Eosinophil depletion with benralizumab is associated with attenuated mannitol airway hyperresponsiveness in severe uncontrolled eosinophilic asthma

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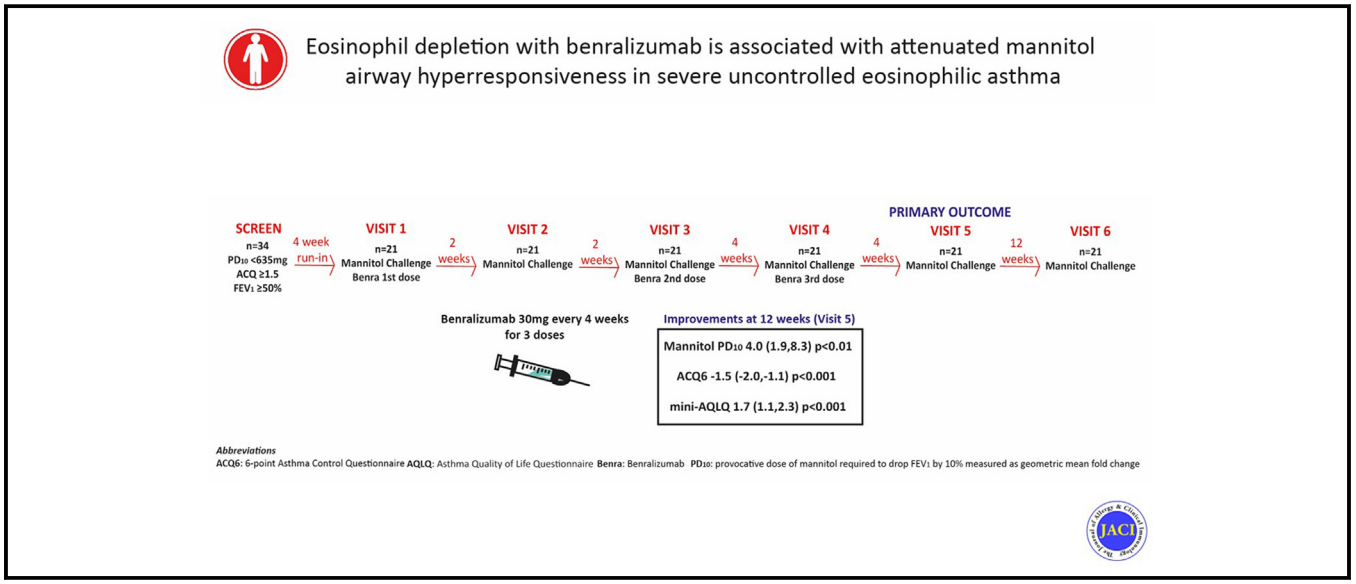
GRAPHICAL ABSTRACT



Background: Airway hyperresponsiveness (AHR) and eosinophilia are hallmarks of persistent asthma.

Objective: We investigated whether eosinophil depletion with benralizumab might attenuate indirect mannitol AHR in severe uncontrolled asthma using a pragmatic open-label design.

Methods: After a 4-week run-in period with provision of usual inhaled corticosteroids and/or long-acting β-agonist (baseline), adults with mannitol-responsive uncontrolled severe eosinophilic asthma received 3 doses of open-label benralizumab 30 mg every 4 weeks, followed by 16 weeks' washout after the last dose. The primary outcome was doubling difference (DD) in provocative dose of mannitol required to decrease FEV_1 by 10% (PD_{10}) at the end point after 12 weeks, powered at 90% with 18 patients required to detect 1 DD. Secondary outcomes included measures assessed by the asthma control questionnaire and mini-asthma quality of life questionnaire.

Results: Twenty-one patients completed 12 weeks' benralizumab therapy at the end point at week 12. Mean (SEM) age was 53 (4) years, and FEV_1 80.2% (4.1%) inhaled corticosteroid dose was 1895 (59) μg, with 12 receiving long-acting muscarinic antagonist and 13 leukotriene receptor

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antagonists. Improvement in AHR was significant by 8 weeks, with a mean 2.1 DD (95% confidence interval 1.0, 3.3; $P < .01$) change in PD_{10} at week 12, while mean changes in asthma control questionnaire and mini-asthma quality of life questionnaire were significant by week 2 and sustained over 12 weeks, both exceeding the minimal important difference. Peripheral blood eosinophils were depleted by 2 weeks (439 to 6 cells/μL). No significant improvement occurred in lung function after 12 weeks. Domiciliary peak flow and symptoms also improved with benralizumab.

Conclusion: Eosinophil depletion results in clinically meaningful attenuated AHR in severe uncontrolled asthma patients. (J Allergy Clin Immunol 2022;■■■:■■■-■■■.)

