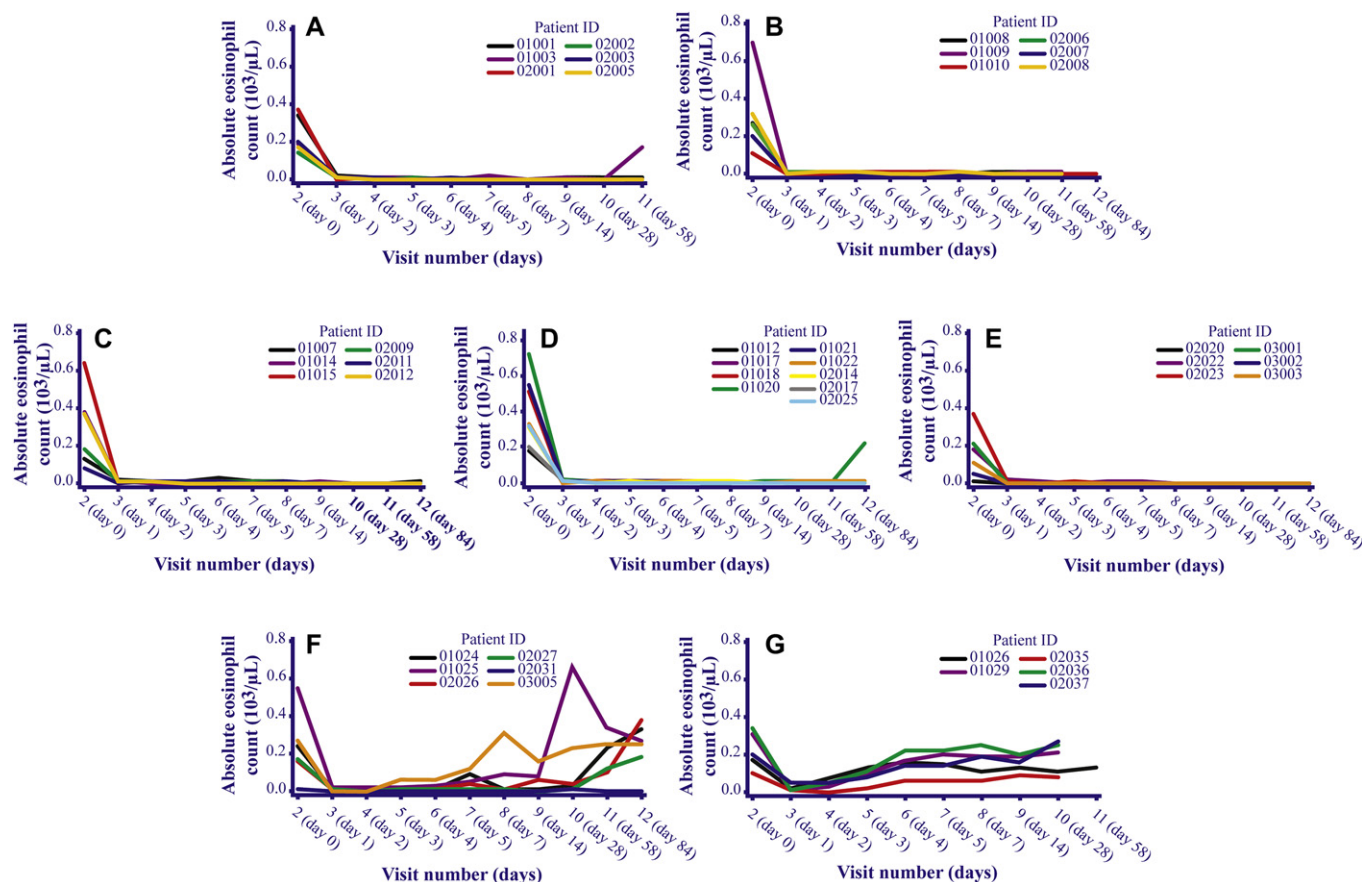


FIG 1. Absolute eosinophil counts by cohort dose level: 0.03 mg/kg (cohort 1, **A**); 0.1 mg/kg (cohort 2, **B**); 0.3 mg/kg (cohort 3, **C**); 1 mg/kg (cohort 4, **D**); 3 mg/kg (cohort 5, **E**); 0.003 mg/kg (cohort 6, **F**); 0.0003 mg/kg (cohort 7, **G**). *ID*, Identification.

