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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Postbronchodilator lung function improvements with benralizumab for patients with severe asthma

To the Editor,

Airway remodeling and loss of lung function are prominent features of asthma.[1] Activated eosinophils release an array of inflammatory cytokines and lipid mediators that play a role in airway remodeling.[2] Imaging studies using quantitative computed tomography have demonstrated correlations between parameters of airway remodeling and postbronchodilator forced expiratory volume in 1 second (FEV_1) as well as changes over time.[3,4] Therefore, we sought to determine whether a reduction in eosinophils could affect airway remodeling by examining postbronchodilator FEV_1.

SIROCCO and CALIMA phase III trials demonstrated that benralizumab, an interleukin (IL)-5 receptor alpha–directed cytolytic monoclonal antibody that depletes eosinophils via enhanced antibody-dependent cell-mediated cytotoxicity,[5] reduces exacerbations, lessens asthma symptoms, and augments prebronchodilator lung function for patients with severe, eosinophilic asthma.[6,7] Post hoc analysis of pooled SIROCCO and CALIMA data identified subgroups of patients with long-term oral corticosteroid (OCS) use, nasal polyposis (based on medical history), prebronchodilator forced vital capacity (FVC) <65% predicted at baseline, ≥3 asthma exacerbations in the year prior to enrollment, or age at diagnosis ≥18 years with demonstrated enhanced clinical response to benralizumab.[8,9]

In this analysis, we examined whether subcutaneous benralizumab 30 mg every 8 weeks (first three doses every 4 weeks) also improved postbronchodilator lung function for these same enhanced responder subgroups, providing further evidence that benralizumab may alter inflammation-induced airway changes. Methods, statistics (including confidence intervals for reported results), and study limitations are provided in the Supplement. All *P*-values are nominal, with $P < .05$ considered nominally significant. The demographics and baseline clinical characteristics were similar between benralizumab and placebo arms (Table S1).[8]

At the end of treatment (EOT), postbronchodilator FEV_1 improvements from baseline were greater with benralizumab (n = 693) than placebo (n = 717) for all patients (least squares [LS] mean difference vs placebo 0.09 L, $P = .0001$) and for each enhanced responder subgroup (Figures 1 and S1, Tables S2 and S3). The greatest differences vs placebo were for patients with nasal polyps (0.33 L, $P < .0001$), long-term OCS use (0.26 L, $P = .0001$), and baseline FVC < 65% predicted (0.23 L, $P = .0001$).

Improvements in postbronchodilator FEV_1 were similar for patients with baseline blood eosinophil counts ≥300 and <300 cells/μL, with numerically greater changes for those with ≥300 cells/μL (Figure 2, Table S3). Of patients with baseline blood eosinophil counts <300 cells/μL, the greatest increases in postbronchodilator FEV_1 with benralizumab vs placebo were for those with nasal polyps (LS mean difference vs placebo 0.34 L, $P = .0031$) and those with ≥3 exacerbations in the previous year (LS mean difference vs placebo 0.14 L, $P = .0219$).

Greater improvements in postbronchodilator FVC and forced expiratory flow 25%-75% predicted (FEF_{25-75}) from baseline to EOT were observed with benralizumab compared with placebo (Tables S4 and S5). For all patients, mean postbronchodilator FVC improved

