Original Article

Corticosteroid Responsiveness Following Mepolizumab in Severe Eosinophilic Asthma—A Randomized, Placebo-Controlled Crossover Trial (MAPLE)

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What is already known about this topic? Oral corticosteroids reduce inflammation and improve symptoms in biologic-naive patients with severe eosinophilic asthma, but oral corticosteroid response in those receiving anti−IL-5 treatment is less clear.

What does this article add to our knowledge? In patients with severe eosinophilic asthma receiving treatment with mepolizumab, oral corticosteroids further reduce inflammation but have limited clinical benefits. Heterogeneity in oral corticosteroid response whilst on mepolizumab is likely driven by existing subphenotypes of eosinophilic asthma.

How does this study impact current management guidelines? Residual corticosteroid-responsive disease persists in people with severe eosinophilic asthma receiving anti−IL-5 treatment with mepolizumab. However, alternative corticosteroid-sparing therapy should be considered instead of additional oral corticosteroids because the clinical benefits are limited.

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This study was funded jointly by the Medical Research Council (MRC) UK (grant no. *MR/M016579/1) and industrial partners within the MRC Refractory Asthma Stratification Program consortium. None of the industry partners had a role in the design or conduct of the study, drafting, or approval of the manuscript.

Conflicts of interest: J. Busby has received honoraria for attending advisory panels for NuvoAir. L. G. Heaney is Academic Lead for the UK MRC Consortium for Stratified Medicine in Severe Asthma − Industrial Pharma partners Amgen, AstraZeneca (AZ), Medimmune, Janssen, Novartis, Roche/Genentech, GlaxoSmithKline (GSK), and Boehringer Ingelheim; prior project grant funding from Medimmune, Novartis UK, Roche/Genentech, and GSK; has taken part in advisory boards/lectures supported by Novartis, Roche/Genentech, GSK, Teva, Theravance, and Vectura; has travel funding support to international respiratory meetings (AZ, Chiesi, Novartis, Boehringer Ingelheim, Teva, and GSK); and has taken part in asthma clinical trials (GSK, Schering Plough, Synairgen, Novartis, and Roche/Genentech) for which his institution was remunerated. I. D. Pavord has received speaker's honoraria for speaking at sponsored meetings from AZ, Boehringer Inglehiem, Aerocrine, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini, and GSK and payments for organizing educational events from AZ, GSK, Sanofi/Regeneron, and Teva; has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AZ, Boehringer Ingelheim, GSK, Novartis, Teva, Merck, Circassia, Chiesi, and Knopp and payments to support Food and Drug Administration approval meetings from GSK; has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, AZ, Teva, and Chiesi; has received a grant from Chiesi to support a phase 2 clinical trial in Oxford; is copatent holder of the rights to the Leicester Cough Questionnaire and has received payments for its use in clinical trials from Merck, Bayer, and Insmed; and in 2014-2015 was an expert witness for a patent dispute involving AZ and Teva. C. E. Brightling declares grant payments to his institution from GSK, AZ, Novartis, Chiesi, Sanofi, Genentech, Merck, Mologic, 4DPharma, and Gossamer; consulting fees paid to his institution from GSK, AZ, Boehringer Ingelheim, Novartis, Chiesi, Sanofi, Genentech, Merck, Mologic, 4DPharma, Gossamer, TEVA, Regeneron, Roche, and CSL Behring, all in the 36 months before manuscript submission. P. Bradding has received research funding from Genentech via the University Hospitals of Leicester NHS Trust; consultancies for Boehringer Ingelheim and Genentech via the University of Leicester; and support to attend scientific meetings from Chiesi, Teva, and Sanofi Genzyme. R. Chaudhuri has received lecture fees from GSK, AZ, Teva, Chiesi, Sanofi, and Novartis; honoraria for advisory board meetings from GSK, AZ, Teva, Chiesi, and Novartis; sponsorship to attend international scientific meetings from Chiesi, Napp, Sanofi, Boehringer, GSK, and AZ; and a research grant to her institute from AZ for a UK multicenter study.