WBC was as follows: lymphocytes (12.2%) > neutrophils (7.0%) > eosinophils (4.4%) > monocytes (1.6%).

Because CPK values may vary naturally over time, a multilinear regression analysis of all CPK values and those from a subgroup of subjects with peak CPK values >3 times the upper limit of normal were analyzed. The results failed to demonstrate a dose-dependent or time-dependent correlation with peak CPK values (data not shown). Two subjects in the 3 mg/kg cohort had normal CPK values at baseline, but they developed high peak values (1515 U/L and 2009 U/L) on day 7. Only 1 of these subjects developed electrocardiographic (transient ST segment) changes during the trial; however, these were considered clinically insignificant. Neither subject experienced chest or muscle weakness or pain during this period.

According to a protocol amendment, all subjects with AEs related to elevated CPK values ($n = 7$) had concomitant creatine kinase, muscle, and brain enzyme levels measured. All creatine kinase, muscle, and brain/CPK ratios from these subjects were consistent with a peripheral muscle origin (creatinine phosphokinase, muscle and brain [CPKMB]/CPK <2%).^{20,21} All subjects with elevated peak CPK values were asymptomatic.

Mean $\pm$ SD CRP levels increased ~ 5.5 fold from 0.37 ± 0.9 mg/dL at baseline to 2.0 ± 6.8 mg/dL at 24 hours postdose; levels returned to baseline by study end (0.57 ± 1.4 mg/dL; [Table II](#)). Mean $\pm$ SD peak IL-6 levels increased from 2.7 ± 7.9 pg/mL at baseline to 10.4 ± 14.2 pg/mL at 6 hours postdose and

12.7 ± 19.7 pg/mL at 12 hours postdose; by 24 hours postdose, mean $\pm$ SD IL-6 levels decreased to 5.7 ± 10.3 pg/mL ([Table II](#)). These transient spikes in CRP and IL-6 levels were neither dose-dependent nor associated with any clinical symptoms in the majority of subjects, except for the 4 subjects described below.

A total of 4 subjects had ≥ 4 acute (<24 hours) infusion-related AEs (0.03 mg/kg, $n = 2$; 3 mg/kg, $n = 2$). All AEs were transient; 2 subjects required minimal medical intervention (acetaminophen and/or ibuprofen). One subject in cohort 1 (0.03 mg/kg) developed chills, sweating, nasal congestion, and a dry sore throat 23 hours after the infusion. The second subject in cohort 1 developed mild flushing, fatigue, tension, and a generalized sensation of warmth 4 to 6 hours after the infusion. In cohort 5 (3 mg/kg), 1 subject experienced nausea, light-headedness, fever, chills, and headache approximately 7 hours after the infusion. A second subject in cohort 5 developed a headache approximately 5 hours postinfusion, followed by fever, chills, flushing, rigors, and fatigue 3 to 4 hours later. To minimize the probability of acute infusion-related AEs, subsequent infusions were extended to 30 minutes. Thus, 3 additional subjects in the 1 mg/kg group and 2 subjects in the 3 mg/kg group were infused over 30 minutes. Because of the much lower dose involved, the last 2 dose de-escalation cohorts, 6 (0.003 mg/kg) and 7 (0.0003 mg/kg), were infused over 5 minutes. Neither these subjects nor those infused over 30 minutes experienced a similar cluster of acute infusion-related AEs as described for the 4 subjects in cohorts 1 and 5.

