ORIGINAL ARTICLE

Benralizumab for *PDGFRA*-Negative Hypereosinophilic Syndrome

F.L. Kuang, F. Legrand, M. Makiya, J.A. Ware, L. Wetzler, T. Brown, T. Magee, B. Piligian, P. Yoon, J.H. Ellis, X. Sun, S.R. Panch, A. Powers, H. Alao, S. Kumar, M. Quezado, L. Yan, N. Lee, R. Kolbeck, P. Newbold, M. Goldman, M.P. Fay, P. Khoury, I. Maric, and A.D. Klion

ABSTRACT

BACKGROUND

Hypereosinophilic syndrome is a group of diseases defined by marked eosinophilia in blood or tissue and eosinophil-related clinical manifestations. Benralizumab is a monoclonal antibody against interleukin-5 receptor α, which is expressed on human eosinophils.

METHODS

In this randomized, double-blind, placebo-controlled, phase 2 trial, we administered a series of three monthly subcutaneous injections of either benralizumab (at a dose of 30 mg) or placebo in 20 symptomatic patients who had *PDGFRA*-negative hypereosinophilic syndrome and an absolute eosinophil count of at least 1000 cells per cubic millimeter; all the patients were receiving stable therapy (drugs or dietary changes) for this disease. This regimen was followed by an open-label phase, during which the patient's background therapy could be tapered as tolerated, and an extension phase. The primary end point of the randomized phase was a reduction of at least 50% in the absolute eosinophil count at week 12.

RESULTS

During the randomized phase, the primary end point occurred in more patients in the benralizumab group than in the placebo group (9 of 10 patients [90%] vs. 3 of 10 patients [30%], P=0.02). During the open-label phase, clinical and hematologic responses were observed in 17 of 19 patients (89%) and were sustained for 48 weeks in 14 of 19 patients (74%); in the latter group, in 9 of 14 patients (64%), background therapies could be tapered. Bone marrow and tissue eosinophilia were also suppressed with benralizumab therapy. The most common drug-related adverse events, headache and an elevated lactate dehydrogenase level, occurred in 32% of the patients after the first dose of benralizumab and resolved within 48 hours in all patients. Other adverse events occurred with similar frequency in the two groups. Of the many potential predictors of response that were examined, only clinical disease subtype appeared to be associated with the initial response or relapse.

CONCLUSIONS

In this small phase 2 trial, patients with *PDGFRA*-negative hypereosinophilic syndrome who received benralizumab for 12 weeks had lower absolute eosinophil counts than those who received placebo. During the open-label phase, clinical and hematologic responses were sustained for 48 weeks in 74% of the patients. Adverse events did not limit treatment. (Funded by the National Institute of Allergy and Infectious Diseases; ClinicalTrials.gov numbers, NCT00001406 and NCT02130882.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Kuang at the National Institutes of Health, Bldg. 4, Rm. B1-27, 4 Memorial Dr., Bethesda, MD 20892, or at feili.kuang@nih.gov.

N Engl J Med 2019;380:1336-46.
DOI: 10.1056/NEJMoa1812185

Hypereosinophilic syndrome is a group of rare chronic disorders that are defined by an absolute eosinophil count (i.e., the number of eosinophils in blood) of at least 1500 cells per cubic millimeter and evidence of eosinophil-related clinical manifestations that can include intractable pruritus, pulmonary infiltrates, eosinophilic gastroenteritis, endomyocardial fibrosis, and thromboembolism. The goal of treatment is a reduction in blood and tissue eosinophilia, thereby preventing further organ damage.[1] Conventional therapies, including glucocorticoids and immunomodulatory and cytotoxic therapies, have variable efficacy and substantial toxic effects.[2] Despite promising early trial results with antibodies against interleukin-5,[3,4] the only therapy for this disorder that has been approved by the Food and Drug Administration (FDA) is imatinib mesylate, a tyrosine kinase inhibitor that is effective in the treatment of primary myeloid forms of the disease, including myeloid neoplasms associated with the gene encoding platelet-derived growth factor receptor alpha (*PDGFRA*)[5]; new therapies that can help establish disease control in *PDGFRA*-negative hypereosinophilic syndrome are needed.[6]

The interleukin-5 receptor is expressed on human eosinophils, their precursors, basophils, and mast cells.[7-11] Benralizumab (MEDI-563; Fasenra, MedImmune/AstraZeneca) is a humanized, afucosylated monoclonal antibody against interleukin-5 receptor α (IL5RA) that targets IL5RA-bearing cells for enhanced antibody-dependent cellular cytotoxicity.[8] Benralizumab safely depleted eosinophils in the sputum and blood of patients with asthma.[12,13] In phase 3 clinical trials involving patients with severe eosinophilic asthma, benralizumab reduced exacerbations and improved lung function.[14,15] On the basis of these trials, in November 2017, the FDA approved the drug for use as add-on maintenance therapy for patients with severe eosinophilic asthma. Our current phase 2 trial was designed to assess the efficacy of benralizumab in reducing eosinophilia in patients with *PDGFRA*-negative hypereosinophilic syndrome.

METHODS

PATIENTS

We used a protocol that was designed to evaluate patients with eosinophilia to screen adults with hypereosinophilic syndrome (ClinicalTrials.gov number, NCT00001406). A diagnosis of hypereosinophilic syndrome was determined by a history of persistent blood eosinophilia of at least 1500 cells per cubic millimeter without a known secondary cause and evidence of end-organ manifestations attributable to the eosinophilia. Symptomatic adults with a *PDGFRA*-negative hypereosinophilic syndrome and an absolute eosinophil count of at least 1000 cells per cubic millimeter while receiving stable therapy (drugs or dietary changes) for this disease for at least 1 month were eligible to participate in the trial. Full inclusion and exclusion criteria are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. Control samples for in vitro experiments were collected from healthy volunteers under protocols (NCT00090662; National Institutes of Health [NIH] protocol 93-CC-0094) that had been approved by institutional review boards at the NIH.

