

Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia

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Background: Many asthmatic patients exhibit sputum eosinophilia associated with exacerbations. Benralizumab targets eosinophils by binding IL-5 receptor α , inducing apoptosis through antibody-dependent cell-mediated cytotoxicity.

Objectives: We sought to evaluate the safety of benralizumab in adults with eosinophilic asthma and its effects on eosinophil counts in airway mucosal/submucosal biopsy specimens, sputum, bone marrow, and peripheral blood.

Methods: In this multicenter, double-blind, placebo-controlled phase I study, 13 subjects were randomized to single-dose intravenous placebo or 1 mg/kg benralizumab (day 0; cohort 1), and 14 subjects were randomized to 3 monthly subcutaneous doses of placebo or 100 or 200 mg of benralizumab (days 0, 28, and 56; cohort 2). Cohorts 1 and 2 were consecutive.

Results: The incidence of adverse events was similar between groups. No serious adverse events related to benralizumab occurred. In cohort 1 intravenous benralizumab produced a median decrease from baseline of 61.9% in airway mucosal eosinophil counts (day 28; placebo: +19.6%; $P = .28$), as well as an 18.7% decrease (day 21) in sputum and a 100% decrease

(day 28) in blood counts. Eosinophils were not detectable in bone marrow of benralizumab-treated subjects (day 28, $n = 4$). In cohort 2 subcutaneous benralizumab demonstrated a combined (100 + 200 mg) median reduction of 95.8% in airway eosinophil counts (day 84; placebo, 46.7%; $P = .06$), as well as an 89.9% decrease (day 28) in sputum and a 100% decrease (day 84) in blood counts.

Conclusion: Single-dose intravenous and multiple-dose subcutaneous benralizumab reduced eosinophil counts in airway mucosa/submucosa and sputum and suppressed eosinophil counts in bone marrow and peripheral blood. The safety profile supports further development. Additional studies are needed to assess the clinical benefit in asthmatic patients. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Eosinophils, IL-5, IL-5 receptors, asthma, antibody-dependent cell-mediated cytotoxicity

Eosinophils are thought to play an important role in the pathogenesis and severity of asthma. The clinical relevance of eosinophils in asthmatic patients has been confirmed in longitudinal studies demonstrating a reduction in acute exacerbations in subjects who maintained sputum eosinophil counts of less than 2%,¹ less than 3%,² or less than 8%³ with adjustments in inhaled corticosteroids (ICSs) versus those whose ICSs were modified per standard clinical asthma guidelines. However, a subset of patients with refractory asthma has persistent airway eosinophilia despite chronic high-dose ICS treatment.⁴

IL-5 is a cytokine secreted predominantly by T lymphocytes, mast cells, and eosinophils and is involved in regulating the differentiation, proliferation, and activation of eosinophils through the IL-5 receptor (IL-5R).⁵ Several studies with anti-IL-5 mAbs in patients with eosinophilic asthma have shown clinical promise. In a pilot study 12 monthly infusions of 750 mg of mepolizumab reduced severe exacerbations by 41% ($P = .02$ vs placebo).⁶ These results were reproduced in a larger phase IIb study, with exacerbations reduced by 39% to 52% ($P < .001$ vs placebo).⁷ Mepolizumab also demonstrated a steroid-sparing effect in a 6-month study, allowing subjects with prednisone-dependent eosinophilic asthma to reduce oral prednisone dose by 84% compared with 48% on placebo ($P = .04$).⁸ Although underpowered for this end point, a reduction in asthma exacerbations ($P = .08$) was shown with reslizumab, another anti-IL-5 mAb.⁹ These studies provide compelling evidence that targeting the IL-5 pathway in patients with eosinophilic asthma has therapeutic potential.

Benralizumab is a humanized afucosylated mAb designed to target IL-5R α expressed on eosinophils and basophils.^{10,11} Lack of a fucose sugar moiety on the oligosaccharide core enhances the

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Disclosure of potential conflict of interest: D. L. Gossage is a former employee of MedImmune and is currently employed by Gilead Science. G. Gauvreau, R. Leigh, and R. Katial have received research support from MedImmune. W. W. Busse is a member of the advisory board for Merck; has consultant arrangements with Amgen, Novartis, GlaxoSmithKline, MedImmune, and Genentech; and receives research support from the National Institutes of Health/National Institute of Allergy and Infectious Diseases and the National Heart, Lung, and Blood Institute. S. Wenzel has consultant arrangements with TEVA and has received research support from GlaxoSmithKline and MedImmune. Y. Wu is employed by MedImmune and has stock in AstraZeneca. V. Datta is employed by MedImmune and has stock in AstraZeneca. N. A. Molfino is a former employee of MedImmune and is currently employed by KaloBios Pharmaceuticals. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication December 21, 2012; revised May 21, 2013; accepted for publication May 23, 2013.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2013.05.020>

Abbreviations used

ADCC:	Antibody-dependent cell-mediated cytotoxicity
AE:	Adverse event
ATP:	According to protocol
CPK:	Creatine phosphokinase
CRP:	C-reactive protein
H&E:	Hematoxylin and eosin
ICS:	Inhaled corticosteroid
IL-5R:	IL-5 receptor
IQR:	Interquartile range
SABA:	Short-acting β_2 -agonist

binding affinity of benralizumab to Fc γ RIII α and augments antibody-dependent cell-mediated cytotoxicity (ADCC), inducing apoptosis of target cells.¹² In an open-label study in patients with mild atopic asthma, a single intravenous dose of benralizumab had an acceptable safety profile and resulted in marked reductions in peripheral blood eosinophil counts within 24 hours of dosing.¹³

This phase I study evaluated single (intravenous) or multiple subcutaneous doses of benralizumab in adults with eosinophilic asthma. The primary objectives were to evaluate the safety profile of benralizumab and the effect of benralizumab on eosinophil counts in airway mucosal/submucosal biopsy specimens 28 days after dosing. Exploratory objectives included evaluation of eosinophil counts in sputum and bone marrow and eosinophil and basophil counts in peripheral blood.

METHODS**Study design**

This was a multicenter, randomized, double-blind, placebo-controlled study (ClinicalTrials.gov identifier: NCT00659659) conducted from April 2008 through April 2011 (Fig 1). Subjects were recruited from 3 US and 4 Canadian medical centers. All subjects signed an informed consent form before any study-related activities. The protocol was approved by local ethics committees for each site along with the US Food and Drug Administration and Health Canada.

Eligible subjects aged 18 to 65 years had a documented diagnosis of asthma supported by at least 1 of the following criteria: (1) 12% or greater increase in FEV₁ after inhalation of 400 μ g of albuterol during screening, (2) history of 12% or greater FEV₁ reversibility within 1 year of randomization, or (3) history of 20% reduction in FEV₁ in response to a provocative methacholine challenge (PC₂₀) of less than 8 mg/mL within 1 year of randomization. In addition, subjects had sputum eosinophil counts of 2.5% or greater, postbronchodilator FEV₁ of 65% or greater, prebronchodilator FEV₁/forced vital capacity ratio of less than age-adjusted norms,¹⁴ and an asthma therapeutic regimen that was unchanged for 4 weeks before randomization and maintained from screening to the first follow-up airway mucosal/submucosal biopsy. Key exclusion criteria were lung disease other than asthma, smoking within 2 years of baseline or a history of 10 or more pack years, a clinically significant medical condition or acute infection, current use of immunosuppressive drugs (other than oral corticosteroids), positive serology to HIV, hepatitis, history of tuberculosis, or positive tuberculosis test result without a complete course of treatment. Subjects were asked to maintain their regular asthma medication during the study.

Subjects were randomized to receive an intravenous infusion of 1 mg/kg benralizumab or placebo (2:1) on day 0 (cohort 1) or 100 or 200 mg of benralizumab or placebo (1:1:1) delivered in 4 subcutaneous injections on days 0, 28, and 56 (cohort 2). Group assignment was determined by using block randomization through an interactive voice-response system. Cohort 2 was recruited subsequent to cohort 1. Subjects were followed up for at least 84 days after the last dose of study medication.

Safety assessments

Safety was assessed by monitoring treatment-emergent adverse events (AEs), serious AEs, physical examinations, vital signs, and laboratory test results. Any significant changes identified during physical examinations were considered AEs.

Clinical procedures

A baseline bronchoscopy with airway mucosal/submucosal biopsies was performed according to standard procedures at least 7 days after screening sputum induction and within 2 days before day 0. Subjects were pretreated with an inhaled β_2 -agonist. Airway mucosal/submucosal biopsies were repeated on days 28 (cohort 1) and 84 (cohort 2; optional for cohort 1, not reported). The number of eosinophils per square millimeter in airway biopsy specimens was determined by using an immunofluorescence assay with anti-major basic protein 1 antibody (US Biological, San Antonio, Tex). Hematoxylin and eosin (H&E)-stained sections were also prepared to assess the quality of the biopsy specimen.