TABLE II. Safety parameters levels (mean $\pm$ SD) by dose cohort*

Parameter	0.0003 mg/kg (n = 5)	0.003 mg/kg (n = 6)	0.03 mg/kg (n = 6)	0.1 mg/kg (n = 6)	0.3 mg/kg (n = 6)	1 mg/kg (n = 9)	3 mg/kg (n = 6)	Total (n = 44)
CRP (mg/dL)								
Baseline	0.38 $\pm$ 0.4	0.09 $\pm$ 0.1	0.17 $\pm$ 0.2	0.16 $\pm$ 0.2	0.32 $\pm$ 0.4	0.26 $\pm$ 0.3	1.27 $\pm$ 2.3	0.37 $\pm$ 0.9
Day 1	0.29 $\pm$ 0.3	0.34 $\pm$ 0.3†	0.37 $\pm$ 0.2	0.32 $\pm$ 0.2	0.58 $\pm$ 0.4	0.96 $\pm$ 0.6	11.2 $\pm$ 16.5	2.02 $\pm$ 6.8‡
End of study	0.30 $\pm$ 0.5§	0.08 $\pm$ 0.1	NA	NA	0.36 $\pm$ 0.4†	0.22 $\pm$ 0.3	1.83 $\pm$ 2.9	0.57 $\pm$ 1.4¶
ECP (μg/L)								
Baseline	19.7 $\pm$ 14.8	23.0 $\pm$ 17.0	30.3 $\pm$ 17.9	15.7 $\pm$ 10.5	29.9 $\pm$ 32.0	19.8 $\pm$ 10.8	12.1 $\pm$ 9.6	21.4 $\pm$ 17.2
Day 1	9.8 $\pm$ 5.5	5.6 $\pm$ 2.4	10.9 $\pm$ 3.5	12.0 $\pm$ 7.3	10.8 $\pm$ 5.0	9.4 $\pm$ 5.2	13.7 $\pm$ 14.6	10.3 $\pm$ 7.0
End of study	15.7 $\pm$ 14.3§	13.2 $\pm$ 9.1	NA	NA	6.5 $\pm$ 3.6†	7.9 $\pm$ 9.2	6.1 $\pm$ 11.0	9.2 $\pm$ 9.4¶
IL-6 (pg/mL)								
Baseline	0.58 $\pm$ 0.4	0.48 $\pm$ 0.6	1.9 $\pm$ 1.5	1.3 $\pm$ 0.8	1.2 $\pm$ 0.7†	7.9 $\pm$ 16.9	2.3 $\pm$ 2.5	2.7 $\pm$ 7.9‡
6 hours#	12.0 $\pm$ 13.8	1.6 $\pm$ 1.2	6.0 $\pm$ 4.1	8.3 $\pm$ 7.0†	6.6 $\pm$ 4.9†	18.5 $\pm$ 24.4	16.3 $\pm$ 16.2	10.4 $\pm$ 14.2**
12 hours#	9.7 $\pm$ 15.4††	1.9 $\pm$ 1.8	7.4 $\pm$ 3.8	17.5 $\pm$ 26.1	5.3 $\pm$ 4.6††	23.0 $\pm$ 31.3	15.5 $\pm$ 15.4	12.7 $\pm$ 19.7**
Day 1	1.9 $\pm$ 1.1	1.6 $\pm$ 1.1	5.0 $\pm$ 3.4	6.0 $\pm$ 4.3	3.7 $\pm$ 4.1†	10.4 $\pm$ 18.3	7.6 $\pm$ 14.9	5.7 $\pm$ 10.3‡

CRP, Measured predose, 24 hours $\pm$ 1 hour postdose, and on days 4, 7, 14, 28, 58, and 84; IL-6, measured 6, 12, and 24 hours $\pm$ 1 hour postdose; NA, not available.

*Consisted of all the subjects who received the study drug.

†n = 5.

‡n = 43.

§n = 3.

||n = 8.

¶n = 28.

#Postdose.

**n = 41.

††n = 4.