TRIAL DESIGN

This single-center, investigator-initiated, phase 2 trial had three sequential stages: a randomized, double-blind, placebo-controlled phase (12 weeks), an open-label phase (12 weeks), and an open-label extension phase (24 weeks) (Fig. 1A). During the randomized phase, patients received benralizumab (at a dose of 30 mg) or placebo subcutaneously every 4 weeks for three doses while they were receiving stable therapy for hypereosinophilic syndrome. The trial team and patients were unaware of eosinophil counts during this phase. At week 12, all the patients began to receive subcutaneous injections of 30 mg of benralizumab every 4 weeks. From week 13 forward, the absolute eosinophil count was unblinded and background therapy was tapered, as tolerated. Patients who had a clinical or hematologic response at week 24 were eligible to continue to receive benralizumab during the open-label extension. The trial-group assignments and eosinophil counts before week 13 were unblinded only after the last patient had completed the week 24 visit and the database had been locked.

PROCEDURES AND OUTCOME MEASURES

All clinical evaluations were performed at the NIH Clinical Center. After one of the patients in the benralizumab group (Patient 2) had a post-treatment reaction, the remaining patients were admitted for observation for at least 24 hours following the first dose of benralizumab or pla-

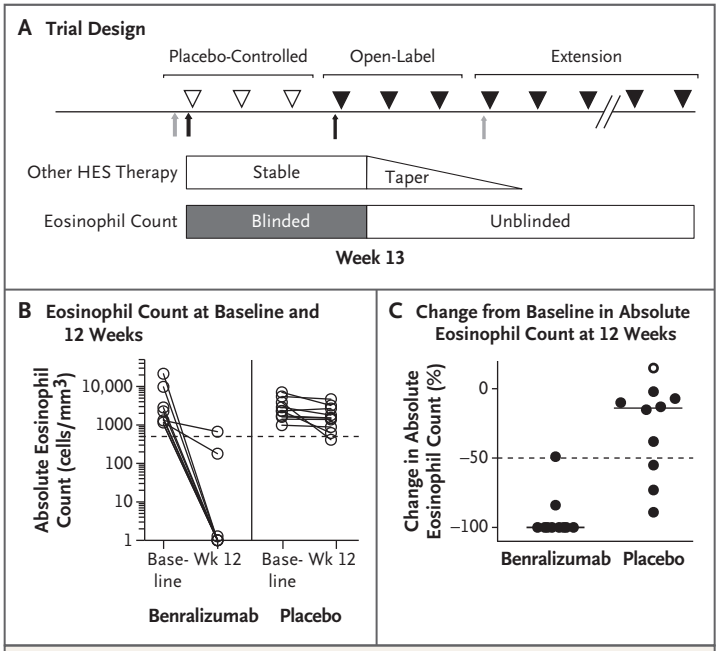


Figure 1. Trial Design and Effect of Benralizumab on Eosinophil Counts.

Panel A shows the design of the trial, in which 20 patients with hypereosinophilic syndrome (HES) were randomly assigned to receive subcutaneous injections of benralizumab (30 mg) or placebo every 4 weeks for 12 weeks (open triangles). Patients continued to receive stable therapy (drugs or dietary changes) for hypereosinophilic syndrome, and the absolute eosinophil counts were blinded until week 13 of the trial. The placebo-controlled, randomized phase was followed by open-label and extension phases, during which all the patients received 30 mg of benralizumab every 4 weeks (closed triangles). Bone marrow biopsies (black arrows) and peripheral-tissue biopsies (gray arrows) were performed at the indicated time points. Panel B shows the eosinophil counts at baseline and at week 12 in patients in the two groups during the randomized phase of the trial. The dotted line indicates an eosinophil count of 500 cells per cubic millimeter. Panel C shows the percent change from baseline to week 12 in the eosinophil count for each patient after three doses of benralizumab or placebo ($P=0.02$ by Fisher's exact test). The dotted line indicates the cutoff for the primary end point (a reduction of ≥50% in the eosinophil count). Week 6 data were used for Patient 17, who had withdrawn from the trial before week 12 (open circle).

cebo and the first dose of open-label benralizumab (at week 12). Adverse events were scored at each visit with the use of the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4. Bone marrow aspirates and biopsy samples were obtained at baseline and at week 12. When possible, tissue biopsy samples were obtained at baseline and at week 24.

The primary end point was the percentage of patients who had a reduction of at least 50% in the absolute eosinophil count at week 12. Secondary end points included a reduction in the absolute eosinophil count at 12 weeks, the frequency and severity of adverse events, changes in bone marrow and tissue eosinophilia, and reductions in concomitant therapy at 48 weeks. Exploratory end points included an assessment of clinical and laboratory predictors of response. Details regarding trial procedures and end points are provided in the Supplementary Appendix.

TRIAL OVERSIGHT

The trial protocol, which was designed by the last author with input from coauthors, is available at NEJM.org and was approved by the institutional review board at the National Institute of Allergy and Infectious Diseases (NIAID). NIAID was the trial sponsor and obtained benralizumab under a cooperative research and development agreement with MedImmune/AstraZeneca. All the patients provided written informed consent.

Adverse events were reviewed every 6 months by an independent data and safety monitoring board. Representatives of the manufacturer who served as coauthors performed and analyzed the pharmacokinetic and antidrug-antibody assays (Fig. S6 in the Supplementary Appendix) but were not otherwise involved in data collection or drafting of the initial version of the manuscript. All the authors had full access to the data and vouch for the completeness and accuracy of the data and for the adherence of the trial to the protocol. All the authors reviewed the manuscript, and the first and last authors made the decision to submit the manuscript for publication.