Key words: *Airway hyperresponsiveness, benralizumab, mannitol, severe asthma, asthma control, quality of life*

Abbreviations used

ACQ: Asthma control questionnaire
AHR: Airway hyperresponsiveness
CI: Confidence interval
DD: Doubling difference
EDN: Eosinophil-derived neurotoxin
FENO: Fractional exhaled nitric oxide
ICS: Inhaled corticosteroids
MCID: Minimal clinically important difference
Mini-AQLQ: Mini-Asthma Quality of Life Questionnaire
PD_{10}: Provocative dose of mannitol required for 10% fall in FEV_1
PEF: Peak expiratory flow
RDR: Response dose ratio
SEA: Severe eosinophilic asthma

Together with type 2 eosinophilic airway inflammation and variable airflow obstruction, airway hyperresponsiveness (AHR) plays a key role in the pathophysiology of severe asthma.[1] Indirect airway challenges such as mannitol measure AHR by promoting the release of endogenous mediators from airway inflammatory cells, resulting in bronchoconstriction.[2,3] As opposed to direct challenge, such as methacholine or histamine acting on airway smooth muscle, indirect AHR is more closely associated with airway inflammation in patients with persistent asthma.[4] Indirect bronchial challenge test with mannitol identifies asthma with a high degree of specificity.[5]

Therapy with biologics has revolutionized the management of severe asthma, especially in relation to improvements in severe exacerbations, asthma control, and quality of life.[6] However, there remains a paucity of data regarding the effect of biologics on AHR. One study determined that IL-13 and IL-4, but not IL-5, induce histamine-, carbachol-, and leukotriene D_4–mediated AHR in isolated human small airways.[7] However, another *ex vivo* study on passively sensitized human airways showed that the anti–IL-5Rα monoclonal antibody benralizumab was significantly more potent than anti–IL-5 mepolizumab in attenuating direct AHR to histamine *ex vivo*.[8] Regardless, the effect of benralizumab on mannitol AHR in patients with severe eosinophilic asthma (SEA) is currently unknown.

We therefore used a pragmatic clinical trial design to investigate if eosinophil depletion due to benralizumab might attenuate indirect AHR with mannitol challenge as the primary outcome, with key secondary outcomes including asthma control and quality of life. In addition, we wished to see if such effects are maintained at 16 weeks after stopping benralizumab.

METHODS

Benralizumab in severe asthma, or BISA, was a single-arm open-label phase 4 proof-of-concept clinical trial (EudraCT 2019-003763-22) that was conducted in the Scottish Centre for Respiratory Research between December 2020 and May 2022, primarily screening patients with uncontrolled SEA. Eligible patients were those with an asthma control questionnaire (ACQ) score of ≥1.5, blood eosinophils ≥300 cells/μL, or the presence of chronic rhinosinusitis with nasal polyps or fixed airway obstruction and eosinophils ≥150 cells/μL, FEV_1 ≥50%, and receipt of inhaled corticosteroids (ICS)/long-acting β-agonist at a dose of ≥1000 μg beclomethasone dipropionate equivalence. All patients screened in this study had a secondary care respiratory physician diagnosis of severe asthma according to Global Initiative for Asthma criteria. Furthermore, those eligible for the study (n = 21) had their diagnosis verified by a positive mannitol bronchial challenge test,[9] signified by the provocative dose required to decrease FEV_1 by 10% less than 635 mg. Such patients entered into a 4-week run-in period where they received standard-of-care therapy (baseline) and subsequently received 3 doses of subcutaneous benralizumab 30 mg every 4 weeks in addition to standard of care over a 12-week treatment period followed by a washout period where no benralizumab was provided for 16 weeks after the last dose (see Fig E3 in this article's Online Repository at www.jacionline.org).

The primary outcome was the doubling difference (DD; log_2 transformed) in the provocative dose of mannitol required to decrease FEV_1 by 10% (PD_{10}) after 12 weeks of benralizumab therapy. Although PD_{15} is usually used in clinical practice,[5] we elected to use the previously validated PD_{10} threshold[3,10,11] as a result of ethical concerns raised about the potential for severe bronchoconstriction in patients with uncontrolled severe asthma. Bronchial challenges were performed using mannitol dry powder (Aridol, Pharmaxis, Sydney, Australia) as previously described.[3,10]

Key secondary outcomes included ACQ and mini-asthma quality of life questionnaire (mini-AQLQ). Other secondary outcomes of interest included peripheral blood eosinophils, eosinophil-derived neurotoxin (EDN), fractional exhaled nitric oxide (FENO), and spirometry and oscillometry. FENO was measured using NIOX VERO (Circassia, Oxford, United Kingdom) according to the manufacturer's instructions and American Thoracic Society guidelines.[12] Spirometry (Micromedical, Chatham, United Kingdom) was performed according to European Respiratory Society/American Thoracic Society guidelines.[13] Oscillometry was measured using TremoFlo (Thorasys, Montreal, Quebec, Canada), with measurements performed in triplicate according to the European Respiratory Society technical standards; oscillometry was always performed before spirometry.[14] Accuracy of resistance measurements was confirmed on each day with a standard 0.2 kPa/L/s resistance mesh.

Statistical analysis was performed by SPSS v27 (IBM, Armonk, NY), and graphs were prepared by GraphPad Prism v6 (GraphPad Software, La Jolla, Calif). Data were assessed for outliers and for normality with normality plots and Shapiro-Wilks test before analysis. An initial overall repeated-measures analysis of variance was performed to evaluate any significant differences between the various time points. This was followed by Bonferroni-corrected pairwise comparisons for each time point versus baseline as well as a separate comparison for week 12 versus 24, with a 2-tailed alpha error set at 0.05. Values are presented as arithmetic means (95% confidence interval [CI]), except for PD_{10} and response dose ratio (RDR), which are provided as geometric means (95% CI). Ethical approval was obtained via the East of Scotland Research Ethics Service, and written informed consent was obtained from patients before data collection began.