TABLE III. Frequency of adverse events*

Parameter	No. (%) of subjects with adverse events							
	0.0003 mg/kg (n = 5)	0.003 mg/kg (n = 6)	0.03 mg/kg (n = 6)	0.1 mg/kg (n = 6)	0.3 mg/kg (n = 6)	1 mg/kg (n = 9)	3 mg/kg (n = 6)	Total (N = 44)
WBC count decrease	0 (0.0)	4 (66.7)	3 (50.0)	1 (16.7)	1 (16.7)	5 (55.6)	1 (16.7)	15 (34.1)
Nasopharyngitis	0 (0.0)	2 (33.3)	3 (50.0)	2 (33.3)	1 (16.7)	2 (22.2)	2 (33.3)	12 (27.3)
Blood creatine phosphokinase increase	1 (20.0)	0 (0.0)	1 (16.7)	2 (33.3)	2 (33.3)	3 (33.3)	2 (33.3)	11 (25.0)
Headache	0 (0.0)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	1 (11.1)	5 (83.3)	11 (25.0)
CRP increase	1 (20.0)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	3 (33.3)	2 (33.3)	10 (22.7)
Neutrophil count decrease	0 (0.0)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	3 (33.3)	1 (16.7)	10 (22.7)
Urine protein presence	2 (40.0)	2 (33.3)	1 (16.7)	1 (16.7)	0 (0.0)	2 (22.2)	1 (16.7)	9 (20.5)
Nasal congestion	1 (20.0)	1 (16.7)	1 (16.7)	0 (0.0)	1 (16.7)	2 (22.2)	1 (16.7)	7 (15.9)
Blood potassium decrease	0 (0.0)	1 (16.7)	0 (0.0)	2 (33.3)	1 (16.7)	1 (11.1)	1 (16.7)	6 (13.6)
Triglycerides increase	0 (0.0)	1 (16.7)	0 (0.0)	2 (33.3)	0 (0.0)	2 (22.2)	1 (16.7)	6 (13.6)
Fatigue	1 (20.0)	0 (0.0)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	6 (13.6)
Hematocrit decrease	1 (20.0)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	1 (11.1)	0 (0.0)	6 (13.6)
Percent monocyte increase	1 (20.0)	4 (66.7)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	0 (0.0)	6 (13.6)

*Adverse events reported by >13% of the total population are presented.

Pharmacokinetic profile

At doses of 0.03 mg/kg to 3 mg/kg, the mean maximum concentration (1 μ g/mL to 82 μ g/mL) and mean area under the curve (5 μ g*d/mL to 775 μ g*d/mL) were approximately dose-proportional (Table IV; Fig 2). The faster systemic clearance and shorter half-life observed with MEDI-563 at the 0.03 mg/kg dose level was likely attributed to the pharmacokinetic levels falling below the assay limit of quantitation in the terminal elimination phase. Two additional cohorts were dosed at 0.003 mg/kg and 0.0003 mg/kg; however, because of the limited sensitivity of the pharmacokinetic assay, MEDI-563 plasma concentrations at these dose levels could not be quantified.

MEDI-563 mean volume of distribution (52 mL/kg to 93 mL/kg) was greater than the plasma volume, suggesting potential

binding of MEDI-563 to IL-5R α –expressing blood cells and/or moderate penetration into extravascular tissues.

DISCUSSION

MEDI-563 produced depletion of circulating PB eosinophil levels within 24 hours at all doses administered. This effect persisted for at least 2 to 3 months in subjects dosed in the 0.03 mg/kg to 3 mg/kg range. Basophil counts were not reported in this study because of the known difficulties of obtaining a reliable measurement using standard techniques.²² Future studies will use flow cytometry to assess changes in basophils counts. In addition to the biological effect observed, no deterioration of lung function was detected after administration of single-dose MEDI-563. This

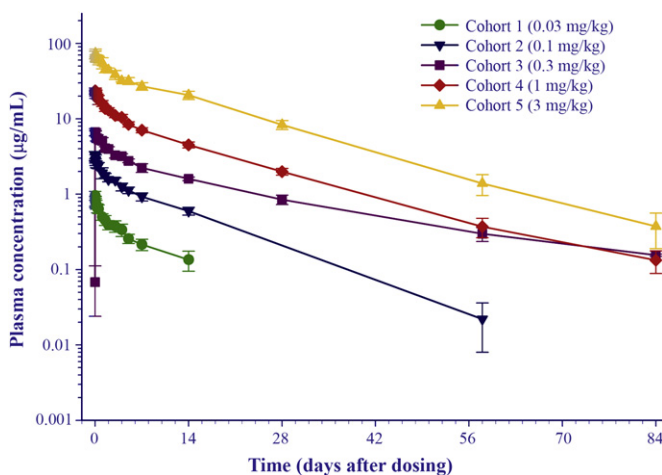
TABLE IV. Noncompartmental pharmacokinetic parameters summary*