Received for publication January 19, 2022; revised June 10, 2022; accepted for publication June 28, 2022.
Available online July 19, 2022.

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2213-2198

https://doi.org/10.1016/j.jaip.2022.06.050

Abbreviations used
ACQ- Asthma Control Questionnaire
AQLQ- Asthma Quality of Life Questionnaire
FEF_{25-75}- forced expiratory flow at 25% and 75%
FENO- fractional exhaled nitric oxide
OCS- oral corticosteroid
ppb- parts per billion
SEA- severe eosinophilic asthma
SGRQ- St George's Respiratory Questionnaire
T2- type 2
VAS- Visual Analogue Scale

BACKGROUND: Mepolizumab inhibits IL-5 activity and reduces exacerbation frequency and maintenance oral corticosteroid (OCS) dosage in patients with severe eosinophilic asthma (SEA). Some patients remain dependent on OCS despite anti−IL-5 treatment, suggesting residual corticosteroid-responsive mechanisms.
OBJECTIVE: To determine the clinical and anti-inflammatory effects of OCS in patients with SEA on mepolizumab.
METHODS: We conducted a randomized, triple-blind, placebo-controlled crossover trial of prednisolone (0.5 mg/kg/d, maximum 40 mg/d, for 14 ± 2 days) in adults with SEA after 12 or more weeks of mepolizumab. We compared change in asthma symptoms, quality of life, lung function measured by spirometry and airwave oscillometry, fractional exhaled nitric oxide, and blood and sputum eosinophil cell count after prednisolone and placebo treatment.
RESULTS: A total of 27 patients completed the study. Prednisolone did not improve 5-item Asthma Control Questionnaire (mean difference in change for prednisolone vs placebo, −0.23; 95% CI, −0.58 to 0.11), mini-Asthma Quality of Life Questionnaire (0.03; 95% CI, −0.26 to 0.42), St. George's Respiratory Questionnaire (0.24; 95% CI, −3.20 to 3.69), or Visual Analogue Scale scores for overall asthma symptoms (0.11; 95% CI, −0.58 to 0.80). The mean difference for FEV_1 in favor of prednisolone was 105 mL (95% CI, −4 to 213 mL); forced expiratory flow at 25% and 75% 484 mL/s (95% CI, 151 to 816 mL/s); fractional exhaled nitric oxide reduction 41% (95% CI, 25% to 54%); blood eosinophil count reduction 49% (95% CI, 31% to 62%); and percentage of sputum eosinophil reduction 71% (95% CI, 26% to 89%).
CONCLUSIONS: OCS improved small-airway obstruction and reduced biomarkers of type 2 inflammation but had no significant effect on symptoms or quality of life in patients with SEA receiving treatment with mepolizumab. **(J Allergy Clin Immunol Pract 2022;10:2925-34)**

Key words: *Asthma; Eosinophils; Severe eosinophilic asthma; IL-5; Mepolizumab; Type 2 inflammation; T2; Prednisolone; Oral corticosteroids; Corticosteroid resistance*

BACKGROUND

Severe asthma leads to significant health and socioeconomic burden.[1-3] Oral corticosteroids (OCSs) were the most effective treatment for severe asthma before the introduction of biologic therapies. Prolonged or frequent use of OCS is no longer considered acceptable because of its high potential for side effects compared with biologic treatment; however, OCSs continue to have a prominent role in severe asthma management because some patients remain dependent on maintenance or rescue OCS despite mAb therapies.[4-6]