STATISTICAL ANALYSIS

For the primary analysis, we used a two-sided Fisher's exact test to compare the percentages of patients who had a reduction of at least 50% in the eosinophil count in the two groups at 12 weeks. We determined that the enrollment of 10 patients in each group would provide a power of more than 90% to show a difference in the primary response, assuming a response rate of 1% in the placebo group and 70% in the benralizumab group. We performed analyses using the intention-to-treat principle in anticipation of few withdrawals on the basis of previous trials involving patients with this rare disease. Analyses for the secondary and exploratory end points are described in the Supplementary Appendix.

RESULTS

PATIENTS

From May 2014 through January 2017, a total of 24 patients were screened (Fig. S1 in the Supplementary Appendix). Three patients were excluded because they had an absolute eosinophil count of less than 1000 cells per cubic millimeter, and 1 was excluded because of unstable disease. Baseline demographic and clinical characteristics, including clinical disease subtype and end-organ involvement, were similar in the two groups (Table 1, and Table S1 in the Supplementary Appendix). All the patients had not had an adequate response to a number of therapies (median number, 3.5; range, 1 to 11) and had shown a variety of clinical manifestations.

EFFICACY

During the first 12 weeks of the trial, a reduction of at least 50% in the absolute eosinophil count at week 12 (the primary end point) occurred in more patients in the benralizumab group than in the placebo group (9 of 10 patients [90%] vs. 3 of 10 patients [30%], P=0.02) (Fig. 1B and 1C), for a between-group difference of 60 percentage points (95% confidence interval [CI], 8 to 89). One patient in the placebo group (Patient 17) withdrew at week 6 because of an inability to complete the trial visits, so the last available absolute eosinophil count was used for this patient. All the other patients were followed for 48 weeks. (Details regarding sensitivity and secondary efficacy analyses are provided in the Supplementary Appendix.)

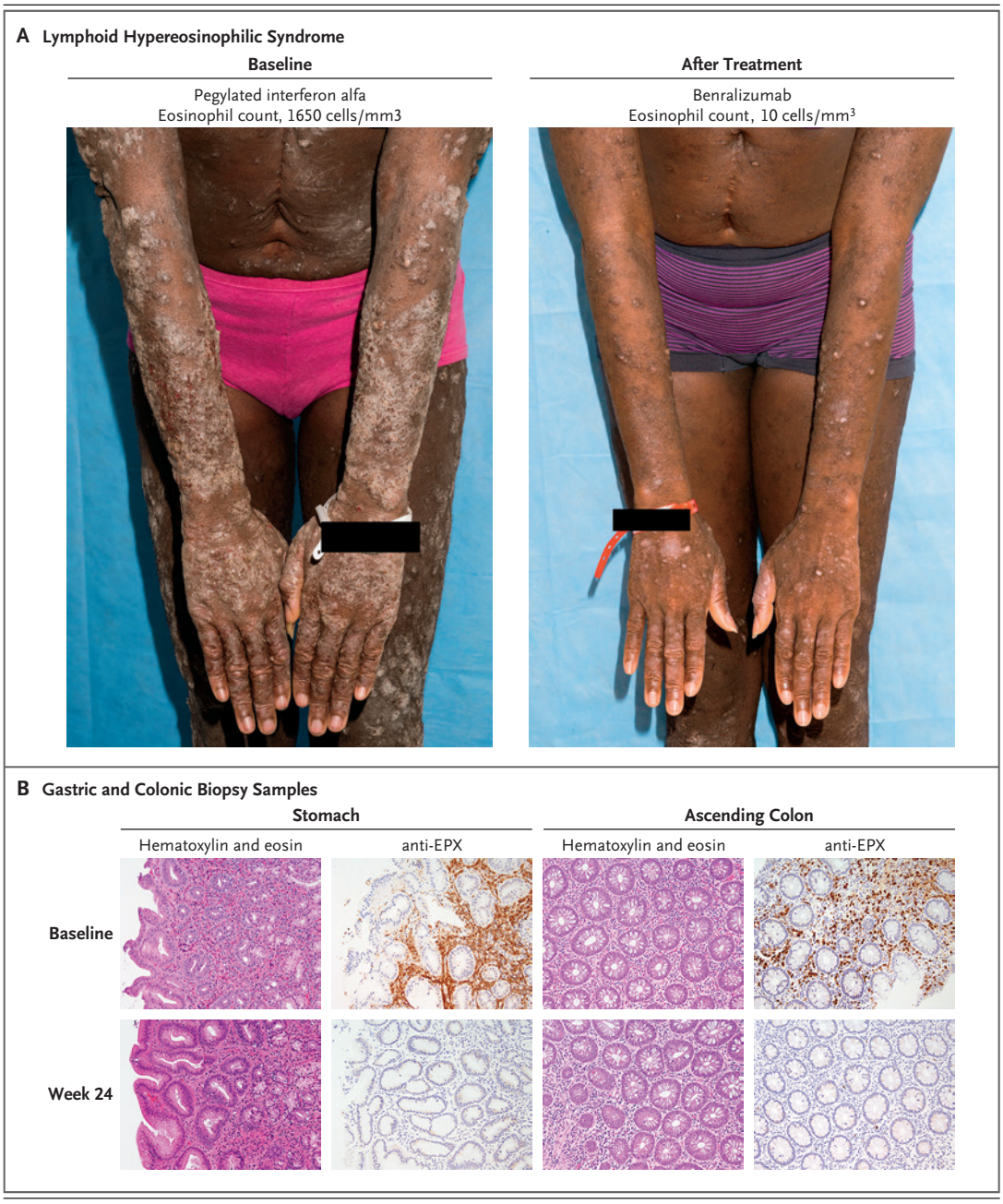
One week after the first dose of open-label benralizumab was administered (week 13), the absolute eosinophil count was less than 200 cells per cubic millimeter in 17 of 19 patients (89%) (Fig. S2 in the Supplementary Appendix). Patients 10 and 12 were identified as having had no response to treatment, so benralizumab was discontinued in these patients at weeks 16 and 24, respectively. Patients 1, 3, and 6 had a clinical and hematologic relapse after 12 to 24 weeks of response, which resulted in the discontinuation of benralizumab therapy. The remaining 14 of 19 patients (74%) continued to receive benralizumab through week 48 of the open-label phase and week 96 of the extension phase. On the basis of an assumption that Patient 17, who had withdrawn from the trial after receiving two doses of placebo, did not have a response, the median duration of response (≥50% reduction in the absolute eosinophil count) was 84 weeks (range, 0 to 96 weeks).