RESULTS

Mean baseline demographic data are presented in Table E1 in the Online Repository at www.jacionline.org. Mean (95% CI)

TABLE I. Mean values for mannitol PD_{10}, RDR, spirometry, oscillometry, ACQ, mini-AQLQ, and type 2 biomarkers at weeks 0, 2, 4, 8, 12, and 24

Characteristic	Baseline (after run-in)	Week 2	Week 4	Week 8	Week 12 (after benralizumab)	Geometric mean fold difference (CI) at week 12	Week 24
Mannitol PD_{10} (mg)	67	142	157	185*	266**	4.0 (1.9, 8.3)	169*
Mannitol RDR (%/mg)	0.1418	0.0679	0.0630	0.0490*	0.0345**	0.2 (0.1, 0.5)	0.0435*
						Arithmetic mean difference (CI) at week 12	
FEV_1 (L)	2.37	2.56	2.50	2.48	2.49	0.12 (−0.15, 0.39)	2.58
FEF_{25-75} (L/s)	1.48	1.58	1.56	1.51	1.53	0.05 (−0.27, 0.37)	1.59
FVC (L)	3.62	3.83	3.78	3.79	3.78	0.16 (−0.05, 0.37)	3.89*
FEV_1/FVC	65.5	66.6	66.4	65.8	66.5	1.0 (−1.9, 3.9)	66.8
R5-R20 (kPa/L/s)	0.14	0.14	0.15	0.14	0.14	0.00 (−0.04, 0.04)	0.13
X5 (kPa/L/s)	−0.28	−0.24	−0.22	−0.25	−0.24	0.04 (−0.04, 0.12)	−0.23
AX (kPa/L)	2.77	2.42	2.34	2.49	2.30	−0.46 (−1.43, 0.50)	2.14
PBE (cells/μL)	439	6****			13****	−426 (−574, −277)	33***
EDN (ng/ml)	65.6	28.5****			15.3****	−50.3 (−62.2, −38.5)	19.9****
FENO (ppb)	51	53	50	65	59	8 (−11, 28)	51
ACQ-6	2.6	1.8*	1.5***	1.3****	1.1****	−1.5 (−2.0, −1.1)	1.5**†
Mini-AQLQ	3.6	4.6***	5.0***	5.2****	5.3****	1.7 (1.1, 2.3)	4.8*

Values are shown at 2 and 4 weeks (week 2, 4) after the first dose, 4 weeks after the second (week 8) and third doses (week 12), and 16 weeks after the third dose (week 24). *AX,* Area under reactance curve; *FEF_{25-75},* forced expiratory flow rate between 25% and 75% of FVC; *PBE,* peripheral blood eosinophil; *R5-R20,* difference in resistance between 5 and 20 Hz.

Bonferroni-corrected *P* values versus baseline: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

†*P* value versus week 12 ($P < .05$).

baseline bronchodilator reversibility was 270 mL (179, 361) and 11.4% (8.3, 14.4) for FEV_1, whereas the postbronchodilator FEV_1/FVC ratio was 0.69 (0.64, 0.73). There were no significant differences comparing pre- and post–run-in (baseline) values for any outcomes (see Table E2 in the Online Repository), although FENO fell nonsignificantly by 8 ppb, perhaps as a result of improved ICS adherence during this period. However, mannitol challenge was only performed after the run-in.

In total, 132 mannitol bronchial challenges were performed during the trial. The geometric mean baseline PD_{10} was 67 (95% CI 34, 135). Significant changes in mannitol PD_{10} as the geometric mean fold difference occurred after 8 weeks (Table I). After week 12 at the primary end point, a mean 2.1 (95% CI 1.0, 3.3) DD in PD_{10} (Bonferroni $P < .01$) and 2.0 (95% CI 0.9, 3.1) DD in RDR (Bonferroni $P < .01$) were observed (Fig 1). Twelve of 21 patients experienced ≥1.0 DD in PD_{10} and RDR (see Fig E1 in the Online Repository at www.jacionline.org) at week 12. The disease of 5 patients subsequently became unresponsive to mannitol at 12 weeks.

Significant improvements in ACQ-6 and mini-AQLQ scores were demonstrated by week 2 and were sustained over 12 weeks (Table I). Notably, response analysis showed 18 of 21 and 17 of 21 participants experienced a ≥0.5-unit improvement in ACQ and mini-AQLQ, respectively, at week 12, exceeding the minimal clinically important difference (MCID) (Fig 2). After 12 weeks, 5 patients had an ACQ score of <0.75, indicating good disease control, whereas 13 patients had intermediate disease control, as evidenced by an ACQ score of ≥0.75 to 1.5. All 12 patients with disease that responded to mannitol at 12 weeks also had improvements in ACQ and mini-AQLQ exceeding the MCID. For the 9 patients with disease that did not respond to mannitol at 12 weeks (Fig E1), when represented as a change in PD_{10} within

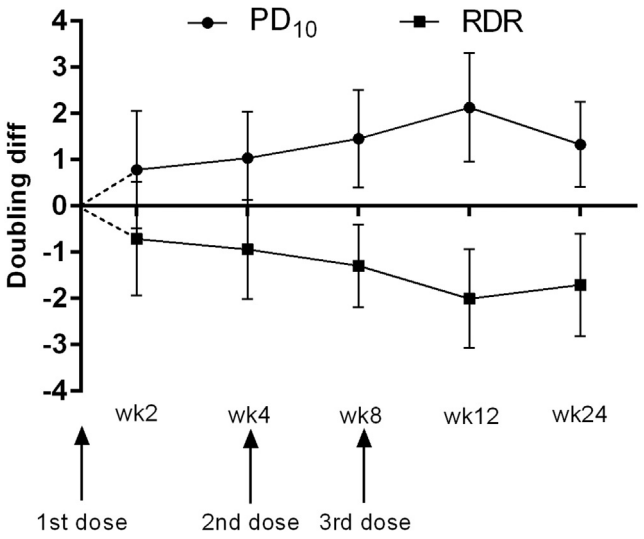

FIG 1. Mean (95% CI) DD for PD_{10} and RDR at serial time points after benralizumab therapy.

the biological variability of ±1.0 DD, 9 and 6, respectively, still experienced a ≥0.5-unit improvement in their ACQ and mini-AQLQ scores. The mean improvements in ACQ and mini-AQLQ scores amounted to 1.6 and 1.7 for mannitol nonresponse, which were comparable to respective improvements of 1.4 and 1.7 for mannitol response.