Parameter	Dose† (mg/kg)				
	0.03 (n = 6)	0.1 (n = 6)	0.3 (n = 6)	1 (n = 9)	3 (n = 5)
C _{max} (μg/mL)	1 ± 0.3	4 ± 1	8 ± 2	23 ± 9	82 ± 18
AUC _∞ (μg*d/mL)	5 ± 2	26 ± 5	79 ± 17	185 ± 34	775 ± 110
CL (mL/kg/d)	7 ± 3	4 ± 1	4 ± 0.7	4 ± 3	4 ± 0.6
V _{ss} (mL/kg)	65 ± 28	68 ± 28	93 ± 12	52 ± 45	71 ± 18
T _{1/2} (d)	7 ± 2	13 ± 4	19 ± 4	12 ± 4	16 ± 3

AUC_∞, Area under the curve from time 0 extrapolated to infinite time; CL, systemic clearance; C_{max}, maximum concentration; T_{1/2}, elimination half life; V_{ss}, volume of distribution at steady state.

*Parameters are presented as mean ± SD.

†Observations for cohort 6 (0.003 mg/kg) and cohort 7 (0.0003 mg/kg) were below the limit of quantitation.

**FIG 2.** Mean (±SD) MEDI-563 concentration-time profile. Mean MEDI-563 plasma concentration after a single intravenous dose of 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, and 3 mg/kg.

is noteworthy because lung function was designed to detect potential changes signaling the presence of an AE. Similarly, the overall mean daily asthma symptom scores and albuterol use were low during the study. These results were not unexpected given that the population studied had mild asthma.

Mature eosinophils are terminally differentiated blood cells that rapidly undergo cell death in the absence of IL-5, IL-3, GM-CSF, or other eosinophil-active cytokines. Thus, theoretically, MEDI-563 could be eliminated via a target-mediated clearance pathway, in which the antibody binds to IL-5R expressed on the eosinophil membrane and is catabolized along with either IL-5R internalization or eosinophil destruction. However, such target-mediated clearance effect on MEDI-563 pharmacokinetics was not observed in this study, most likely because of the rapid shrinking of the antigen pool (PB eosinophil counts) upon MEDI-563 dosing. The MEDI-563 pharmacokinetics were apparently dose-linear with a mean elimination half-life of approximately 2 to 3 weeks, which is typical for human IgG antibodies. Limitations of the pharmacokinetic assay sensitivity precluded the characterization of the terminal phase at the 0.03 mg/kg dose level and the quantitation of MEDI-563 plasma concentrations after the 0.0003 mg/kg or 0.003 mg/kg doses.

At the dose levels tested (0.0003 mg/kg to 3 mg/kg), MEDI-563 had an acceptable safety profile in subjects with mild atopic asthma. Reduced WBC counts, nasopharyngitis, and increased CPK levels were the most frequently reported AEs, with no dose-dependency or serious AEs observed. Four subjects experienced ≥ 4 acute infusion-related AEs (0.03 mg/kg, n = 2; 3 mg/kg, n = 2) apparently associated with the rapid infusion rate. A similar cluster of acute infusion-related AEs was not observed, however, in any other subjects after modifications were made to the dosing regimen. An intravenous infusion rate of at least 30 minutes will therefore be recommended for all future studies.

The mechanisms responsible for the transient, nonspecific decrease in WBC counts remain unclear. In preclinical studies, mild, transient neutropenia was noted in 2 cynomolgus monkeys that received multiple 30 mg/kg intravenous doses of MEDI-563, whereas no decrease in WBC counts was observed in monkeys treated with the same dose of MEDI-563 via subcutaneous injection (MedImmune, data on file). These data suggest that route of MEDI-563 administration may influence potential effects on WBC counts.

The etiology of the observed elevated CPK levels at baseline and after treatment in a subset of subjects is unknown. Elevated serum CPK values have been reported in patients with asthma with exacerbations.^{23,24} Moreover, treatment with β_2 -agonists or nonasthma drugs, such as statins, has been shown to increase serum CPK levels.²⁵⁻²⁷ Yet none of the subjects developed asthma exacerbations or used statins in this study. The lack of a dose or time correlation with peak CPK values does not seem to support a relationship between the administration of MEDI-563 and the elevated CPK levels observed. However, the data do not exclude the possibility that impressive PB eosinophil depletion by MEDI-563 could lead to mediator release from eosinophils undergoing direct cytolysis, thus affecting neighboring cells in tissues such as skeletal muscle. A recent study supports the capacity of eosinophils to promote bronchial smooth muscle injury via release of ECP in asthma.²⁸

If direct drug effects are excluded, the more likely explanation for the mild to moderate sporadic increases in the CPK levels is recreational exercise.²⁹ The majority of subjects in this trial were young adults with a mean age of 24 years (range, 19-41 years). Unfortunately, no exercise diaries were maintained during the study to confirm this effect. Also, because the study did not include a placebo group, an estimate of the prevalence of CPK elevations could not be determined. Further investigations including serial measurements of serum troponin are underway in ongoing clinical studies of MEDI-563 to address the effects observed on CPK levels.