All 17 patients who had an initial hematologic response to benralizumab also reported having clinical improvement in their symptoms. (Clinical vignettes are provided in the Supplementary Appendix, and photographs of a patient before and after therapy are shown in Fig. 2A.) Concomitant therapies other than benralizumab were tapered on the basis of absolute eosinophil counts and clinical manifestations beginning at

Table 1. Characteristics of the Patients at Baseline (Intention-to-Treat Population).*

Characteristic	Benralizumab (N=10)	Placebo (N=10)
Median age (range) — yr	46 (23–67)	44 (28–74)
Male sex — no.	4	3
Background therapy — no.†		
Glucocorticoids	9	4
Interferon alfa	1	3
Hydroxyurea	2	1
Cyclophosphamide	0	1
Geometric mean eosinophil count (range) — cells/mm^3	2331 (1050–21,580)	2535 (1000–7250)
Clinical subtype — no.		
Myeloid variant‡	1	1
Lymphoid variant	3	3
Single-organ overlap	4	2
Idiopathic	2	4
Organ involvement — no.		
Dermatologic	4	5
Gastrointestinal	5	2
Pulmonary	6	5
Neurologic	1	1
Musculoskeletal	2	3
Cardiac	1	2
Constitutional	10	10

* There was no significant difference between the two groups.
† Some patients were receiving more than one agent for background therapy.
‡ The two patients with myeloid variant disease had genetic changes in *JAK2* (one with the V617F mutation and one with a complex insertion or deletion in exon 13 [codon 583–586]).



week 13. At the end of the trial (week 48), 9 of 14 patients who were still receiving benralizumab were able to taper their concomitant therapies, and 7 of those 9 patients were in stable condition while receiving benralizumab monotherapy (Table S2 in the Supplementary Appendix). At week 48, the therapy regimen was unchanged in Patients 14 and 16; Patients 13 and 15 did not receive concomitant drug therapy during the trial. At week 48, Patient 2 was receiving an increased dose of prednisone for treatment of sarcoidosis that was unmasked during prednisone tapering. All 3 patients who had a relapse after an initial response to benralizumab (Patients 1, 3, and 6) had been able to transiently taper their concomitant therapy before relapse. However, despite clinical improvement with resumption of the baseline prednisone dose (along with interferon alfa in Patient 2), these 3 patients ultimately received additional therapy.

Figure 2 (facing page). Clinical Improvement and Resolution of Tissue Eosinophilia.

Panel A shows a 37-year-old woman with a lymphoid form of hypereosinophilic syndrome before and after treatment with benralizumab. At baseline, the patient's severe spongiotic dermatitis was complicated by superinfections and bacteremia, resulting in multiple hospitalizations. Previous therapies included oral prednisone (40 mg daily), phototherapy, methotrexate, cyclosporine, mycophenolate, thalidomide, interferon alfa, and various topical agents, none of which adequately controlled her symptoms or eosinophilia. The baseline eosinophil count was 1650 cells per cubic millimeter while the patient was receiving subcutaneous pegylated interferon alfa (90 μg weekly). Since she had been assigned to the placebo group, she did not receive benralizumab until week 12 during the open-label phase of the trial. At week 13, the eosinophil count had dropped to 30 cells per cubic millimeter and declined further to 0 cells per cubic millimeter by week 15. After three monthly doses of benralizumab, during which the pegylated interferon alfa was discontinued, there was a great reduction in the number of skin lesions and associated infections. (A more detailed case vignette about this patient is provided in the Supplementary Appendix.) Panel B shows representative gastric and colonic biopsy samples (hematoxylin and eosin staining and immunostaining against eosinophil peroxidase [anti-EPX]) obtained from two patients with eosinophilic gastrointestinal disorders (Patients 7 and 13) at baseline (top row) and after benralizumab therapy at week 24 (bottom row). In the two patients, biopsy samples showed an eosinophil count of more than 200 cells per high-power field at baseline and a count of 0 at week 24.

The numbers of bone marrow eosinophils, eosinophil precursors, and blood and bone marrow basophils were significantly decreased at week 12 in all the patients in the benralizumab group, but the number of mast cells and serum tryptase levels were unchanged (Fig. 3, and Fig. S3 in the Supplementary Appendix). Tissue samples obtained at week 24 showed nearly complete depletion of eosinophils (≤1 eosinophil per high-power field) in a total of 52 gastrointestinal biopsy samples obtained from the seven patients with gastrointestinal eosinophilia (Table S3 in the Supplementary Appendix). Immunohistochemical staining for eosinophil peroxidase (anti-EPX) confirmed the absence of tissue eosinophils and eosinophil granules in samples obtained from two patients (Fig. 2B). Bronchoalveolar lavage that was performed at baseline and at week 24 in a patient with a history of eosinophilic pulmonary infiltrates (Patient 9) showed resolution of bronchoalveolar lavage eosinophilia while the patient was receiving benralizumab therapy (data not shown). Patients 4 and 19, who had previous evidence of eosinophilia in skin-biopsy samples, underwent repeated biopsy during the course of the trial to evaluate new or worsening rash. Eosinophils were absent in both biopsy samples obtained during benralizumab therapy, and the rash resolved in the two patients.