Mean individual components of the mini-AQLQ response are shown in Table E3 in the Online Repository at www.jacionline.org. This demonstrated significant improvement in all domains after 2 weeks that were sustained over 12 weeks except for activity. Table E4 in the Online Repository presents a

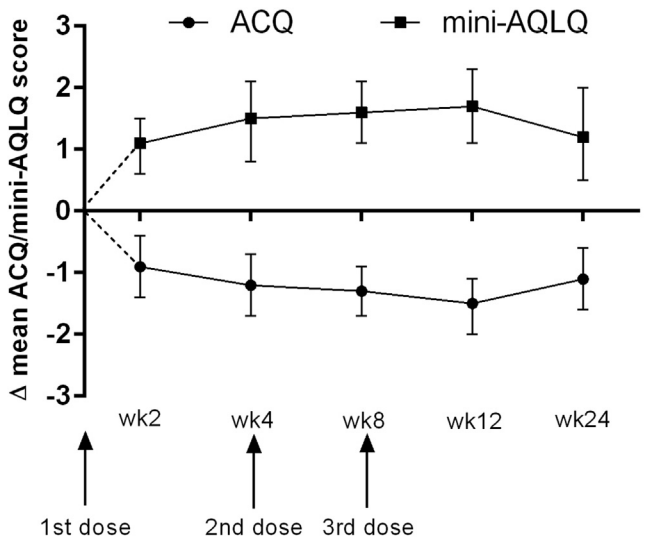

FIG 2. Mean (95% CI) improvements in ACQ and mini-AQLQ scores at various time points after benralizumab therapy. Measures at weeks 12 and 24 were performed 4 and 16 weeks after the third and final dose.

comparison in baseline demographics, asthma control, type 2 biomarkers, and FEV_1 in patients whose mannitol PD_{10} response exceeded 1.0 DD after 12 weeks of benralizumab versus those whose mannitol PD_{10} response did not.

No significant changes in spirometry or oscillometry results were observed after 12 weeks (Table I). Peripheral blood eosinophils were significantly depleted by week 2 and sustained over 12 weeks, whereas numerical increases in F_{ENO} over 12 weeks were not significant (Table I). Serum EDN levels significantly fell from baseline to weeks 2 (57% decrease) and 12 (77% decrease) (see Figs E4 and E5 in the Online Repository at www.jacionline.org).

Peak expiratory flow (PEF), symptoms, and relief via salbutamol therapy from patient diary cards all significantly improved after 3 doses of benralizumab—that is, week 12 versus baseline (Table II). Notably, the lower CI for the change in PEF at week 12 compared to baseline exceeded the MCID of 19L/min (see Fig E2 in the Online Repository at www.jacionline.org).

After the 12-week washout period, there was a numerical nonsignificant trend toward worsening of mannitol PD_{10} when comparing values between 4 months and 1 month after the last dose of benralizumab—that is, week 24 versus week 12, amounting to a mean −0.7 DD (95% CI −1.6, 0.3) difference. For the same time point comparison, a significant worsening in ACQ and a worsening in mini-AQLQ equaling MCID was observed (Table I) despite there being no differences in peripheral blood eosinophils. However, values for ACQ and mini-AQLQ remained significantly better when comparing week 24 to baseline. No differences in spirometry or oscillometry measurements were detected between weeks 12 and 24, while changes in PEF persisted 4 months after the final dose was provided at week 24.

DISCUSSION

In this pragmatic study, we found that eosinophil depletion due to benralizumab was associated with clinically relevant attenuation of indirect AHR at the end point after 12 weeks in patients with uncontrolled SEA. This was accompanied by significant improvements in asthma control and quality of life. Notably, at 12 weeks, the lower 95% CI for DD shift in mannitol PD_{10} was 1.0 compared to biological variability of ±1.0 DD. As a reference, the within-subject biological variabilities in the placebo arm for mannitol AHR in the CASCADE[15] and UPSTREAM[16] studies were 0.57 and 1.0 DD, respectively. Therefore, we opted to use 1.0 DD to represent the minimal change that must be exceeded for a clinically relevant treatment effect. The lower 95% CIs for ACQ exceeded the MCID of 0.5 at weeks 4, 8, and 12. Indeed, by 12 weeks, the lower 95% CI exceeded twice the MCID at >1.0. These findings are especially clinically relevant because it has previously been determined that each 1.0-unit increase in ACQ is associated with an approximately 50% increased risk of asthma exacerbation.[17] For mini-AQLQ scores, the lower 95% CIs exceeded 0.5 at 2, 4, 8, and 12 weeks (Fig 2).

We observed significantly improved PEF at week 12 versus baseline, with the lower CI for the change exceeding the MCID of 19 L/min.[18] One likely explanation for the apparent disconnect between improvements in PEF but not FEV_1 is that serial values for PEF were averaged during the 7 days before the particular study visit. Thus, serial measures of peak flow are always more likely to detect subtle changes in airway caliber, as opposed to spot laboratory measures using spirometry.