Sputum samples were induced, collected, and analyzed for eosinophils at screening (within 14 days before day 0) and at days 21, 56, and 77 (cohort 1) and days 28, 77, and 140 (cohort 2).

Subjects who consented had bone marrow aspiration at screening and day 28. Bone marrow aspirates stained with Wright-Giemsa stain were analyzed.

Blood samples were taken at screening; days 0, 7, 28, 56, and 84 (both cohorts); and days 21, 119, and 140 (cohort 2 only) for evaluation of eosinophil counts by using an automated hematology analyzer. Basophils were evaluated from whole blood by means of flow cytometry with antibodies to the IgE high-affinity receptor (eBioscience, San Diego, Calif), CD123, CCR3 (CD193), and chemoattractant receptor of type 2 helper (CRTH2; CD294; BD Biosciences, San Jose, Calif) at days 0, 1, 7, 28, 56, 84, and 140 (cohort 2 only).

Further details of the procedures used to obtain and prepare samples of airway mucosa, peripheral blood, bone marrow, and sputum are provided in the *Methods* section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Twelve randomized subjects per cohort were considered to be sufficiently powered to assess the effect of benralizumab on eosinophil counts in airway mucosal/submucosal biopsy specimens. All subjects randomized into the study were included in the intent-to-treat population. The according-to-protocol (ATP) population was defined as subjects who received all planned doses of study drug, had airway mucosal/submucosal biopsy results at screening and day 28 (cohort 1) or day 84 (cohort 2), and did not receive a burst of corticosteroids within 28 days before the second airway biopsy. For cohort 2, subjects were excluded from the ATP population if they had an upper or lower respiratory tract infection requiring treatment within 28 days before the second airway biopsy. The safety population included all subjects who received at least 1 dose of study drug. Cell count evaluations were based on the ATP population, and safety evaluations were based on the safety population.

Baseline was defined as the day 0 value before administration of study medication. If the day 0 value was missing, invalid, or measured after study drug administration, the baseline value was determined as the latest assessment before day 0.

The primary end points were safety and the effect of benralizumab on eosinophil counts in airway mucosal/submucosal biopsy specimens 28 days after completion of dosing. A 2-sided Wilcoxon rank sum test ($\alpha = .05$) was used to analyze differences in median percentage change in airway mucosal/submucosal absolute eosinophil counts between placebo and benralizumab (cohort 1) and placebo and the combined (100 + 200 mg) benralizumab groups (cohort 2).

A *post hoc* analysis was performed using the Sign test to compare the within-group median percentage change from screening with that 28 days after the last dose of study medication in airway mucosal absolute eosinophil counts. A second *post hoc* analysis was conducted using a Wilcoxon rank sum test to compare median percentage change from screening with that 28 days after the last dose of study medication in airway mucosal/submucosal, sputum, and peripheral blood absolute eosinophil counts between placebo (cohorts

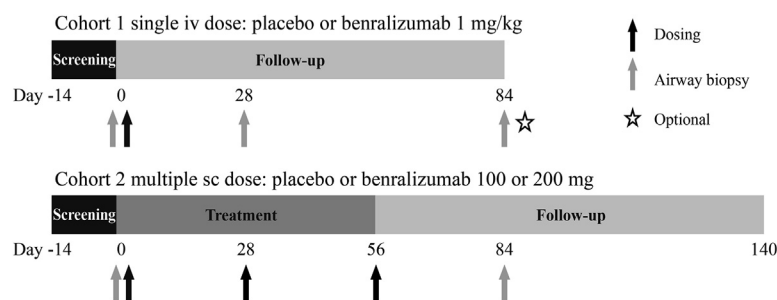


FIG 1. Study design. Cohorts 1 and 2 were consecutive. *iv*, Intravenous; *sc*, subcutaneous.

TABLE I. Subjects' demographics and baseline characteristics (ITT population)

Characteristic*	Cohort 1 (single-dose IV), † n = 13		Cohort 2 (multiple-dose SC), † n = 14			
	Placebo (n = 5)	Benralizumab, 1 mg/kg (n = 8)	Placebo (n = 5)	Benralizumab, 100 mg (n = 4)	Benralizumab, 200 mg (n = 5)	Benralizumab, total (n = 9)
Age (y), mean (SD)	34.4 (9.7)	38.9 (14.7)	40.2 (14.5)	41.0 (14.9)	37.2 (14.4)	38.9 (13.8)
Sex (no.), male/female	3/2	2/6	1/4	3/1	2/3	5/4
Race, no. (%)						
White	4 (80.0)	8 (100)	5 (100)	4 (100)	4 (80.0)	8 (88.9)
Other	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (11.1)
Weight (kg), mean (SD)	72.2 (10.8)	72.9 (17.5)	74.6 (19.9)	92.4 (14.5)	80.4 (12.6)	85.7 (14.1)
≥1 Asthma controller medication, no.	4	6	5	2	4	6
ICS dose (budesonide equivalent [μg/d]), median (range)	400 (0-2000)	550 (0-1300)	800 (400-3520)	400 (0-2400)	800 (0-1600)	800 (0-2400)
FEV						
FEV ₁ (L), mean (SD)	2.5 (0.8)	2.3 (0.6)	1.9 (0.4)	3.0 (1.2)	2.2 (0.6)	2.6 (1.0)
Predicted FEV ₁ (%), ‡ mean (SD)	67.2 (9.1)	70.5 (15.6)	60.5 (6.6)	72.5 (13.1)	65.7 (10.2)	68.7 (11.4)
FEV ₁ /FVC ratio (%), ‡ mean (SD)	66.7 (10.0)	70.7 (9.8)	67.0 (12.7)	69.6 (5.9)	67.2 (4.2)	68.3 (4.8)
Sputum eosinophil counts (%), mean (SD)	13.9 (10.3)	6.6 (2.2)	34.1 (32.7)	10.5 (7.7)	4.9 (2.4)	7.4 (5.8)

FVC, Forced vital capacity; ITT, intent to treat; IV, intravenous; SC, subcutaneous.

*Subjects' baseline values were recorded at the visit occurring just before receiving the first dose of study medication; this might have been day 0 or screening.

†Note that cohorts were consecutive (nonparallel) in this study.

‡Screening postbronchodilator FEV₁ percent predicted and screening prebronchodilator FEV₁/forced vital capacity ratio (as a percentage) values were used as part of the eligibility criteria.

1 and 2 combined) and benralizumab (cohorts 1 and 2 combined). All other parameters were summarized descriptively.

(cohort 2, receiving placebo) was taking oral corticosteroids at baseline.

RESULTS

Subjects

Twenty-seven adults with asthma were randomized to receive placebo or benralizumab (administered intravenously or subcutaneously), and all subjects were included in all analyses. Subject disposition is shown in Fig E1 in this article's Online Repository at www.jacionline.org. Twenty-six subjects completed the study. One subject randomized to 1 mg/kg intravenous benralizumab withdrew consent after receiving study drug (no reason was given) but was followed up to day 56; this subject was randomized, received study drug, and had airway biopsies at screening and day 28 and was thus included in all study populations. Subjects were 20 to 62 years old, and most were non-Hispanic (88.9%) and white (92.6%), with a slightly higher percentage of female subjects (59.3%, Table I). Actual and predicted baseline FEV₁ values were similar across groups and in both cohorts. ICSs were taken at baseline by 6 of 8 benralizumab-treated and 4 of 5 placebo-treated subjects in cohort 1 and 6 of 9 benralizumab-treated and all 5 placebo-treated subjects in cohort 2. In addition, 1 subject

Safety

The incidence of all AEs was similar between the placebo and benralizumab groups after both intravenous and subcutaneous administration (Table II). The most common AEs in the benralizumab groups were nasopharyngitis (25.0%) and headache (25.0%) in cohort 1 and nasopharyngitis (22.2%) and nausea (22.2%) in cohort 2. All AEs reported in the placebo and benralizumab groups in cohort 1 and all those in the placebo group and approximately two thirds of those in the benralizumab groups in cohort 2 were mild to moderate in severity.