SAFETY

A total of 238 adverse events were reported during the randomized phase of the trial; the number of adverse events (including grade 3) and the number of patients reporting an adverse event were similar in the two groups (Table 2). The only serious adverse event (hypotension) occurred in a patient receiving placebo. Six patients in the benralizumab group had transient, mild lymphocytopenia after the first dose, and persistent lymphocytopenia developed in one patient in the placebo group (P=0.06).

Adverse events that were deemed by investigators to be possibly, probably, or definitely related to benralizumab during the entire 48 weeks of the trial are listed in Table S4 in the Supplementary Appendix. No deaths were reported, and no patient discontinued benralizumab because of an adverse event. Two grade 3 serious adverse events were deemed to be possibly related to benralizumab: eosinophilia that occurred after discontinuation of the drug (because of a lack of response) in Patient 10 and ureteral obstruction from a kidney stone in Patient 2, who had an elevated serum uric acid level. Serum uric acid levels were monitored every 3 months for 1 year and yearly thereafter in subsequent patients and were in the normal range in all the patients tested.

Eight patients had a constellation of symptoms, including fever, chills, headache, nausea, and fatigue, approximately 6 hours after the first dose of benralizumab (Table S5 in the Supplementary Appendix). These self-limited episodes did not recur with subsequent doses. Serum lactate dehydrogenase levels increased in 16 of 19 patients on the day after the first dose of benralizumab; a greater increase was observed in patients who had post-injection reactions than in those who did not (median increase, 72 ng per milliliter and 12 ng per milliliter, respectively; P=0.006). None of the variables that were examined, which included the clinical disease

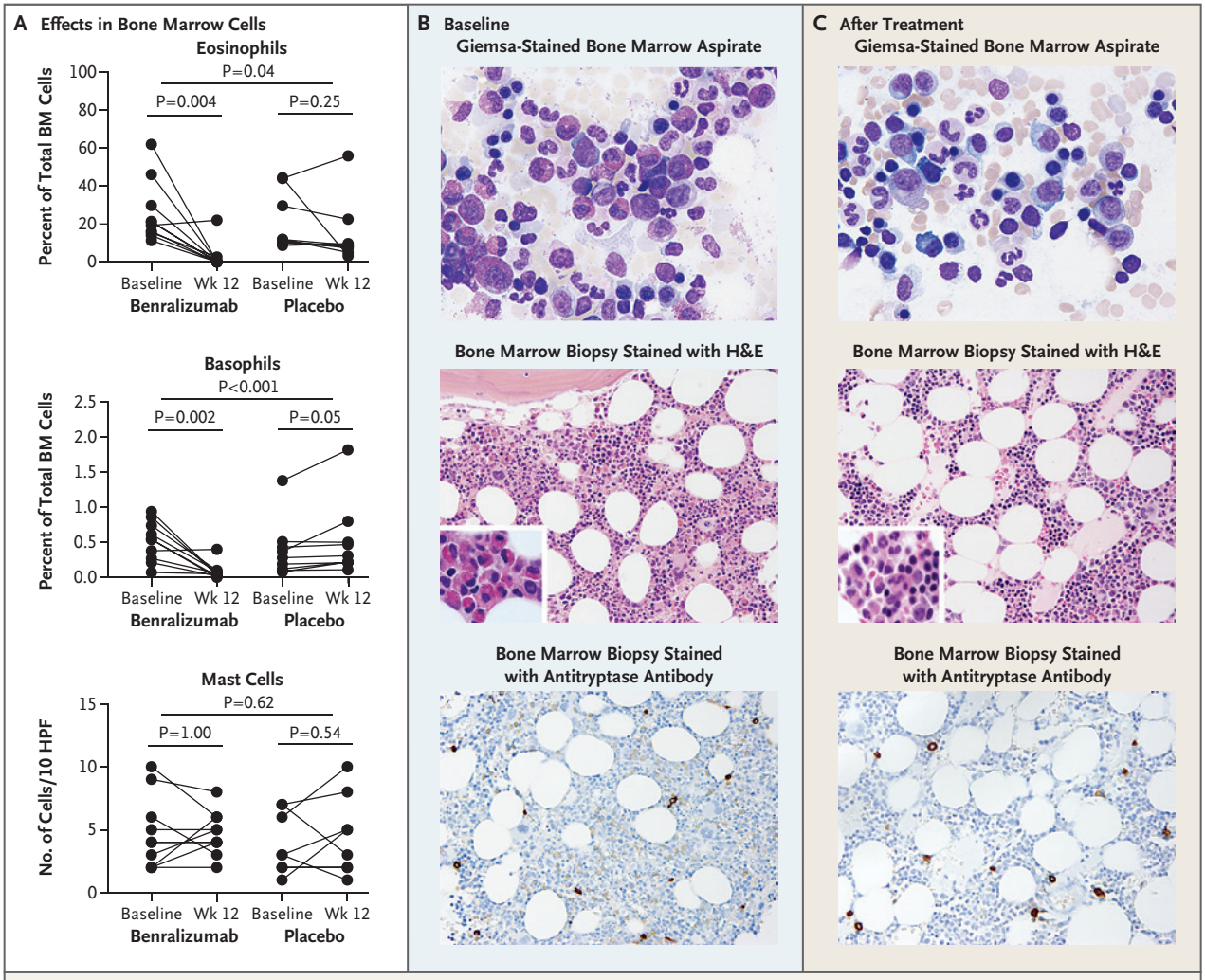


Figure 3. Effect of Benralizumab in Bone Marrow.