The putative mechanism for benralizumab on indirect mannitol challenge is likely to be mediated via intraepithelial eosinophils in attenuating endogenous AHR in asthma, at least in part by leukotriene D_4 release from depleted eosinophils.[19,20] Mast cells also play a key role in indirect AHR via IL-33 signaling,[21] with early evidence suggesting that eosinophils may regulate mast cell function.[19] Previous studies with mepolizumab reported no effect on direct-acting challenge to either methacholine or histamine, suggesting that blocking IL-5 has no effect on airway smooth muscle.[22,23]

EDN is an eosinophil degranulation protein that better reflects asthma control compared to blood eosinophils.[24] Furthermore, EDN is associated with acute asthma exacerbations and AHR.[25] In this study, we demonstrated that although peripheral blood eosinophil counts neared complete depletion by week 2 after benralizumab, EDN levels progressively drifted downward from baseline to week 12, where there was 77% suppression (Table I and, in the Online Repository at www.jacionline.org, Fig E5). This incomplete suppression of EDN could potentially be explained by a persistent reservoir of airway eosinophils that are not entirely depleted from benralizumab therapy.[26]

We appreciate there are potential limitations associated with our study. First, it was not placebo controlled. Accepting a putative 1.0 DD change in AHR with placebo, we do not think that the 2.1 DD change in AHR with benralizumab represents regression to the mean over 12 weeks compared to baseline, especially when using rigorous Bonferroni-corrected *P* values. It is also perhaps worth mentioning that the 5 of 21 patients with non–mannitol-responsive disease at week 12 (ie, $PD_{10} \geq 635$ mg) had their PD_{10} values censored at the maximum dose (635 mg) for statistical analysis, therefore likely underestimating the true effect of benralizumab in attenuating AHR in such patients.

TABLE II. Mean PEF rate, patient subjective symptoms, and relief therapy in the 7 days before each respective visit

Characteristic	Baseline	Week 2	Week 4	Week 8	Week 12	Mean difference (95% CI) at week 12	Week 24
PEF (L/min)	357	388*	400**	398**	404**	48 (21, 74)	404*
Symptoms	1.7	1.3*	1.3**	1.1***	1.0****	−0.7 (−0.9, −0.5)	0.9***
Relief therapy	3	2*	2*	1**	1*	−2 (−3, −0)	2*

Bonferroni-corrected P values versus baseline: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

Procedures for this study were particularly difficult to execute because they coincided with the peak of the coronavirus disease 2019 pandemic, associated with stringent protective measures involving aerosol-generating procedures, especially early in the pandemic. We hope we might have mitigated the lack of a placebo-controlled arm by calculating biological variability values during the run-in period[27] that can be used as surrogates for the minimal change that must occur for a clinically significant treatment effect.

Furthermore, we acknowledge that it is increasingly difficult to justify randomizing patients with severe uncontrolled asthma, whom we were initially shielding, onto a placebo when there is abundant evidence for the efficacy of benralizumab. This would not be the case for evaluation of a novel biologic drug that cannot be accessed via the National Health Service. Our particular strategy was to inform patients before enrollment that they would be referred onto the severe asthma multidisciplinary team at the conclusion of the trial if there was evidence of a good response to benralizumab.

We included a washout period to assess if the effects of benralizumab might have started to wear off after a period of 4 months of the last dose. Despite no differences in peripheral blood eosinophils when comparing 4 and 1 months after the last dose of benralizumab (week 24 vs week 12), our patients experienced a small but significant worsening of both their ACQ and mini-AQLQ scores, while mannitol PD_{10} demonstrated a numerical nonsignificant worsening. Nonetheless, values for week 24 remained significantly better than baseline for mannitol PD_{10}, RDR, and ACQ and mini-AQLQ. We duly acknowledge that blood eosinophils may not necessarily reflect lung eosinophils even though persistent depletion in blood was observed at week 24.

We think that improvements in mannitol AHR were unlikely to be due to increased patient adherence to ICS, as one would otherwise expect FENO values to fall over 12 weeks.[28] Instead, FENO levels increased nonsignificantly by 8 ppb from baseline to week 12. Notably, we did not document ICS and/or long-acting β-agonist adherence in the present study. Finally, although the subanalysis in Table E4 is likely underpowered, we noted a significantly higher baseline EDN level in patients who experienced improvement in mannitol PD_{10} of <1.0 DD at 12 weeks, possibly warranting further investigation in future studies.

In conclusion, we have shown that eosinophil depletion with benralizumab attenuates indirect mannitol AHR while also improving domiciliary peak flow, asthma control, and quality of life in patients with severe uncontrolled eosinophilic asthma.

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Key messages

- **Previous studies have shown that eosinophil suppression with IL-5 does not improve direct AHR to histamine or methacholine.**
- **Eosinophil depletion with benralizumab, an anti–IL-5Rα monoclonal antibody, resulted in effects on indirect AHR that occurred in association with improvements in peak flow, disease control, and quality of life.**
- **Targeting blood eosinophils through the IL-5 pathway may play a key role in indirect AHR in SEA.**