The incidence of AEs was generally higher in the benralizumab groups than in the placebo group and in cohort 1 than in cohort 2. In cohort 1 there were no AEs in the placebo group, and 17 AEs were reported by 3 of 8 subjects in the intravenous benralizumab group. One subject who received 1 mg/kg intravenous benralizumab reported 15 of these AEs (chills, headache, asthenia [loss of energy and weakness], nausea, dysgeusia, tremor, dizziness, hot flush, hyperhidrosis, and swelling on day 0, with a decreased white blood cell count [$2.3 \times 10^3/\mu\text{L}$], decreased neutrophil count [$1.1 \times 10^3/\mu\text{L}$], and increased C-reactive protein (CRP)

TABLE II. Overall summary of AEs (safety population)

AEs	Cohort 1 (single-dose IV),* n = 13		Cohort 2 (multiple-dose SC),* n = 14			
	Placebo (n = 5)	Benralizumab, 1 mg/kg (n = 8)	Placebo (n = 5)	Benralizumab, 100 mg (n = 4)	Benralizumab, 200 mg (n = 5)	Benralizumab, total (n = 9)
Total no. of AEs	17	27	20	15	10	25
Subjects reporting ≥ 1 AE, no. (%)	5 (100)	5 (62.5)	5 (100)	4 (100)	2 (40.0)	6 (66.7)
AEs reported by ≥ 2 subjects, no. (%)	5 (100)	5 (62.5)	5 (100)	4 (100)	2 (40.0)	6 (66.7)
Nasopharyngitis	0 (0.0)	2 (25.0)	4 (80.0)	2 (50.0)	0 (0.0)	2 (22.2)
Nausea	1 (20.0)	1 (12.5)	0 (0.0)	1 (25.0)	1 (20.0)	2 (22.2)
Asthma	0 (0.0)	1 (12.5)	1 (20.0)	0 (0.0)	1 (20.0)	1 (11.1)
Headache	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cough	1 (20.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (11.1)
Dyspnea	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (11.1)
Fatigue	1 (20.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (11.1)
Oropharyngeal pain	0 (0.0)	1 (12.5)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rash	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	1 (11.1)
Tremor	1 (20.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia	0 (0.0)	0 (0.0)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)

IV, Intravenous; SC, subcutaneous.

*Cohorts 1 and 2 were consecutive.

level [1.61 mg/dL] measured on day 1 after dosing). All these AEs were considered to be moderate in severity apart from the decreased neutrophil count, which was mild, and most resolved the following day. In cohort 2, 6 AEs occurred in 1 of 5 subjects in the placebo group, and 1 AE occurred in 1 of 9 subjects in the combined subcutaneous benralizumab group. One subject with a prior history of hyperthyroidism who received 200 mg of benralizumab administered subcutaneously presented with symptoms consistent with a thyroid storm, was hospitalized for 8 days, and subsequently completed the study. The incident occurred 50 days after the first dose and 23 days after the last dose. The serious AE was considered to be severe but not treatment related. No discontinuations because of AEs or deaths occurred.

There were no apparent trends or markedly abnormal findings from serum chemistry (see Table E1 in this article's Online Repository at www.jacionline.org), hematology, or laboratory tests in either cohort, except for the expected low blood eosinophil counts in subjects receiving benralizumab. Details of safety variables are provided in the Results section in this article's Online Repository at www.jacionline.org.

Airway mucosal/submucosal eosinophils

Airway mucosal/submucosal eosinophil counts decreased from screening to end point for most subjects who received benralizumab (cohort 1, 6/8 [75.0%]; cohort 2, 8/9 [88.9%]; Fig 2, A and B).^{4,15} Eosinophil counts in biopsy specimens determined by using immunofluorescence at screening and day 84 are shown in Fig 2, C. The photomicrographs demonstrate increased mucosal and submucosal eosinophil counts in the placebo-treated subject. In contrast, no eosinophils are observed at day 84 in the benralizumab-treated subject. Corresponding H&E-stained photomicrographs from the same biopsy sections can be found in Fig E2 in this article's Online Repository at www.jacionline.org.

In cohort 1 median absolute eosinophil counts increased in the placebo group from 89.3/mm² (range, 30.4-185.6/mm²) at screening to 107.6/mm² (range, 6.7-166.7/mm²) at day 28 (median change, +19.6%) compared with a decrease in the intravenous benralizumab group from 43.3/mm² (range, 11.0-165.3/mm²) at screening to 15.6/mm² (range, 4.7-61.6/mm²) at day 28 (median

change, -61.9%). The difference in median percentage change from screening to day 28 between the placebo and benralizumab groups was not statistically significant (Table III).

In cohort 2 median absolute eosinophil counts decreased in the placebo group from 42.0/mm² (range, 8.0-126.3/mm²) at screening to 22.4/mm² (range, 0.7-91.3/mm²) at day 84 (median change, -46.7%), and in the combined 100 plus 200 mg of subcutaneous benralizumab groups, they decreased from 63.6/mm² (range, 4.7-127.3/mm²) at screening to 2.7/mm² (range, 0-32.0/mm²) at day 84 (median change, -95.8%). The difference in median percentage change from screening to day 84 between the placebo and benralizumab groups was not statistically significant (Table III).

Data from the *post hoc* analysis combining data from cohorts 1 and 2 are shown in Table III. The difference between the placebo and benralizumab groups in median percentage change in airway mucosal/submucosal eosinophil counts from screening to 28 days after the last dose was statistically significant ($P = .02$). For the combined placebo cohorts (n = 10), there was a 4.7% increase in airway mucosal/submucosal eosinophil counts 28 days after the last dose (interquartile range [IQR], -64.1% to +84.3%) compared with -83.1% (IQR, -95.8% to -57.6%) for the combined benralizumab cohorts (n = 17).

Sputum eosinophils

Induced sputum eosinophil counts were reduced in response to benralizumab in both cohorts (Fig 3). In cohort 1 median sputum eosinophil counts in the placebo group were 13.0% (range, 4.8% to 31.0%) at screening and 20.8% (range, 2.5% to 33.3%) at day 21 (median change, +146.2%) but decreased in the intravenous benralizumab group from 5.7% (range, 4.3% to 11.0%) at screening to 4.5% (range, 0% to 16.5%) at day 21 (median change, -18.7%). At day 77, median counts were 11.3% (range, 5.0% to 27.3%; median change, -16.7%) for the placebo group and 4.4% (range, 1.5% to 18.0%; median change, -17.7%) for the intravenous benralizumab group. In cohort 2 median sputum eosinophil counts were reduced in the placebo group from 16.8% (range, 2.9% to 73.9%) at screening to 6.4% (range, 1.9% to 20.0%) at day 28 (median change, -66.6%) and in the combined 100 plus 200 mg of subcutaneous benralizumab groups