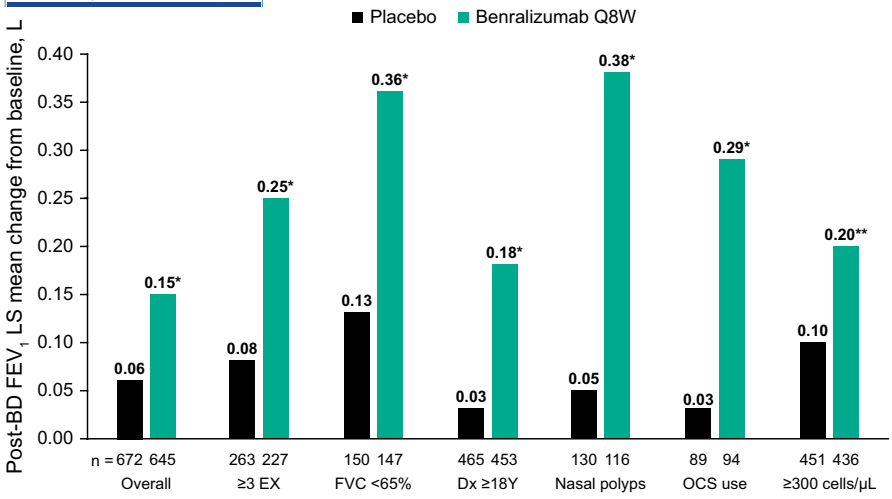

FIGURE 1 Postbronchodilator FEV_1 Improvements with Benralizumab for Subgroups (Full Analysis Set). ≥300 cells/µL, blood eosinophil counts ≥300 cells/µL; BD, bronchodilator; Dx, age at diagnosis; EX, exacerbations; FEV_1, forced expiratory volume in 1 second; FVC <65%, prebronchodilator forced vital capacity <65% predicted; LS, least squares; OCS, oral corticosteroids; Q8W, every 8 weeks; Y, years. Estimates calculated via a mixed-effects model for repeated measures analysis with adjustment for study code, treatment, baseline value, region, OCS use at the time of randomization (except for OCS use subgroup), visit, and visit × treatment. *Nominal $P \leq .0001$ benralizumab vs placebo. **Nominal $P = .0008$ benralizumab vs placebo

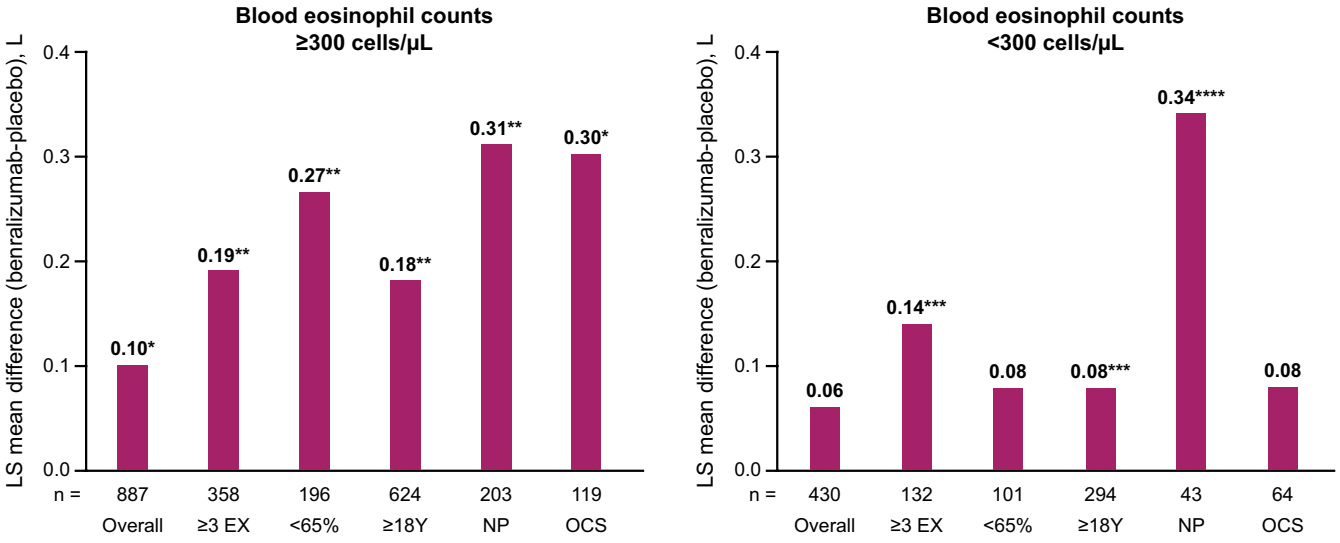

FIGURE 2 Postbronchodilator FEV_1 Differences Between Benralizumab and Placebo at EOT for Patients with Blood Eosinophil Counts ≥300 and <300 cells/µL (Full Analysis Set). ≥18Y, age at diagnosis ≥18 years; <65%, prebronchodilator forced vital capacity <65% predicted; EOT, end of treatment; EX, exacerbations; FEV_1, forced expiratory volume in 1 second; LS, least squares; NP, nasal polyps; OCS, oral corticosteroids. n values are the total numbers of patients with EOT data. *Nominal $P < .001$ benralizumab vs placebo. **Nominal $P < .0001$ benralizumab vs placebo. ***Nominal $P < .05$ benralizumab vs placebo. ****Nominal $P < .01$ benralizumab vs placebo