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METHODS

As per aerosol-generating procedural guidance for coronavirus disease 2019, all bronchial challenges were performed after donning full personal protective equipment, including a fluid-resistant surgical gown, scrub cap, visor, FFP3 face mask, and gloves. Using the supplied dry powder inhaler device, patients serially inhaled doubling doses (0, 5, 10, 20, 40, 80, 160, 160, and 160 mg) of mannitol until a total cumulative of 635 mg was attained. FEV_1 was measured 60 seconds after each inhalation, with the highest value of two recorded. The test ended once a 10% fall in FEV_1 was observed or when the maximum dose of 635 mg had been provided. The PD_{10} could then be calculated using log-linear interpolation of the dose–response curve. Patients without PD_{10} after the full protocol (ie, $PD_{10} \geq 635$ mg) had their values censored at 635 mg for the purposes of statistical analysis. The within-subject biological variability of mannitol AHR is ± 1 DD shift such that values exceeding this shift from baseline in response to benralizumab were considered as being clinically relevant. Withholding times for asthmatic therapies before mannitol challenge were as follows: antihistamines, theophylline, and leukotriene receptor antagonists, 2 days; long-acting β-agonists and long-acting muscarinic antagonists, 1 day; and salbutamol or terbutaline, 6 hours. Patients were administered nebulized salbutamol 2.5 mg immediately after challenge to aid recovery.

Blood testing was performed for peripheral blood eosinophils and circulating levels of specific IgE antibodies with fluorescence enzyme-linked immunoassay (Phadia ImmunoCAP 250, Phadia, Uppsala, Sweden) to define common allergens including house dust mite, grass, cat, dog, and silver birch. Serum EDN levels were measured by commercially available ELISA kits (MBL Medical and Biological Laboratories, Nagoya, Japan) for human EDN. All samples were systematically diluted by 1:5 when needed, and assayed following the manufacturer's instructions. The assay range after dilution was 3.0 to 200 ng/mL, and the minimum detection limit was 0.62 ng/mL. Samples with an intra-assay coefficient of variation ≥15% were excluded from the analysis. Asthma control was determined using the 6-point ACQ,[E1] while quality of life was measured using the 15-point mini-AQLQ.[E2] Patients were supplied study diary cards to document their daily receipt of salbutamol for relief and at-home early-morning PEF readings using a Mini-Wright peak flow meter (Clement Clarke, Harlow, United Kingdom), noting the best-of-3 value. Patients were also asked to rate their early-morning asthma symptoms using a 4-point nominal scale: 0, none; 1, mild; 2, moderate; and 3, severe. Relief therapy, PEF, and symptoms from the previous week were averaged for analysis. The MCID for PEF is 19 L/min.[E3]

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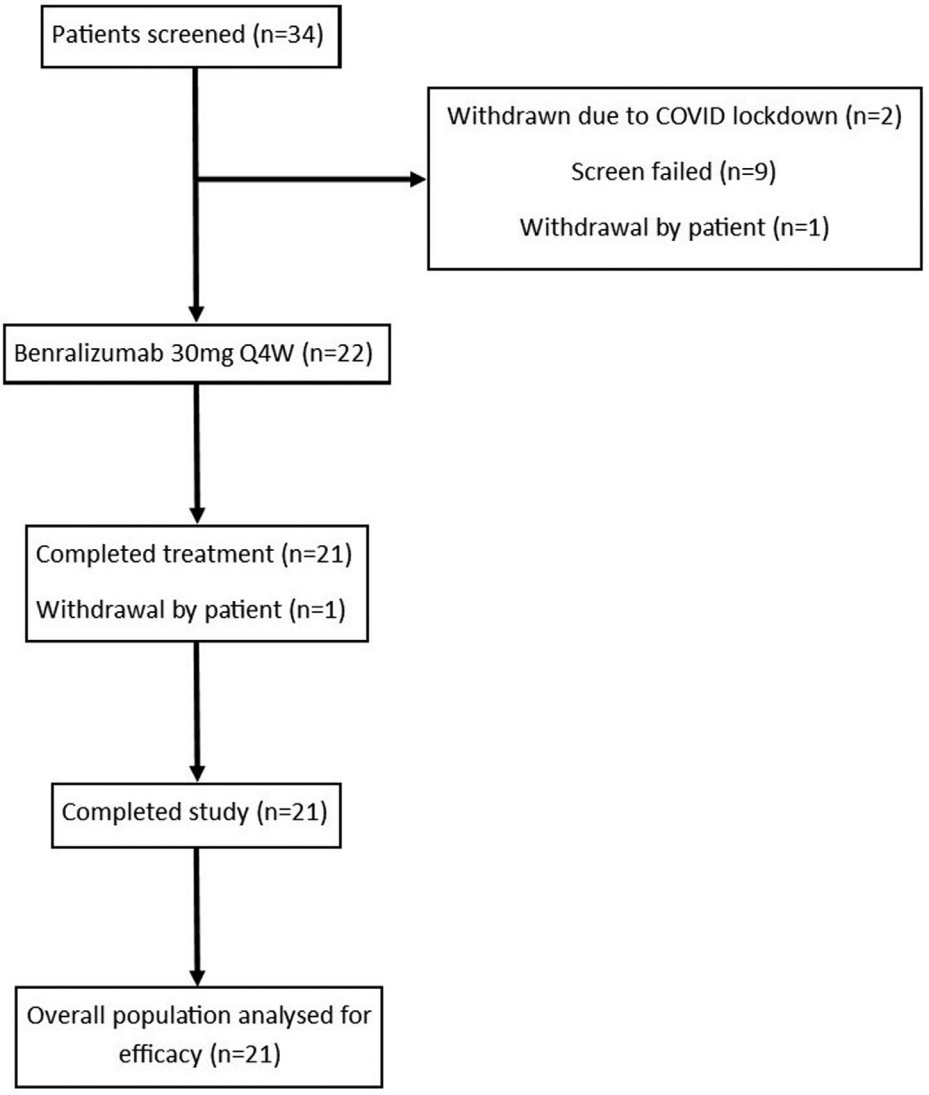

FIG E1. Patient disposition.