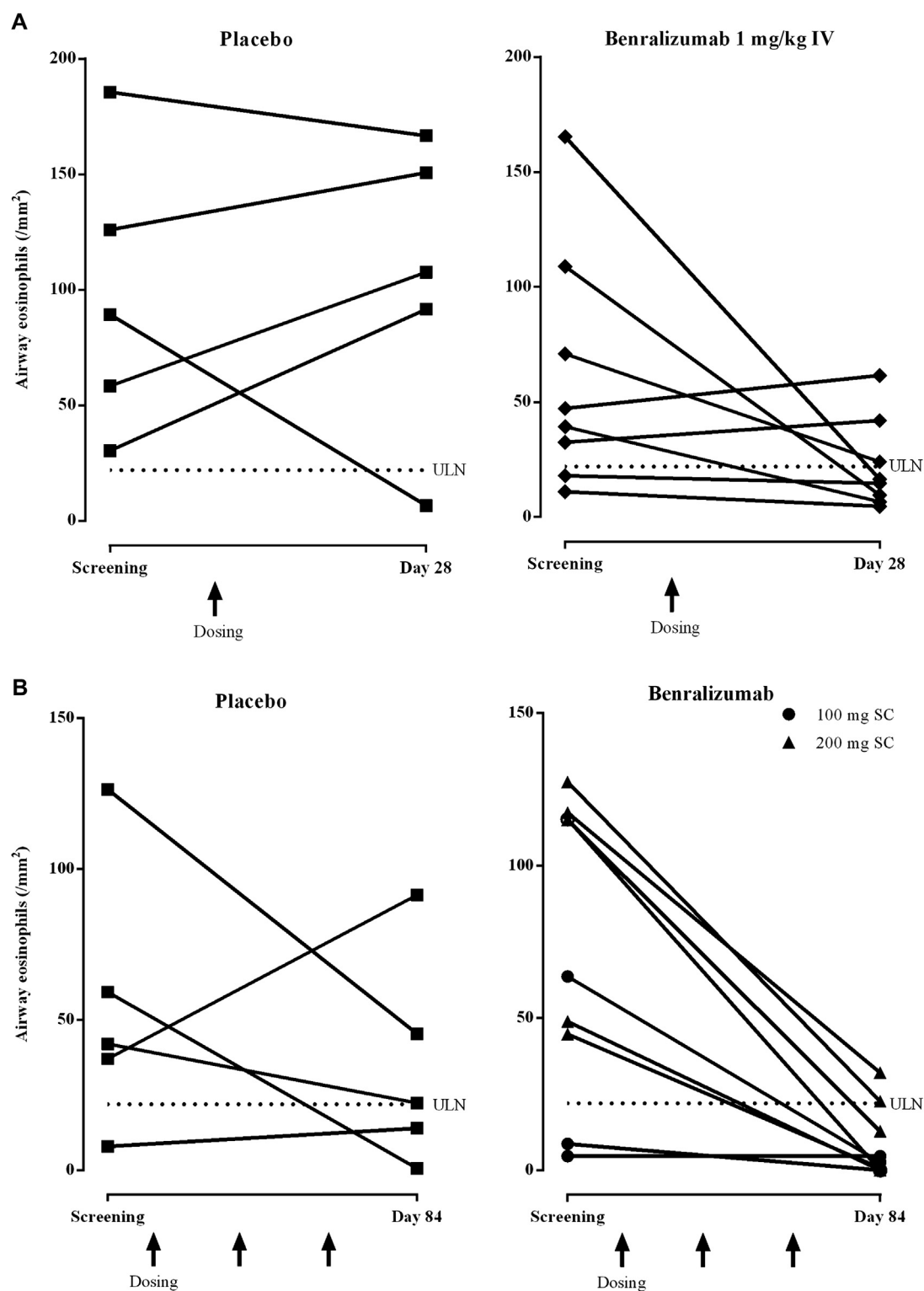


FIG 2. Airway eosinophils (per square millimeter) for individual subjects (ATP population). **A**, Cohort 1 (intravenous [IV]): placebo, $n = 5$; 1 mg/kg benralizumab, $n = 8$. **B**, Cohort 2 (subcutaneous [SC]): placebo, $n = 5$; 100 mg of benralizumab, $n = 4$; 200 mg of benralizumab, $n = 5$. *Dashed lines* represent the upper limit of normal (ULN) for airway eosinophil counts ($22/\text{mm}^2$).^{4,15} **C**, Cohort 2 (subcutaneous): representative immunofluorescence photomicrographs showing mucosal and submucosal tissue sections obtained from 1 placebo-treated subject and 1 benralizumab-treated subject at screening and day 84. *Green* indicates anti-major basic protein 1-stained eosinophils, and *blue* indicates 4',6-diamidino-2-phenylindole-stained nuclei. The insets show sections at $\times 400$ magnification.

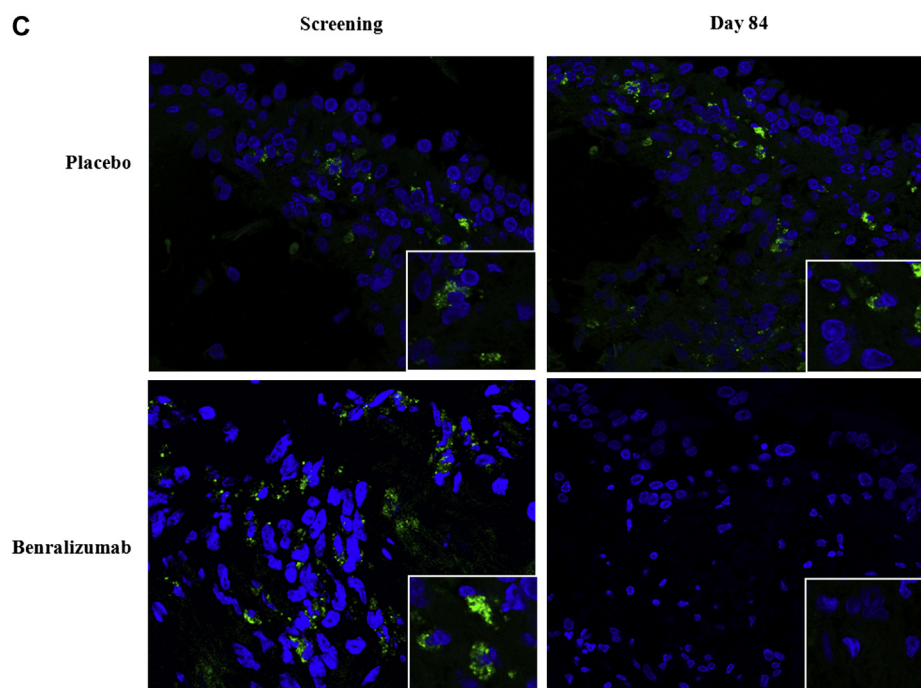


FIG 2. (Continued)

TABLE III. Airway mucosal eosinophil counts (percentage change from baseline [ATP population])

Cohort	Group, end point	No.	Median % change from baseline	P value, benralizumab vs placebo	P value, baseline vs end point
1 (IV)	Placebo, day 28	5	19.6		1
	Benralizumab, 1 mg/kg, day 28	8	−61.9	.28*	.29†
2 (SC)	Placebo, day 84	5	−46.7		1
	Benralizumab, 100 + 200 mg, day 84	9	−95.8	.06*	.01†
1+2	Placebo, 28 days after last dose	10	4.7		1
	Benralizumab, 28 days after last dose	17	−83.1	.02‡	.0023†

IV, Intravenous; SC, subcutaneous.

*Prespecified Wilcoxon rank sum test of percentage change from baseline.

†Post hoc Sign test of absolute eosinophil counts.

‡Post hoc Wilcoxon rank sum test of percentage change from baseline.

from 4.6% (2.5% to 20.8%) at screening to 0.6% (0% to 3.5%) at day 28 (median change, −89.9%).

For the combined cohorts (*post hoc* analysis), the median percentage change from baseline to 21 days after the last dose was −46.4% (IQR, −89.5% to +171.7%) for the placebo group (n = 8) and −95.1% (IQR, −100% to −6.6%) for the benralizumab group (n = 16, *P* = .04 vs the placebo group).

Bone marrow eosinophils and neutrophils

Five subjects in cohort 1 (1 receiving placebo and 4 receiving 1 mg/kg intravenous benralizumab) and 1 subject in cohort 2 (receiving 100 mg of subcutaneous benralizumab) consented to

bone marrow aspiration and provided evaluable samples at screening and day 28 (cohort 1) or day 84 (cohort 2). Eosinophil precursors and mature eosinophils were not detectable at day 28 in the bone marrow of all subjects who received intravenous benralizumab or at day 84 in the subject who received 100 mg of subcutaneous benralizumab (Fig 4, A). Intravenous benralizumab produced no clear effect on neutrophil counts (Fig 4, B).

Peripheral blood eosinophils and basophils

Peripheral blood eosinophil counts were less than the level of detection by 1 day after administration of benralizumab and remained depleted through the end of the study in both cohorts (Fig 5, A and B). Within cohort 1, median peripheral blood eosinophil counts did not change in the placebo group from $0.40 \times 10^3/\mu\text{L}$ (range, $0.2\text{--}0.7 \times 10^3/\mu\text{L}$) at baseline (day 0) to $0.40 \times 10^3/\mu\text{L}$ (range, $0.3\text{--}0.5 \times 10^3/\mu\text{L}$) at day 28 and decreased in the intravenous benralizumab group from $0.15 \times 10^3/\mu\text{L}$ (range, $0.1\text{--}0.6 \times 10^3/\mu\text{L}$) at baseline to $0 \times 10^3/\mu\text{L}$ (range, $0\text{--}0 \times 10^3/\mu\text{L}$) at day 28. This effect continued to day 84. In cohort 2 median peripheral blood eosinophil counts for placebo were $0.30 \times 10^3/\mu\text{L}$ (range, $0.1\text{--}0.6 \times 10^3/\mu\text{L}$) at baseline and $0.20 \times 10^3/\mu\text{L}$ (range, $0.1\text{--}0.8 \times 10^3/\mu\text{L}$) at day 84. For the combined subcutaneous benralizumab groups, there was a decrease from $0.40 \times 10^3/\mu\text{L}$ (range, $0.2\text{--}0.9 \times 10^3/\mu\text{L}$) at baseline to $0 \times 10^3/\mu\text{L}$ (range, $0\text{--}0 \times 10^3/\mu\text{L}$) at day 84.

For the combined cohorts (*post hoc* analysis), the difference in median percentage change from baseline to 28 days after the last dose was significantly lower for the benralizumab group versus the placebo group (*P* < .0001): 0% (IQR, −50.0% to +20.3%) for the placebo group (n = 7) and −100% (IQR, −100% to −100%) for the benralizumab group (n = 14).