Panel A shows the numbers of eosinophils, basophils, and mast cells in bone marrow obtained from trial patients at baseline and at 12 weeks after the receipt of either benralizumab or placebo. Eosinophils and basophils are expressed as the percentage of total bone marrow (BM) cells in bone marrow aspirates. Mast cells were quantified as the average number of tryptase-positive cells in 10 high-power fields (HPF) at 40× magnification in bone marrow biopsy samples stained with antibody against tryptase. Two-sample t-testing (Welch's method) of the within-patient differences was used to determine whether there was a treatment effect for benralizumab as compared with placebo. Unadjusted P values are shown. P values that were adjusted with the use of Holm's correction for multiple comparisons are P=0.07 for eosinophils, P<0.001 for basophils, and P=0.62 for mast cells. Also shown are representative histopathological samples of bone marrow obtained from Patient 19 before the initiation of benralizumab (Panel B) and after 12 weeks of treatment (Panel C), including Giemsa-stained aspirates and biopsy samples stained with hematoxylin and eosin (H&E) and antitryptase antibody.

subtype, baseline therapy, pretreatment numbers of eosinophils and natural killer (NK) cells, and the activation status of NK cells, were associated with the development of post-treatment reactions (Table S6 in the Supplementary Appendix).

Although this trial was not designed to explore the long-term effects of eosinophil depletion in humans, exploratory studies in the benralizumab group revealed no evidence of impaired recall response to tetanus vaccination (in 14 patients) or altered glucose metabolism as assessed by measurement of the glycated hemoglobin level (in 19 patients).

FACTORS ASSOCIATED WITH CLINICAL OUTCOMES

The 2 patients who did not have a response to benralizumab had received the diagnosis of a primary myeloid hypereosinophilic syndrome

Table 2. Adverse Events during the 12-Week Randomized Phase.*

Event	Benralizumab (N=10)		Placebo (N=10)	
	Episodes	Patients	Episodes	Patients
	no.	*no. (%)*	*no.*	*no. (%)*
Serious adverse event				
Any	0		1	1 (10)
Hypotension	0		1	1 (10)
Adverse event				
Any grade	124	10 (100)	114	10 (100)
Grade 1	79	9 (90)	68	7 (70)
Grade 2	37	10 (100)	34	10 (100)
Grade 3	8	6 (60)	12	5 (50)
Grade 4	0		0	
Grade 3 adverse event				
Fatigue	3	3 (30)	1	1 (10)
Hyponatremia	3	2 (20)	0	
Abdominal pain	1	1 (10)	1	1 (10)
Headache	1	1 (10)	0	
Eosinophilia	0		5	3 (30)
Diarrhea	0		2	2 (20)
Back pain	0		1	1 (10)
Dyspnea	0		1	1 (10)
Hypotension	0		1	1 (10)
Adverse event				
Any	124	10 (100)	114	10 (100)
Lymphocytopenia	6	6 (60)	1	1 (10)
Headache	7	5 (50)	4	4 (40)
Elevated serum lactate dehydrogenase	5	5 (50)	1	1 (10)
Anemia	7	4 (40)	4	2 (20)
Fatigue	5	4 (40)	4	3 (30)
Chills	5	3 (30)	1	1 (10)
Nausea	4	3 (30)	4	3 (30)
Abdominal cramps	3	3 (30)	0	
Elevated creatinine	3	3 (30)	0	
Pain in hip	3	3 (30)	1	1 (10)
Upper respiratory infection	3	3 (30)	1	1 (10)
Fever	4	2 (20)	0	
Decreased appetite	3	2 (20)	0	
Hyponatremia	3	2 (20)	0	
Neutrophilia	2	2 (20)	4	3 (30)
Hypophosphatemia	2	2 (20)	4	2 (20)
Malaise	2	2 (20)	1	1 (10)
Pruritic rash	2	2 (20)	3	2 (20)
Elevated urinary white-cell count	2	2 (20)	1	1 (10)
Elevated C-reactive protein	2	2 (20)	0	

* Shown are the number of episodes and the number of affected patients (% of group). All serious adverse events and grade 3 adverse events that occurred during the randomized phase of the trial (baseline to week 12) are shown. Grade 1 and grade 2 adverse events that were reported in two or more patients in the benralizumab group are also reported. Adverse events were categorized according to codes used in the *Medical Dictionary for Regulatory Activities*, versions 16.1 through 20.1.

with a *JAK2* mutation. All 3 patients who had a relapse after an initial response had lymphoid hypereosinophilic syndrome with an aberrant clonal population of CD3−CD4+ T cells, although an additional patient with the same clinical subtype had a sustained clinical response (absolute eosinophil count, 0 cells per cubic millimeter) at 48 weeks. Surface expression of IL5RA (the target of benralizumab) was detected on eosinophils obtained from all 17 patients who were tested before benralizumab therapy (geometric mean IL5RA antibodies bound per cell, 2287; range, 891 to 3802) (Fig. S4A in the Supplementary Appendix); in 18 of 19 patients, serum levels of soluble IL5RA at baseline were within the upper range of levels measured in healthy controls (3.45 ng per milliliter; range, 0.72 to 78.10) (Fig. S4B in the Supplementary Appendix). The pretreatment absolute eosinophil counts were similar in the patients who had a response and in those who did not have a response (Fig. S5A in the Supplementary Appendix).

Since benralizumab action depends on the activity of NK cells, we assessed the number of NK cells, the ratio of NK cells to eosinophils in whole blood, and *CD16* polymorphisms associated with altered efficiency of NK-mediated antibody-dependent cellular cytotoxicity. At baseline, we quantified benralizumab-induced NK-mediated killing of eosinophils in vitro using autologous eosinophils and NK cells. None of these measurements appeared to be associated with an initial clinical response or relapse (Fig. S5 in the Supplementary Appendix).