by 0.06 L ($P = .0084$) vs placebo at EOT. Patients with nasal polyposis again had the greatest improvement (0.30 L, $P < .0001$) (Table S4). Of patients with baseline blood eosinophil counts ≥300 or <300 cells/µL, those with nasal polyps had the greatest improvements from baseline to EOT in postbronchodilator FVC with benralizumab vs placebo (Table S4). Postbronchodilator FEF_{25-75} improved from baseline to EOT with benralizumab vs placebo for all patients by 0.11 L/s ($P = .0016$). Treatment effects for patients with baseline OCS use, nasal polyps, or baseline FVC <65% predicted were more than double those of the unselected, total population (Table S5). For patients with blood eosinophil counts ≥300 cells/µL, baseline OCS use was associated with the greatest improvement in postbronchodilator FEF_{25-75}. Patients with baseline nasal polyps had the greatest response of those with blood eosinophil counts <300 cells/µL.

Bronchodilator responses, measured after up to 8 puffs of albuterol, were similar between patients receiving placebo and benralizumab at EOT relative to baseline, regardless of baseline clinical characteristics, including blood eosinophil counts (Figure S2 and Table S2). For the overall population of benralizumab-treated patients, mean reversibility changed from 0.41 L (standard deviation

[SD] = 0.36) at baseline to 0.25 L (SD = 0.24) at EOT. For placebo, mean reversibility changed from 0.41 L (SD = 0.36) to 0.26 L (SD = 0.25) at EOT. Thus, the postbronchodilator lung function changes are more likely to result from airway structural changes rather than changes in airway hyperreactivity.

By evaluating postbronchodilator lung function in pooled SIROCCO and CALIMA data, this analysis provides further evidence that benralizumab improves lung function and perhaps modulates airway remodeling pathways related to chronic eosinophil-driven inflammation.[1,2] The presence of nasal polyposis, maintenance OCS use, and baseline FVC <65% predicted were all associated with enhanced benralizumab response and may provide important clues to the pathogenetic effects of uncontrolled eosinophilic inflammation.[2]

Blood eosinophil counts are a practical but indirect and imperfect indicator of airway eosinophilia. In our analysis, patients with nasal polyposis and lesser blood eosinophil counts had numerically greater improvements in postbronchodilator FEV_1 compared with patients with nasal polyposis and greater blood eosinophil counts from baseline to EOT with benralizumab vs placebo. Nasal polyposis is likely a marker for IL-5 and eosinophil-driven inflammation.[2] Thus, nasal polyps may be an important surrogate marker for uncontrolled eosinophilic inflammation in the lower airways or other IL-5-dependent pathways for patients with asthma.

Patients receiving maintenance OCS also had an enhanced postbronchodilator FEV_1 benralizumab response. Similar to the finding with nasal polyps, this suggested that eosinophilic inflammation or other IL-5-dependent pathways persisted in the airways of some patients while OCS use suppressed peripheral eosinophilia. This may be particularly true for patients with declined lung function, because baseline FVC <65% predicted was one of the three strongest clinical characteristics associated with enhanced benralizumab response. Although these findings should be confirmed with a larger population over a longer observation period, they suggest that some patients with asthma have occult eosinophilic inflammation, which may not be detected with blood eosinophil counts alone as the surrogate marker and may result in loss of lung function.

In summary, we demonstrated that benralizumab improves postbronchodilator lung function for patients with eosinophilic asthma. Elevated blood eosinophil counts, nasal polyposis, decreased lung function, and long-term OCS use were all potential indicators of an enhanced response. Benralizumab may improve structural changes associated with eosinophil-driven, chronic airway inflammation, which are not always detected with blood eosinophil counts alone.

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CONFLICT OF INTEREST

Sameer K. Mathur is a consultant for and received honorarium from AstraZeneca and received research funds for his institution from Novartis. Brian D. Modena has received consulting fees from AstraZeneca. Hanneke Coumou has nothing to declare. Peter Barker, James L. Kreindler, and James G. Zangrilli are employees of AstraZeneca.

DATA AVAILABILITY STATEMENT

Data underlying the findings described in this manuscript may be requested in accordance with AstraZeneca's data-sharing policy described at https://astrazenecagroup-dt.pharmacm.com/DT/Home.