Median estimated basophil counts in the placebo group were similar at baseline ($0.075 \times 10^3/\mu\text{L}$ [range, $0.054\text{--}0.089$]

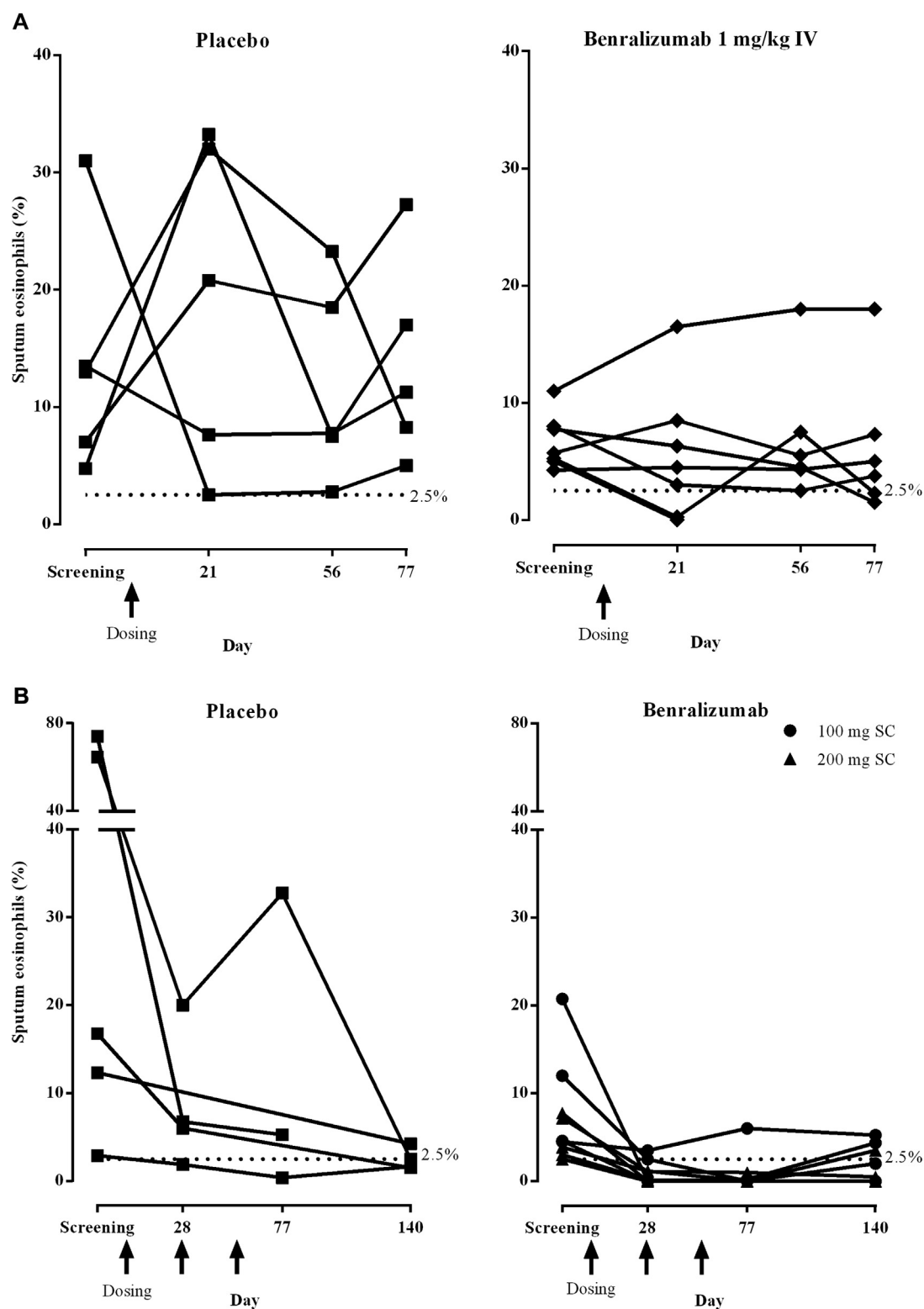


FIG 3. Sputum eosinophil counts (as a percentage) for individual subjects (ATP population). **A**, Cohort 1 (intravenous [IV]): placebo, $n = 5$; 1 mg/kg benralizumab, $n = 7$. One subject in the benralizumab group completed the study but did not have sputum induction after screening and is not included in this figure. An additional subject discontinued the study but provided sputum at screening and day 21; these data are included in this figure. **B**, Cohort 2 (subcutaneous [SC]): placebo, $n = 5$; 100 mg of benralizumab, $n = 4$; 200 mg of benralizumab, $n = 5$. *Dashed lines* represent the inclusion criteria cutoff point for sputum eosinophil counts (2.5%).

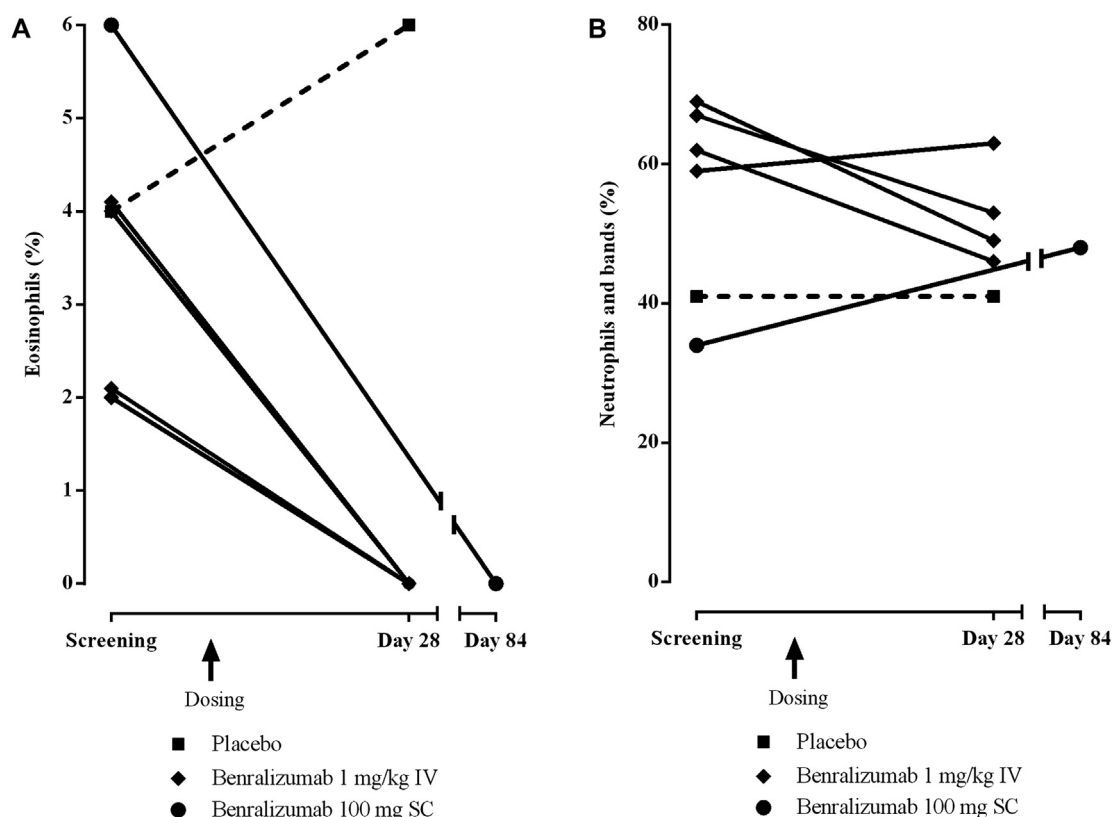


FIG 4. Bone marrow eosinophil and neutrophil counts (as a percentage) for individual subjects (ATP population). Only subjects who consented to bone marrow aspiration and provided evaluable samples at screening and end point are included. **A**, Eosinophils. Cohort 1 (intravenous [IV]): placebo, $n = 1$, square with dashed line; 1 mg/kg benralizumab, $n = 4$, diamond with solid line. Cohort 2 (subcutaneous [SC]): 100 mg of benralizumab, $n = 1$, circle with solid line. **B**, Neutrophils and bands. Cohort 1 (intravenous [IV]): placebo, $n = 1$; 1 mg/kg benralizumab, $n = 4$. Cohort 2 (subcutaneous [SC]): 100 mg of benralizumab, $n = 1$.

$\times 10^3/\mu\text{L}$) and day 84 ($0.073 \times 10^3/\mu\text{L}$ [range, 0.029 – $0.081 \times 10^3/\mu\text{L}$]; median change, $+5.9\%$) but decreased from $0.026 \times 10^3/\mu\text{L}$ (range, 0.011 – $0.081 \times 10^3/\mu\text{L}$) at baseline to $0.006 \times 10^3/\mu\text{L}$ (range, 0.001 – $0.030 \times 10^3/\mu\text{L}$) at day 84 in the combined subcutaneous benralizumab group (median change, -74.2% ; Fig 5, C).

DISCUSSION

This study evaluated the safety profile of benralizumab and its effects on eosinophils in the airways, sputum, bone marrow, and peripheral blood of patients with eosinophilic asthma. Eosinophil counts were reduced in the airway mucosa after both intravenous and subcutaneous benralizumab compared with those seen after placebo. Differences between the benralizumab and placebo groups in percentage change in airway eosinophil counts were not statistically significant within cohorts but reached statistical significance when the 2 cohorts were combined in a *post hoc* analysis. Moreover, benralizumab administration resulted in reduction of sputum eosinophil counts and complete suppression of bone marrow and peripheral blood eosinophil counts. Benralizumab had an acceptable safety profile, although larger studies will be needed to fully assess potential safety issues.