DRUG LEVELS AND ANTIDRUG ANTIBODIES

In most of the patients, all measured serum trough levels of benralizumab were within the 90th prediction interval on the basis of the Monte Carlo simulation[16] derived from the nine clinical trials of benralizumab involving patients with asthma (Fig. S6A, S6B, and S6C in the Supplementary Appendix). Of the 3 patients who had a relapse, 2 (Patients 1 and 6) had declining drug-trough levels at the time of relapse (Fig. S6D in the Supplementary Appendix). The third patient who had a relapse (Patient 3) had undetectable drug trough levels at all time points and measurable antidrug antibodies with rising titers after the third dose of benralizumab, a finding that coincided with relapse. Of the 19 patients who received benralizumab, 3 had detectable antidrug antibodies after receiving the drug; of these patients, 2 (Patients 3 and 9) also had decreased serum drug trough levels (Fig. S6E in the Supplementary Appendix). The frequency of antidrug-antibody development in our trial was similar to that in patients with asthma who had received the same benralizumab regimen.[14,15,17,18]

DISCUSSION

In this small phase 2 trial involving a diverse group of patients with *PDGFRA*-negative hypereosinophilic syndrome with persistent disease or severe side effects after receiving multiple previous therapies, the percentage of patients who had a hematologic and clinical response to benralizumab therapy was 74% at week 48. This observed response rate is similar to those reported for glucocorticoid[2] and mepolizumab[3,19,20] treatment of this disorder. During the 12-week randomized phase of the trial, three patients in the placebo group met the primary end point of a reduction of at least 50% in the absolute eosinophil count. In one of these patients (Patient 8), the improvement was related to the timing of pulsed cyclophosphamide therapy in relation to the trial time points. The reasons for the decreased absolute eosinophil counts in the other two patients are unclear, although neither had resolution of eosinophilia or symptomatic improvement while receiving placebo.

In contrast to eosinophils, which were undetectable in the blood, bone marrow, and tissues after 12 weeks of benralizumab therapy, basophils were reduced in numbers but were not absent in the peripheral blood and bone marrow, and the numbers of mast cells in bone marrow were unchanged. Although the relative contributions of these effector cells to allergic manifestations are controversial,[21] it is noteworthy that benralizumab-treated patients continued to have chronic urticaria, seasonal allergies, and immediate hypersensitivity reactions to food, despite resolution of clinical manifestations of hypereosinophilic syndrome. (Clinical vignettes describing such patients are provided in the Supplementary Appendix.) Whether these reactions were due to the persistence of basophils and mast cells in the blood and tissues is unknown.

As in the trials involving patients with asthma, benralizumab was associated with few side effects in this trial. Mild or moderate post-treatment reactions occurred in eight patients after the first dose of benralizumab, but symptoms

were transient and did not lead to drug discontinuation. Clinically similar reactions were reported in a small number of patients participating in early trials of intravenous benralizumab[12,13] but were absent in subsequent studies with subcutaneous administration.[14,15,17,18] The most likely mechanism of the observed post-treatment reactions is eosinophil killing mediated by antibody-dependent cellular cytotoxicity, given the timing and nature of the symptoms, the presence of elevated lactate dehydrogenase levels, and a lack of other explanations (e.g., no change in serum tryptase levels, which would indicate a mast-cell–mediated process). An alternative explanation is cytokine release by activated NK cells, which has been reported with rituximab.[22]

Of the many clinical and laboratory measurements that we examined, only the clinical disease subtype appeared to be associated with the clinical response. Differing response rates among patients with clinical subtypes of this disorder have also been described for glucocorticoids,[23] imatinib,[5] and mepolizumab,[19,20] which suggests that the mechanism driving the eosinophilia plays an important role in the therapeutic response. The sustained response in Patient 15, who had a lymphoid hypereosinophilic syndrome with an aberrant CD3−CD4+ T-cell population, suggests that clinical disease subtype is not the sole determinant of response. However, Patient 3, who had a relapse, also had a lymphoid form of the disease, high serum levels of soluble IL5RA, and the development of antidrug antibodies, findings that are consistent with the hypothesis that response is multifactorial.

Limitations of this trial include the small sample size and lack of validated clinical outcome measures for this rare disease. The purposeful inclusion of a diverse group of patients with varied disease manifestations allowed us to explore the role of clinical subtype in the response to therapy. However, this heterogeneity also made it more difficult to find a common clinical outcome measure to assess efficacy. Finally, the small number of patients who did not have a response precluded any definitive conclusions with respect to predictors of response to benralizumab.

Despite these limitations, the trial showed that benralizumab therapy is effective in reducing blood and tissue eosinophilia with few or no toxic effects in patients with severe, treatment-refractory hypereosinophilic syndrome, despite having markedly higher eosinophil levels than patients with asthma. Equally important for this chronic and debilitating disorder, the eosinophil-lowering effect of benralizumab was sustained in the majority of patients despite tapering of other therapies that have substantial long-term toxic effects. A larger, well-controlled, multicenter trial of benralizumab is clearly needed to confirm these results and further explore the role of clinical disease subtype and other factors in the treatment response in patients with hypereosinophilic syndrome.

Supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). Benralizumab was provided by MedImmune/AstraZeneca, and representatives of the manufacturer also assessed drug and antidrug-antibody levels under the terms of Cooperative Research and Development Agreement 2013-0697.

Dr. Lee reports being employed by MedImmune; Dr. Kolbeck, being employed by MedImmune and owning shares of stock in AstraZeneca; Dr. Newbold, being employed by and holding stock in AstraZeneca; and Dr. Goldman, being employed by AstraZeneca. No other potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the patients and their families for their participation in the trial, Dr. David Kleiner for providing high-quality photographs of the pathological specimens, Dr. Zengfeng Wang for performing anti-EPX immunohistochemical analyses, the clinical photography department for providing images, and the clinical and administrative staff members of the inpatient units and outpatient clinics at the NIH Clinical Center.