Active depletion of eosinophils by mechanisms involving ADCC through the targeting of the IL-5R α is a novel approach for the treatment of asthma, which may offer potential advantages over therapies directed at the IL-5 molecule alone. It has been shown that inhibition of IL-5 significantly reduces blood and sputum eosinophil counts in patients with asthma. However, the incomplete removal of tissue eosinophils by anti-IL-5 therapy may be a result of other cytokines, such as IL-3 and GM-CSF, which can also promote eosinophil survival.³⁰⁻³² Second, activated eosinophils *in vitro* and in the airways of patients with asthma may express decreased IL-5R α surface expression.^{33,34} Thus, activated eosinophils may be less responsive and therefore less dependent on IL-5 for survival, diminishing the effects of anti-IL-5 mAbs.

The main biological effect of MEDI-563 is the induction of active eosinophil killing through an enhanced ADCC effector function (*in vitro* half maximal effective concentration, 0.9 pmol/L, eosinophils; 0.6 pmol/L, basophils),¹⁹ although it also inhibits IL-5-mediated receptor activation. Because of the high-binding affinity to the FcγRIIIa region, afucosylated IgG₁ mAbs, such as MEDI-563,³⁵ can overcome the inhibitory effects exhibited by serum IgG, which limit the ADCC activity of their fucosylated counterparts.³⁶ In addition, although reduced expression levels of IL-5Rα have been reported in the airway eosinophils of subjects with asthma,³³ ADCC appears to be relatively insensitive to surface density of target receptors.³⁷ It is theoretically plausible, however, that if surface IL-5Rα expression on airway eosinophils falls below the threshold required for ADCC, this mechanism of eosinophil depletion may become inactive. Immunohistochemistry studies of lung biopsies from subjects with mild asthma indicate that MEDI-563 stains more than 90% of eosinophils in the airways, suggesting that receptors for IL-5 are present and can be engaged in that key location.¹⁹ Finally, the ADCC activity in MEDI-563 may circumvent the V/F158 functional polymorphisms associated with the FcγRIIIa region of fucosylated IgG₁ mAbs.³⁸

These theoretical advantages translate into specific pharmacodynamic benefits. For example, MEDI-563 has a faster kinetic rate of PB eosinophil depletion than mepolizumab, with a peak reduction occurring at 24 hours, compared with a gradual reduction reaching a peak at 4 weeks after dosing of mepolizumab.¹⁵ Secondly, the median PB eosinophil levels at 1 month after dosing are lower with MEDI-563 than mepolizumab. A single 750-mg intravenous dose of mepolizumab resulted in eosinophil levels of 45 cells/μL (85% reduction) at 4 weeks.³⁹ In contrast, a single intravenous dose (≥0.03 mg/kg) of MEDI-563 led to median levels of 10 cells/μL (94.5% reduction) at 24 hours and 0 cells/μL (100% reduction) at 4 weeks after dosing. Lastly, dosing with 3 monthly intravenous doses of mepolizumab (750 mg) induced PB eosinopenia that lasted a mean of 9 weeks (range, 4–20 weeks) in one study¹⁵ and 12 weeks in another.³⁹ In contrast, a single intravenous infusion of MEDI-563 (≥0.3 mg/kg; n = 21) resulted in PB eosinopenia that lasted at least 12 weeks in all but 1 subject (4.8%). Prolonged depletion of eosinophils has the potential to improve asthma morbidity. However, 2 reports^{40,41} have described clinical syndromes in individuals deficient in eosinophils and basophils, underscoring the importance of continued safety vigilance in ongoing and future MEDI-563 studies.