The effect of anti-IL-5 mAb therapy on airway mucosal eosinophil counts was first described in steroid-naïve subjects

with mild atopic asthma.¹⁶ In that study, 3 monthly infusions of 750 mg of intravenous mepolizumab resulted in a median decrease in airway mucosal eosinophil counts of 55% ($P < .01$ vs placebo). In a subsequent study, when subjects with refractory eosinophilic asthma received 12 monthly intravenous infusions of 750 mg of mepolizumab, the subepithelial airway eosinophil geometric mean between-group difference was 48% versus placebo ($P = .68$), suggesting that dosing of mepolizumab beyond 3 months might not provide additional airway eosinophil depletion.⁶ Our study extends previous work by demonstrating that benralizumab, which binds with high affinity to IL-5R α and depletes eosinophils and basophils by inducing apoptosis through enhanced ADCC, can produce a 96% median reduction in mucosal airway eosinophil counts after three 100- or 200-mg subcutaneous doses.

After administration of benralizumab, median blood and bone marrow eosinophil counts were reduced by 100% (undetectable) in both cohorts. Median blood eosinophil counts were undetectable from 7 days after the first dose through the end of the study. Previous studies with mepolizumab¹⁶ and reslizumab⁹ also reported median blood eosinophil count reductions of 100% and 80%, respectively. Of the 5 subjects who received benralizumab and consented to bone marrow aspirates, all had undetectable levels of eosinophils and eosinophil precursors at study end point, whereas 3 intravenous mepolizumab doses

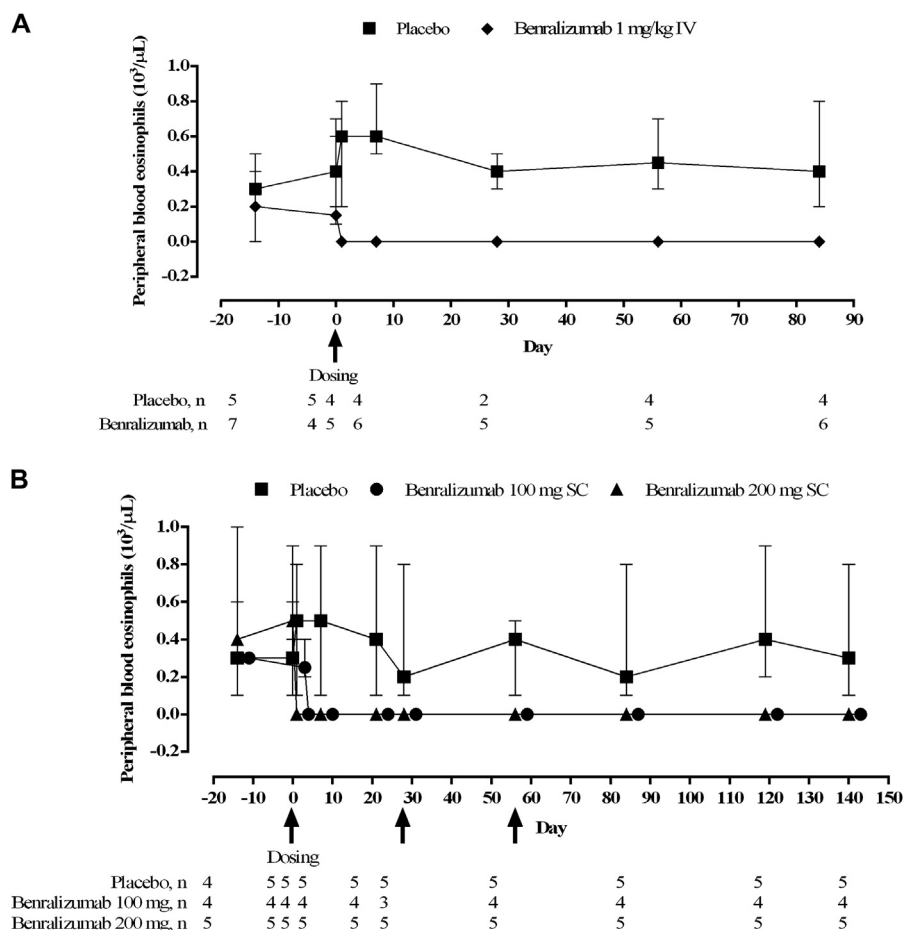


FIG 5. Median (range) peripheral blood eosinophil and basophil counts ($10^3/\mu\text{L}$; ATP population). Subject numbers vary between time points depending on availability of data. At some time points, all subjects in the benralizumab groups had the same value. **A**, Eosinophils: cohort 1 (intravenous [IV]). **B**, Eosinophils: cohort 2 (subcutaneous [SC]). **C**, Basophils: cohort 2 (subcutaneous [SC]).

resulted in a 52% median reduction in bone marrow eosinophil counts.¹⁶

In this study subjects were required to have 2.5% or greater eosinophil counts in sputum for inclusion. After benralizumab administration, sputum eosinophil percentages were reduced, but the results were more variable than in other compartments: median reductions of 18.7% in cohort 1, 89.9% in cohort 2, and 95.1% in cohorts 1 and 2 combined. Results from the combined cohorts are similar to those reported for mepolizumab⁸ and reslizumab.⁹

Basophils also express the IL-5R α chain,¹² making this cell lineage another potential target of benralizumab. Basophils have been shown to be involved in asthma allergen challenges,^{17,18} cold air challenges,¹⁹ asthma exacerbations,²⁰⁻²² and fatal asthma.^{23,24} In an exploratory analysis, estimated blood basophil counts determined by using flow cytometry were reduced by 74%. Assessment of changes in basophil numbers in airway biopsy specimens, sputum, and bone marrow were not feasible because of baseline values of 0 or near 0 (data not shown).

This study has some limitations. The sample size of the study was smaller than published suggested sample sizes for airway biopsy studies.^{25,26} However, *post hoc* analyses performed on combined cohorts 1 and 2 resulted in statistically

significant reductions in airway and sputum eosinophil counts.

Sputum analyses were performed locally at each site, which might have increased the variability of the results. However, all sites had experience in performing induced sputum analysis, and cell counts were performed by experienced cytotechnologists.

Concomitant corticosteroid therapy was not standardized, with doses ranging from none to high-dose ICSs plus oral corticosteroids (1 placebo-treated subject), although the median ICS budesonide equivalent dose was 800 μg for the benralizumab groups in cohorts 1 and 2. This contrasts to mepolizumab airway biopsy studies, which allowed no ICSs¹⁶ or required high-dose ICSs with or without oral corticosteroids.⁶

Lastly, documentation of adherence to ICSs before and during the screening/run-in period was not obtained. Any changes in ICS use during this period could have had an effect on tissue and sputum eosinophil counts.

In conclusion, these results suggest eosinophils and possibly basophils are effectively depleted after administration of benralizumab, an mAb that functions through an ADCC pathway. A potential clinical response to benralizumab was demonstrated in a recent phase II study in which the number and severity of

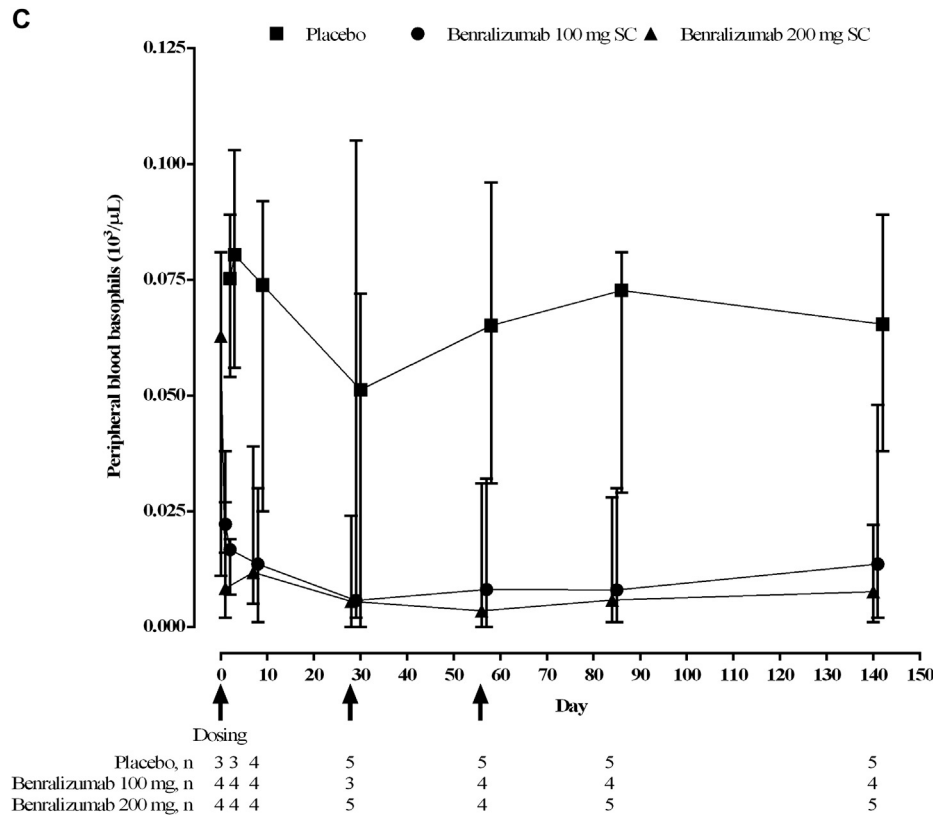


FIG 5. (Continued)

exacerbations were reduced in subjects with acute severe asthma.²⁷ Confirmation of these results and determination of the target population are being explored in an ongoing phase IIb study.