APPENDIX

The authors' full names and academic degrees are as follows: Fei Li Kuang, M.D., Ph.D., Fanny Legrand, Ph.D., Pharm.D., Michelle Makiya, M.Sc., JeanAnne Ware, M.S.N., N.P., Lauren Wetzler, M.H.S., P.A.-C., Thomas Brown, R.N., Tamika Magee, M.H.A., R.N., Brent Piligian, B.S., Pryscilla Yoon, B.A., Jamie H. Ellis, B.S., M.T., Xiaoping Sun, Ph.D., Sandhya R. Panch, M.D., M.P.H., Astin Powers, M.D., Hawwa Alao, M.D., Sheila Kumar, M.D., Martha Quezado, M.D., Li Yan, Ph.D., Nancy Lee, Ph.D., Roland Kolbeck, Ph.D., Paul Newbold, Ph.D., Mitchell Goldman, M.D., Ph.D., Michael P. Fay, Ph.D., Paneez Khoury, M.D., Irina Maric, M.D., and Amy D. Klion, M.D.

The authors' affiliations are as follows: the Laboratory of Parasitic Diseases (F.L.K., F.L., M.M., J.W., L.W., T.B., T.M., B.P., P.Y., P.K., A.D.K.) and Biostatistics Research Branch (M.P.F.), National Institute of Allergy and Infectious Diseases, the Digestive Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases (H.A., S.K.), and the Laboratory of Pathology, National Cancer Institute (A.P., M.Q.), National Institutes of Health (NIH), and the Departments of Laboratory Medicine (J.H.E., X.S., I.M.) and Transfusion Medicine (S.R.P.), NIH Clinical Center, Bethesda, Washington Adventist Hospital, Takoma Park (T.M.), and MedImmune (N.L., R.K.) and AstraZeneca (P.N., M.G.), Gaithersburg — all in Maryland; the Department of Veteran Affairs, Tennessee Valley Healthcare System, Chattanooga (J.H.E.); and MedImmune, South San Francisco, CA (L.Y.).

REFERENCES

1. Klion AD. How I treat hypereosinophilic syndromes. Blood 2015;126:1069-77.
2. Ogbogu PU, Bochner BS, Butterfield JH, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. J Allergy Clin Immunol 2009;124(6):1319-25.e3.
3. Rothenberg ME, Klion AD, Roufosse FE, et al. Treatment of patients with the hypereosinophilic syndrome with mepolizumab. N Engl J Med 2008;358:1215-28.
4. Klion AD, Law MA, Noel P, Kim Y-J, Haverty TP, Nutman TB. Safety and efficacy of the monoclonal anti-interleukin-5 antibody SCH55700 in the treatment of patients with hypereosinophilic syndrome. Blood 2004;103:2939-41.
5. Khoury P, Desmond R, Pabon A, et al. Clinical features predict responsiveness to imatinib in platelet-derived growth factor receptor-alpha-negative hypereosinophilic syndrome. Allergy 2016;71:803-10.
6. Bochner BS, Book W, Busse WW, et al. Workshop report from the National Institutes of Health Taskforce on the Research Needs of Eosinophil-Associated Diseases (TREAD). J Allergy Clin Immunol 2012;130:587-96.
7. Dahl C, Hoffmann HJ, Saito H, Schiøtz PO. Human mast cells express receptors for IL-3, IL-5 and GM-CSF: a partial map of receptors on human mast cells cultured in vitro. Allergy 2004;59:1087-96.
8. Kolbeck R, Kozhich A, Koike M, et al. MEDI-563, a humanized anti-IL-5 receptor alpha mAb with enhanced antibody-dependent cell-mediated cytotoxicity function. J Allergy Clin Immunol 2010;125(6):1344-1353.e2.
9. Bischoff SC, Brunner T, De Weck AL, Dahinden CA. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. J Exp Med 1990;172:1577-82.
10. Wilson TM, Maric I, Shukla J, et al. IL-5 receptor α levels in patients with marked eosinophilia or mastocytosis. J Allergy Clin Immunol 2011;128(5):1086-92.e1-3.
11. Tavernier J, Devos R, Cornelis S, et al. A human high affinity interleukin-5 receptor (IL5R) is composed of an IL5-specific alpha chain and a beta chain shared with the receptor for GM-CSF. Cell 1991;66:1175-84.
12. Laviolette M, Gossage DL, Gauvreau G, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. J Allergy Clin Immunol 2013;132(5):1086-1096.e5.
13. Busse WW, Katial R, Gossage D, et al. Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor alpha antibody, in a phase I study of subjects with mild asthma. J Allergy Clin Immunol 2010;125(6):1237-1244.e2.
14. FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2016;388:2128-41.
15. Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β_2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet 2016;388:2115-27.
16. Wang B, Yan L, Yao Z, Roskos LK. Population pharmacokinetics and pharmacodynamics of benralizumab in healthy volunteers and patients with asthma. CPT Pharmacometrics Syst Pharmacol 2017;6:249-57.
17. Nair P, Wenzel S, Rabe KF, et al. Oral glucocorticoid–sparing effect of benralizumab in severe asthma. N Engl J Med 2017;376:2448-58.
18. Ferguson GT, FitzGerald JM, Bleecker ER, et al. Benralizumab for patients with mild to moderate, persistent asthma (BISE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Respir Med 2017;5:568-76.
19. Roufosse F, de Lavareille A, Schandené L, et al. Mepolizumab as a corticosteroid-sparing agent in lymphocytic variant hypereosinophilic syndrome. J Allergy Clin Immunol 2010;126(4):828-835.e3.
20. Kuang FL, Fay MP, Ware J, et al. Long-term clinical outcomes of high-dose mepolizumab treatment for hypereosinophilic syndrome. J Allergy Clin Immunol Pract 2018;6(5):1518-1527.e5.
21. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2010;125:Suppl 2:S73-S80.
22. Weiner GJ. Rituximab: mechanism of action. Semin Hematol 2010;47:115-23.
23. Khoury P, Abiodun AO, Holland-Thomas N, Fay MP, Klion AD. Hypereosinophilic syndrome subtype predicts responsiveness to glucocorticoids. J Allergy Clin Immunol Pract 2018;6:190-5.