In summary, our study demonstrated that MEDI-563 had an acceptable safety profile in subjects with mild atopic asthma at all doses tested. After a single intravenous dose ≥0.3 mg/kg, PB eosinopenia persisted for at least 12 weeks. Further evaluation is planned to determine whether the changes observed in eosinophil counts will result in clinical improvements of diseases associated with elevated circulating or tissue-infiltrating eosinophils like asthma.

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Clinical implications: MEDI-563 provokes peripheral blood eosinopenia via enhanced antibody-dependent cell-mediated cytotoxic activity and may be a useful therapeutic option for asthma.

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METHODS

Skin testing

Subjects were tested for reactions to a standard battery of seasonal and perennial allergens at the first visit, unless tested within the previous 12 months. Sensitivity was demonstrated by skin prick or intradermal testing. A positive skin test was defined as a wheal ≥ 3 mm larger than the diluent after skin prick testing or 7 mm larger than the diluent following intradermal testing. The Mantoux (purified protein derivative [PPD]) test was conducted at visit 1 in subjects without a previous positive reaction. These subjects were injected (0.5 mL 5 tuberculin units PPD) intradermally in the forearm. Results were reviewed within 48 to 72 hours post-PPD administration. A positive result was defined as a >15 mm induration or by the presence of blisters. All subjects with a positive reaction were excluded from the study.

Pulmonary function, airway inflammation, and hyperresponsiveness

FEV₁ was measured according to the American Thoracic Society guidelines^{E1} before and after administration of albuterol (200 μ g) delivered from a metered-dose inhaler at screening (days -14 to -7), baseline before to dosing, 6 and 12 hours after dosing on day 0, and days 1, 2, 7, 14, 28, 58, and 84. FE_{NO} was measured^{E2} using NIOX MINO (Aerocrine, New Providence, NJ) at a flow rate of 50 mL/s throughout the study before spirometric measurements or 1 hour postevaluation. The methacholine challenge was also performed according to American Thoracic Society guidelines^{E3} in the low-dose (0.0003 mg/kg, 0.003 mg/kg) and high-dose (1 mg/kg, 3 mg/kg) cohorts on days 28, 58, and 84.

Asthma symptom score and β_2 -agonist use

Subjects were asked to record their symptoms and the number of short-acting β_2 -agonist puffs used daily in a diary, and the scores were collected at every visit. Symptoms were rated on a scale of 9 (maximum) to 0 (minimum).

RESULTS

Skin testing

All subjects in the study had a least one positive allergen skin prick test (Table EI).

Pulmonary function, airway inflammation, and asthma control

Pulmonary function and airway inflammation remained constant throughout the study in all 7 cohorts, as demonstrated by the total mean levels of FEV₁ (baseline, 3.47 ± 0.6 L; day 58, 3.48 ± 0.6 L) and FE_{NO} (baseline, 42.25 ± 35.2 parts per billion; day 58, 42.74 ± 33.1 parts per billion). No changes were observed in PC₂₀ during the study in the dose cohorts tested (0.0003 mg/kg, 0.003 mg/kg, 1 mg/kg, 3 mg/kg). The overall mean daily asthma symptom scores after dosing were low in all cohorts (0.41 ± 0.4 [0.0003 mg/kg], 0.50 ± 0.6 [0.003 mg/kg], 0.75 ± 0.6 [0.3 mg/kg], 0.75 ± 1.4 [1 mg/kg], 0.49 ± 0.6 [3 mg/kg]). Likewise, the overall mean daily albuterol use after dosing was low (0.65 ± 0.8 puffs [0.0003 mg/kg], 0.50 ± 0.7 puffs [0.003 mg/kg], 0.45 ± 0.7 puffs [0.3 mg/kg], 0.27 ± 0.5 puffs [1 mg/kg], 0.30 ± 0.4 puffs [3 mg/kg]) during the study.

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TABLE E1. Allergen frequency from skin testing*

Allergen type	Frequency
Cat	n = 22
Ragweed	n = 20
<i>Alternaria</i>	n = 19
<i>Dermatophagoides pteronyssinus</i>	n = 15
<i>Dermatophagoides farinae</i>	n = 13
Timothy grass	n = 11
Bermuda grass	n = 10
Maple/box elder tree	n = 10

*Allergens detected in ≥ 10 subjects are represented here.