We thank Diana Swanson, PhD (MedImmune); Lourdes Briz (MedImmune); and Jennifer Stewart, MSc (QXV Communications, Macclesfield, United Kingdom; funded by MedImmune) for assistance with manuscript preparation. We also thank Laura Richman, DVM, PhD (MedImmune), who read the pathology slides, and Steven Eck, PhD (MedImmune) for flow cytometric analysis of basophils.

Clinical implications: Benralizumab, an mAb that targets the IL-5R and induces ADCC, reduced airway, sputum, bone marrow, and blood eosinophil counts and might provide therapeutic benefit in eosinophilic asthma.

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METHODS

Collection and preparation of airway mucosal samples

Subjects were not to have an influenza vaccine within 14 days before bronchoscopy. Before the bronchoscopy, subjects received 2 to 4 puffs of a short-acting β_2 -agonist (SABA) followed by spirometry. Postbronchodilator FEV₁ had to be 65% or greater to proceed with the bronchoscopy. Before discharge, subjects should have had an FEV₁ of at least 90% of the postbronchodilator value obtained before performing the bronchoscopy. The investigator could administer additional SABAs and observe the subject, as clinically indicated.

Mucosal/submucosal biopsy specimens were obtained from alternating lobes of the lung. The screening mucosal/submucosal biopsy specimens were preferably to be taken from the right lower lobe; the second mucosal/submucosal biopsy specimens (day 28 for cohort 1 or day 84 for cohort 2) were preferably to be taken from the left lower lobe; and the optional third mucosal/submucosal biopsy specimens for cohort 1 subjects, if obtained (day 84), were preferably to be taken from the right lower lobe.

Subjects were pretreated with an inhaled β_2 -agonist before the bronchoscopy. The decision to use anticholinergic medications (atropine or glycopyrrolate [Robinul; Mikart, Atlanta, Ga]), sedatives, and/or analgesics (midazolam, fentanyl, or both) was made jointly by the investigator or qualified designee and the subject (eg, some subjects might have preferred the relaxation provided by sedatives, whereas others tolerated the procedures well without medication and preferred not to undergo the recovery and restrictions associated with conscious sedation). The procedure was done after achievement local anesthesia of the upper and lower airways that had been obtained by using lidocaine with a dose of up to 9 mg/kg or 600 mg total, whichever was less. Airway sampling included 2 to 3 subsegmental and 2 to 3 segmental forceps endobronchial biopsy specimens.

Each biopsy specimen was placed in a labeled formalin cup. Biopsy specimens were kept in formalin for a minimum of 24 and a maximum of 48 hours. Formalin-fixed biopsy specimens were then embedded in paraffin and placed into a prelabeled specimen mold container (plastic box). Samples were sent to MedImmune (Gaithersburg, Md) for analysis.

Immunohistochemistry

Formalin-fixed, paraffin-embedded 5- μ m sections from the diagnostic samples were stained with H&E according to standard histopathologic techniques. Immunohistochemistry was done on formalin-fixed, paraffin-embedded tissue sections mounted on plus-charged microscope slides, air-dried, dewaxed in xylene, and rehydrated with Tris buffer (pH 7.2). The samples were treated with a heat-induced epitope retrieval technique by using citrate buffer (pH 6). Incubation with rabbit anti-major basic protein (catalog no. E3330, US Biological) was conducted for 2 hours at room temperature. Immunodetection was conducted with the Alexa Fluor 488 Goat Anti-Mouse (catalog no. A21121; Invitrogen, Grand Island, NY), followed by 4',6-diamidino-2-phenylindole nuclear staining. Appropriate positive controls were also included. All sections and stains were analyzed with the Leica SP5 confocal microscope system at $\times 100$ and $\times 400$ magnification.

Collection and preparation of peripheral blood samples

Blood samples for assessment of eosinophils were collected in 2.0-mL lavender-top EDTA vacutainers and then gently mixed by inverting the tubes 8 times. Samples were not shaken or put in the centrifuge. Samples were sent to ACM Pivotal Global Central Laboratory in the EDTA tubes at room temperature. The samples were analyzed at ACM as part of the routine hematologic analyses. Hematologic results were reported to the investigator, but eosinophil and basophil counts were blinded in the reports, except if the information was required for management of AEs or for long-term follow-up of eosinophil counts after the last study visit.

Fresh whole-blood samples were collected from subjects in cohort 2 for measurement of basophil counts with flow cytometry. Fresh whole blood was

collected in a 2-mL lithium-heparin Vacutainer (BD, Franklin Lakes, NJ), and 0.4 mL of TransFix (Invitrogen) was added to stabilize and preserve cells for subsequent flow cytometric analysis. Samples were sent by local medical courier to MedImmune the same day they were collected and were analyzed within the established stability for the test (14 days of collection). Because of variable total leukocyte degradation in the leukocyte gate related to the preservation process, basophils were identified from the combined lymphocyte and monocyte population gates, which demonstrated stability with the preservation process. Coexpression of the IgE high-affinity receptor (Fc ϵ RI) and CCR3 with CD123 and chemoattractant receptor of type 2 helper T cells were collectively consulted to identify the basophil cluster in each pairwise comparison, resulting in 3 values that were then averaged to generate the final basophil report value. Values were expressed as a percentage of the combined lymphocyte and monocyte populations, as determined based on side scatter and CD45 expression. The separate values were averaged to generate the final basophil report value.

Collection and preparation of bone marrow aspirates

Bone marrow aspirates were analyzed for mast cells, eosinophil and basophil precursors, and mature cells. The bone marrow aspiration procedure was performed in the right or left iliac crest. Two percent lidocaine was used as a local anesthetic before the procedure.

For sites performing bone marrow aspirate smears, 0.5 to 1 mL of bone marrow was obtained in a 10-mL syringe containing 1 mL of sterile sodium heparin (1000 U/mL). Two aspirate smears were prepared and stained with Wright-Giemsa stain at Charles River Laboratories, Pathology Associates (Frederick, Md). The slides were analyzed by using a manual cell count and bright field microscopy, according to World Health Organization recommendations, by a pathologist at Thomas Jefferson University (Philadelphia, Pa).

Collection and preparation of sputum samples

Before sputum induction, subjects were required to withhold the following:

- SABAs for at least 8 hours;
- long-acting β_2 -agonists and caffeinated food products for at least 12 hours; and
- leukotriene modifiers for at least 24 hours.

Subjects received 2 puffs of SABAs at the site, and spirometry was performed 15 to 20 minutes later. If FEV₁ was less than 60%, an additional 2 puffs of SABAs were administered, and spirometry was repeated 15 to 20 minutes later. If FEV₁ was still less than 60%, sputum induction was rescheduled.

If FEV₁ was 60% or greater, the subject inhaled 3%, 4%, and 5% saline for 7 minutes each. Induction was stopped when an adequate sample was obtained or if FEV₁ decreased by 20% or greater from baseline.

Sputum samples were processed within 2 hours of collection and stored at 4°C (on ice) as much as possible during processing. Samples were emptied into a Petri dish and placed on a black background. Sputum plugs (70–400 mg) free of salivary contamination were selected and transferred into a preweighed 15-mL polypropylene conical tube by using blunt forceps. The weight of the sputum plugs was determined (by subtracting the weight of the 15-mL conical tube), and a volume of Dulbecco PBS equal to 8 times the weight of the sputum plugs was added to the tube. Sputum was dispersed by means of repeated aspiration with a plastic pipette and then vortexed for 15 seconds. Tubes were rocked on ice for 15 minutes with a bench rocker and then centrifuged at 790g for 10 minutes at 4°C.

A volume of 0.2% Sputolysin (Millipore, Temecula, Calif; also known as dithiothreitol) equal to 4 times the weight of the sputum plugs was added. The tube was vortexed for 15 seconds, rocked on ice for 15 minutes with a bench rocker, and then vortexed for another 15 seconds. The solution was filtered through a 48- μ m nylon mesh into a sterile 15-mL polypropylene conical tube and then centrifuged at 500g for 10 minutes at 4°C. The cell pellet was resuspended in 1 mL of PBS and kept on ice until processed.