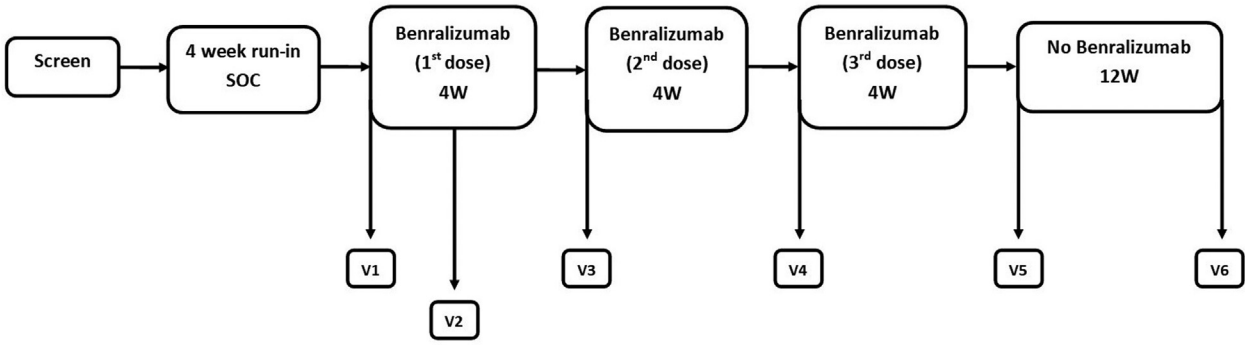

FIG E2. Study flowchart.

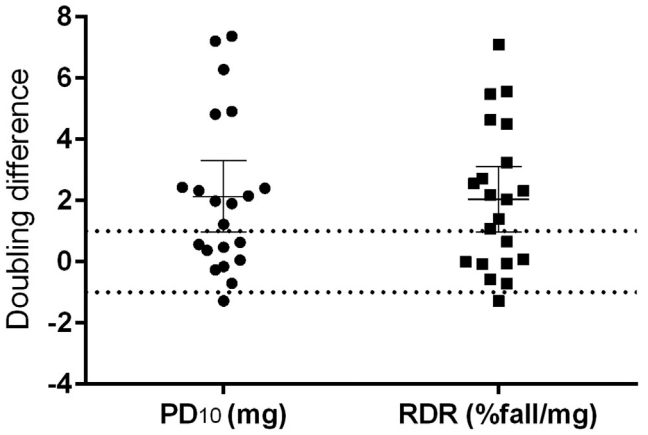

FIG E3. Individual DD for PD_{10} and RDR after 12 weeks of benralizumab therapy as change from baseline. Mean and 95% CI are superimposed. *Dotted lines* depict the within-subject biological variability of ±1 DD.

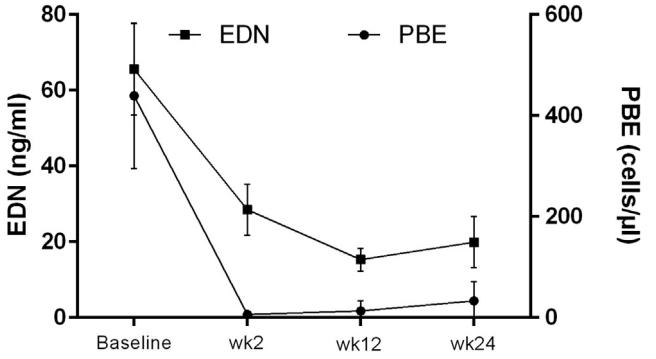

FIG E4. Suppression of peripheral blood eosinophils and EDN after benralizumab.

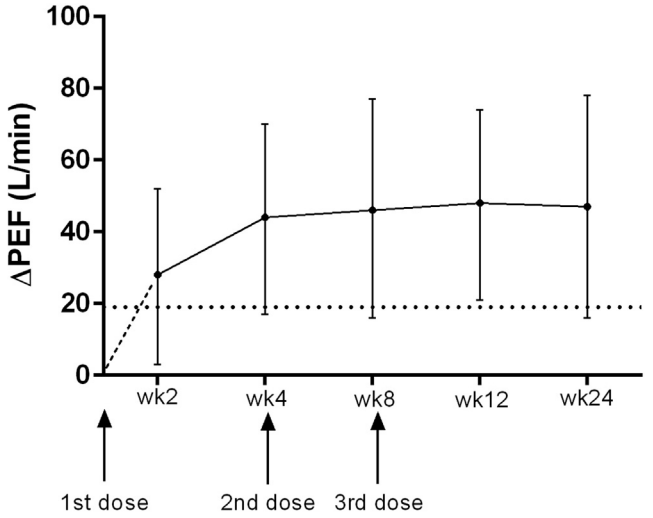

FIG E5. Absolute change in PEF compared to baseline as means and 95% CI. *Broken line* represents MCID for PEF of 19 L/min. Measures at weeks 12 and 24 were performed 4 and 16 weeks after receipt of the third and final dose.