Sameer K. Mathur[1]
Brian D. Modena[2]
Hanneke Coumou[3]
Peter Barker[4]
James L. Kreindler[5]
James G. Zangrilli[6]

[1]*Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA*
[2]*Department of Medicine, National Jewish Health, Denver, CO, USA*
[3]*Department of Pulmonary Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands*
[4]*Research and Development, AstraZeneca, Gaithersburg, MD, USA*
[5]*Respiratory Biologics, AstraZeneca, Wilmington, DE, USA*
[6]*Global Medical Affairs, AstraZeneca, Gaithersburg, MD, USA*

Correspondence
Sameer K. Mathur, Division of Allergy, Pulmonary and Critical Care, Department of Medicine, University of Wisconsin School of Medicine and Public Health, H4/618 Clinical Sciences Center, 600 Highland Avenue, Madison, WI 53792, USA.
Email: skmathur@wisc.edu

ORCID

Sameer K. Mathur https://orcid.org/0000-0001-7656-0256
Brian D. Modena https://orcid.org/0000-0002-4406-8336

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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A new role for “eat me” and “don’t eat me” markers on neutrophils in asthmatic airway inflammation

To the Editor,

The scientific evidence for a role for neutrophils in exacerbation of acute asthma is scarce, but even less is known about their function for the resolution of inflammation in asthmatic airways. Normally, an acute inflammation is initiated within minutes of recognition of a danger signal. The inflammatory process is then generally self-limited and resolved within hours or days. However, in several conditions, including asthma, the inflammation persists. Hence, the naturally protective inflammatory response has become unrestrained threatening to cause prolonged sickness and maybe over time chronic changes to airway structure and function[1].

The inflammatory activity of neutrophils must, like other parts of the inflammatory process, be regulated with precision. This process often starts by induction of apoptosis followed by monocyte-derived macrophage phagocytosis[2]. Monocytes are attracted to the site of resolution through neutrophil production CC chemokines like CCL3/MIP-1α, CCL4/MIP-1β and CCL20/MIP-3A[2]. These chemokines are important for the resolution of a pulmonary inflammation, and a disturbed regulation can increase the susceptibility to infections[3]. The neutrophils also seem to have an immunomodulatory function on the monocytes, creating an anti-inflammatory milieu by cell-to-cell contact[4]. Apoptosis is a well-recognized way of clearing dead cells from the airway tissues. Recent results indicate that also living cells can be the target of phagocytes, often referred to as phagoptosis. Phagoptosis is regulated by exposure of “eat me,” CD43/CD36, and “don’t eat me,”, CD47, signals[5]. So far, next to nothing is known about “eat me” and "don't eat me" signals on neutrophils. However, it is clear that prolonged survival of airway neutrophils is directly related to the asthma severity and that eat me signals generally is a key step in the "appetite" control of macrophage phagoptosis of various cells. The present study focuses on the role of the neutrophil in the resolution of allergic airway inflammation by comparing the differences in markers for phagoptosis on neutrophils from patients with allergic asthma and non-allergic healthy controls.

The expression pattern of "eat me" and "don't eat me" markers on neutrophils at steady state and 60, 90 and 120 minutes after *in vitro* stimulation with LPS and TNFα was analysed with FACS (Figure S1). Neutrophils from the allergic donor blood showed a lower expression of the “eat me” markers CD43 and CD36 and a trend of a higher expression of the “don’t eat me” marker CD47 than neutrophils from the healthy donors. The CD47 expression on the neutrophils from asthmatic donors increased upon *in vitro* stimulation. No such up-regulation was seen in healthy donors, but the CD43 markers decreased (Figure 1A-C). Further, neutrophils from asthmatic donors were found to produce less mRNA for CCL4 and CCL20 than healthy donors after stimulation with LPS and TNFα. There was also a tendency of less CCL3 in asthmatic patients, most pronounced after 120 min (Figure 2A-C) after stimulation with LPS and TNFα. The corresponding protein levels of CCL3 (Figure 2D) was lower in the supernatants from LPS- and TNFα-stimulated neutrophils asthmatic patients compared to the healthy individuals .CCL4 and CCL20 exhibited a tendency towards lower protein levels (Figure 2E,F). The interaction between neutrophils and monocytes was investigated using a monocyte migration assay. Cell-free supernatants from stimulated neutrophils from the control group attracted more monocytes than the supernatants from the asthmatic group. Further, the number of migrating monocytes increased between two and four hours for the healthy group, and this was not seen in the asthmatic group (Figure 2G). There were no differences between neutrophils from healthy and allergic volunteers considering apoptotic (3-10 %) or alive cells (90-97 %) after LPS and TNFα stimulation (data not shown). TLRs are known to recognize microbial components, and