A small aliquot of the resuspended cell pellet was removed for total cell count by using the trypan blue method:

- The hemocytometer was flooded with 10 μ L of cell filtrate mixed thoroughly with 10 μ L of trypan blue.
- Cells were counted in the center square and the four 1-mm corner squares of chamber 1 of the hemocytometer. Cells were classified as viable, nonviable, and squamous (irrespective of whether viable) by using the trypan blue exclusion method. The mean number of cells per square and the percentages of viable leukocytes and squamous cells were calculated.
- The total number of cells and the total cell count (cells per gram of selected sputum) were calculated as follows:
 - Total number of cells ($\times 10^6$) = [Mean number of cells/square $\times 2 \times$ Volume of cell filtrate (mL)]/100.
 - Total cell count (cells $\times 10^6$ /g sputum) = [Mean number of cells/square $\times 2 \times$ Volume of cell filtrate (mL)]/100 $\times$ Weight of selected sputum (g).

On the basis of the total cell number per milliliter, the dilution needed to prepare 20,000 cells on each cytospin slide was determined. At least 2 (and up to 6) cytospin slides were prepared. Two slides were stained with Diff-Quick solution and placed under cover slips with a xylene-based mount; the remaining slides were left unstained. Cells (up to 400) were counted on each of the 2 stained slides, and the counts were averaged. Differential included inflammatory cells only, including neutrophils, eosinophils, lymphocytes, macrophages, and metachromatic cells. Slides (stained and unstained) were also then sent to MedImmune for analysis.

After preparing the slides, 2 aliquots of any remaining cell pellet material were prepared in blue-cap cryovials and frozen immediately in an upright position at a minimum temperature of -70°C . Once frozen, samples were sent to MedImmune for analysis.

Additional safety assessments

Summary data are presented for AEs of interest (infections and infusion or injection site reactions) and measurement of serum CRP, tryptase, and creatine phosphokinase (CPK) levels. Serum CRP samples were drawn twice on days the study drug was administered (day 0 for cohort 1 and days 0, 28, and 56 for cohort 2), with 1 sample drawn just before study drug administration and the other sample drawn 6 hours (cohort 1) or 2 hours (cohort 2) after study drug administration. CRP was assayed with the same standard procedures as for serum laboratory tests. Baseline serum tryptase levels were measured in all subjects in cohort 2 as part of an amendment by using a fluoroenzyme immunoassay kit. If clinically indicated, investigators had the option to order additional serum tryptase tests, although no additional tests were ordered. Serum tryptase is primarily produced and secreted by mast cells, and increased levels have been proposed as a marker to confirm the diagnosis of anaphylaxis.^{E1,E2} CPK levels were measured from samples obtained during routine laboratory tests at screening and days 0, 1, 7, 28, 56, and 84 (or study

termination) for cohort 1. For cohort 2, samples were taken at screening and days 0, 1, 7, 21, 28, 56, 84, 119, and 140 (or study termination).

RESULTS

Additional safety assessments

In cohort 1, 3 infections occurred in 3 of 5 subjects in the placebo group, and 3 infections occurred in 2 of 8 subjects in the benralizumab group. In cohort 2, 6 infections occurred in 4 of 5 subjects in the placebo group, and 3 infections occurred in 3 of 4 subjects in the 100-mg benralizumab group; no infections were reported in the 200-mg subcutaneous benralizumab group. The most commonly reported infection was nasopharyngitis (cohort 1: 2 of 8 benralizumab-treated subjects; cohort 2: 4 of 5 placebo-treated subjects and 2 of 9 benralizumab-treated subjects). All infections in both cohorts were considered mild or moderate in severity.

No infusion-related reactions were reported in cohort 1 during the study.

Within cohort 2, 1 subject who received placebo had AEs of injection-site hematoma, injection-site edema, injection-site erythema, and injection-site swelling. The events were mild or moderate in severity, and most were judged by the investigator as being possibly related to the study drug.

Within cohort 1, no subjects in the placebo group and 1 of 8 subjects in the benralizumab group had an increased CRP level reported as a treatment-related AE. The event was moderate in severity, transient, and judged by the investigator as being probably related to the study drug. A similar transient increase in CRP levels was observed in an additional subject in the benralizumab group but was not reported as a treatment-related AE. No abnormal CRP values were reported as treatment-related AEs in cohort 2. Within cohort 2, there was no apparent change in median serum tryptase levels after benralizumab treatment compared with those after placebo treatment. No anaphylactic AEs occurred.

Increased blood CPK levels were reported in 2 subjects from cohort 2. Both events occurred before administration of benralizumab and continued through the end of the study (Table E1). Neither event was considered related to benralizumab.

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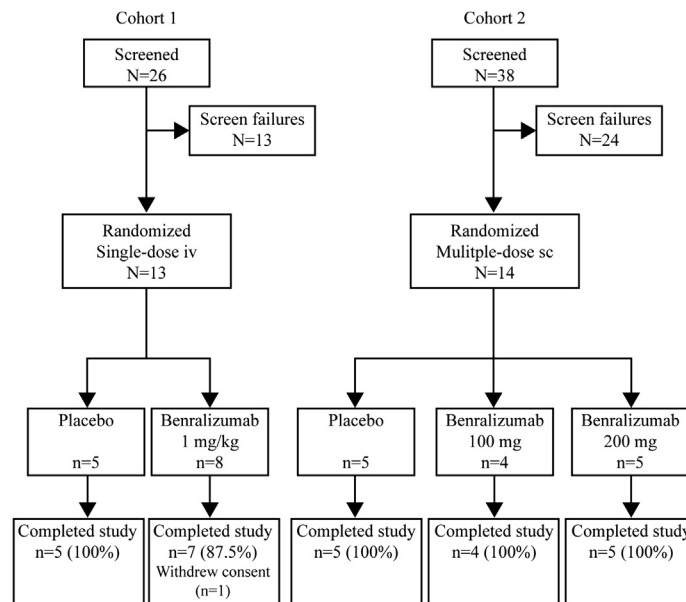


FIG E1. Subject disposition. Cohorts 1 and 2 were consecutive. *iv*, Intravenous; *sc*, subcutaneous.

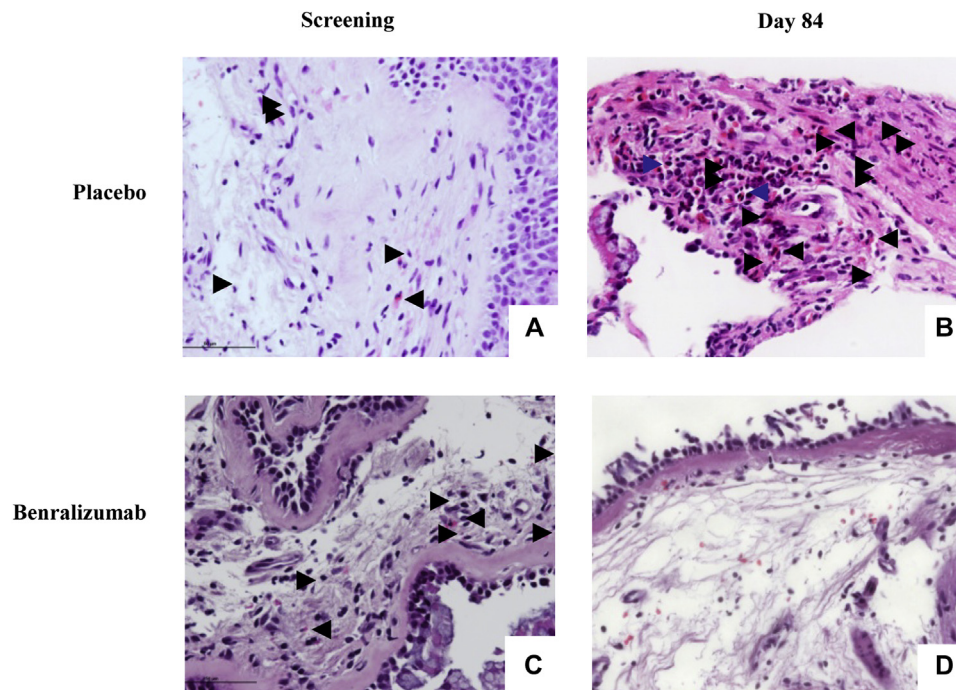


FIG E2. Representative H&E micropictographs from mucosal/submucosal biopsy specimens showing mucosal and submucosal eosinophils (*arrowheads*) in the same subjects at screening and day 84 (cohort 2, subcutaneous). **A** and **B**, Placebo-treated subject. **C** and **D**, Benralizumab-treated subject.

TABLE E1. Serum CPK levels*

Study day	Cohort 2 (SC)	
	Subject A,	Subject B,
	100 mg of benralizumab	200 mg of benralizumab
Day -14 (screening)	148	503
Day 0	2588	474
Day 1	1080	385
Day 7	302	250
Day 28	321	570
Day 84	396	236

*Values are shown as total micrograms per liter (normal range, 0-174 $\mu\text{g/L}$).