TABLE E1. Baseline patient demographics

Characteristic	Value
Sex (F/M)	9/12
Age (years)	53 (4)
BMI (kg/m^2)	30 (1.2)
LABA (%)	95
LAMA (%)	57
LTRA (%)	62
THEO (%)	14
OAH (%)	67
CROMO (%)	5
INAH (%)	14
INS (%)	43
Ex-smoker (%)	38
Current smoker (%)	0
Nasal polyps (Y/N)	8/13
FEV_1 (%)	80.2 (4.1)
FEF_{25-75} (%)	41.5 (4.2)
FVC (%)	100.3 (3.9)
R5 (%)	161 (13)
ICS dose (μg)	1895 (59)
No. of positive-specific IgE	2
Total IgE (kU/L)	409 (180)
OCS exacerbations	4 (2)

Values presented as mean (SEM) except exacerbations, which are presented as median (IQR). *BMI,* Body mass index; *CROMO,* sodium cromoglicate; *FEF_{25-75},* forced expiratory flow rate between 25% and 75% of FVC; *ICS,* ICS–beclomethasone dipropionate equivalent dose; *INAH,* intranasal antihistamine; *INS,* intranasal steroid; *LAMA,* long-acting muscarinic antagonist; *LTRA,* leukotriene receptor antagonist; *OAH,* oral antihistamine; *OCS,* annual oral corticosteroid; *PBE,* peripheral blood eosinophil; *R5,* resistance at 5 Hz; *THEO,* theophylline.

TABLE E2. Changes before and after run-in (baseline)

Characteristic	Mean absolute change (95% CI)	Mean % change (95% CI)	Mean % CV (95% CI)	Biological variability
ACQ-6	−0.3 (−0.7, 0.0)	−11.3 (−23.9, 1.3)	17.9 (9.1, 26.7)	0.4
Mini-AQLQ	0.2 (−0.2, 0.7)	7.0 (−5.8, 19.8)	14.1 (8.5, 19.8)	0.4
FEV_1 (L)	0.003 (−0.098, 0.105)	0.1 (−4.1, 4.4)	4.9 (3.5, 6.3)	0.102
FEF_{25-75} (L/s)	0.110 (−0.074, 0.294)	8.0 (−5.4, 21.4)	13.6 (8.9, 18.3)	0.184
FVC (L)	−0.056 (−0.168, 0.056)	−1.5 (−4.6, 1.5)	4.2 (2.7, 5.7)	0.112
FEV_1/FVC ratio	0.86 (−1.3, 3.0)	−1.3 (−2.0, 4.6)	4.6 (3.4, 5.8)	2.1
R5 (kPa/L/s)	0.00 (−0.04, 0.04)	0.6 (−7.1, 8.2)	9.4 (6.5, 12.4)	0.04
R20 (kPa/L/s)	0.02 (−0.00, 0.05)	6.5 (−0.5, 13.4)	9.4 (7.3, 11.5)	0.03
R5-R20 (kPa/L/s)	−0.02 (−0.05, 0.01)	−12.2 (−31.0, 6.6)	35.4 (15.3, 55.5)	0.03
X5 (kPa/L/s)	−0.01 (−0.05, 0.03)	−4.5 (−18.6, 9.7)	17.1 (8.5, 25.6)	0.04
AX (kPa/L)	−0.02 (−0.51, 0.48)	−0.7 (−18.4, 17.1)	26.9 (14.5, 39.3)	0.50
Fres (Hz)	−0.21 (−2.84, 2.42)	−0.9 (−11.7, 10.0)	11.7 (5.3, 18.2)	2.62
PBE (cells/μL)	−19 (−115, 77)	−4.2 (−25.1, 16.8)	21.2 (13.1, 29.2)	96
FENO (ppb)	−7 (−15, 1)	−12.0 (−25.8, 1.8)	21.0 (14.3, 27.7)	8

Mannitol PD_{10} was only performed after run-in at baseline. The biological variability value was calculated as 1-sided 97.5% CI of the mean absolute change. *AX,* Area under reactance curve; *CV,* coefficient of variation; *FEF_{25-75},* forced expiratory flow rate between 25% and 75% of FVC; *Fres*, resonant frequency; *PBE,* peripheral blood eosinophil; *R5/20,* resistance at 5/20 Hz; *R5-R20,* difference in resistance between 5 and 20 Hz; *X5*, resistance at 5Hz.

TABLE E3. Mean individual components of mini-AQLQ response to benralizumab

Characteristic	Baseline	Week 2	Week 4	Week 8	Week 12	Mean difference (95% CI) at week 12	Week 24
Overall	3.6	4.6***	5.0***	5.2****	5.3****	1.7 (1.1, 2.3)	4.8*
Symptoms	3.4	4.6***	5.2****	5.4****	5.5****	2.0 (1.4, 2.7)	5.0**
Environment	3.4	4.0	4.5*	4.3*	4.7**	1.3 (0.7, 2.0)	4.4
Emotions	3.3	4.2**	4.9**	5.1***	5.1**	1.8 (0.9, 2.7)	4.7
Activity	4.1	5.1*	5.3**	5.6***	4.9	0.8 (0.0, 1.6)	5.1

Bonferroni-corrected comparisons versus baseline: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$.

TABLE E4. Comparison of baseline data according to benralizumab therapy results

Characteristic	$\Delta PD_{10} \geq 1.0$ DD (n = 12)	$\Delta PD_{10} < 1.0$ DD (n = 9)
ICS (μg)	1933	1889
Age (years)	52	54
BMI (kg/m^2)	29.3	31.1
ACQ	2.5	2.8
PBE (cells/μL)	409	478
FENO (ppb)	37	68
EDN (ng/mL)	55.1	79.6*
FEV_1 (%)	74.7	87.6

BMI, Body mass index; *ICS,* ICS–beclomethasone dipropionate equivalent dose; *PBE,* peripheral blood eosinophil.
*$P < .05$.