Mepolizumab was the first available humanized anti−IL-5 mAb treatment for severe eosinophilic asthma (SEA). SEA is driven by type 2 (T2) inflammation and can be effectively treated with therapies targeting the IL-5 pathway.[7] Placebo-controlled trials of mepolizumab showed a 39% to 59% reduction in annual exacerbations and 50% reduction in maintenance OCS dose after 20 weeks. These favorable outcomes were mirrored by other anti−IL-5 and anti−IL-5Rα therapies. However, treatments targeting IL-5 have not achieved complete prevention of exacerbations or withdrawal of maintenance corticosteroids in many patients with SEA[8-12] and improvements in daily symptoms and lung function are modest and inconsistent.[10,13,14] Therefore, IL-5 suppression alone may be inadequate in treating SEA and alternative corticosteroid-responsive inflammatory pathways, such as IL-4/IL-4Rα axis, may be left untreated.

The mechanism of OCS efficacy following circulatory eosinophil depletion via IL-5 inhibition in SEA is unknown. Haldar et al[8] showed that there were no differences in prednisolone response, when measured using Asthma Control Questionnaire, FEV_1, and fractional exhaled nitric oxide (FENO), in patients before and after treatment with mepolizumab.[8] However, improvement in asthma symptoms measured using the Visual Analogue Scale (VAS) were greater in patients not receiving mepolizumab compared with those on mepolizumab.[15] These results were not placebo-controlled but suggest ongoing corticosteroid responsiveness after mepolizumab, albeit with attenuation of symptom response. Current use of OCS while on biologic therapy is guided by practices developed before the availability of targeted therapies. There are no placebo-controlled studies demonstrating the efficacy of OCS use in biologic-treated patients with severe asthma. We conducted a placebo-controlled crossover study of prednisolone in patients treated with mepolizumab. Our hypothesis was that prednisolone would have additional anti-inflammatory effects but attenuated clinical effects.

METHODS

Study population and design

We conducted a randomized, triple-blind, placebo-controlled crossover trial of prednisolone in patients with SEA treated with mepolizumab. Adult patients aged 18 to 80 years with SEA were recruited from 4 specialist UK asthma centers (Glasgow, Oxford, Leicester, and Belfast). All study participants were suitable for mepolizumab as per the National Institute for Health and Care Excellence or Scottish Medicines Consortium Guidelines in 2019.[16,17] The main eligibility criteria include requirement of 4 or more OCS courses for exacerbations over 12 months or continuous OCS equivalent to 5 mg/d for more than 6 months despite good adherence to optimized treatments with at least 1 blood eosinophil count greater than or equal to 300 cells/mL within the last 12 months. Eligibility assessment and decision to start mepolizumab were made by the patients' treating physicians and were independent from participation in the current study. The study was approved by

the Medical Ethics Committee (West of Scotland Research Ethics Service 3, Reference number 18/WS/0060) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All participants provided written informed consent before any study-specific procedures took place. This study is registered on Clinicaltrials.gov (NCT03610685).

Interventions and procedures

All participants had received at least 12 weeks of mepolizumab before randomization. A subgroup of patients underwent a 12-week run-in period before randomization because they entered the study at initiation of mepolizumab. Randomization was conducted using an interactive voice responsive system. The study was triple-blinded, with participants, research staff, and study statistician unaware of treatment allocations. Study participants were randomized (1:1) to prednisolone (0.5 mg/kg/d, maximum dose of 40 mg/d, 14 ± 2 days) followed by a 4-week washout period and then matched placebo or vice versa. Treatment compliance was defined as more than 75% self-reported diary record and laboratory confirmation of measurable serum prednisolone level. To ensure participants were free from OCS effects before treatment periods, all study visits were delayed by 4 weeks from when the participant last received OCSs (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Patients using regular maintenance prednisolone were excluded. All inclusion and exclusion criteria are listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org.

Demographic, medical history, and asthma treatments were recorded at study entry. Participants who were established on mepolizumab at study entry consented to data collection from previous clinical records. Clinical outcomes include asthma symptoms, quality of life, and lung function and were assessed by the 5-item Asthma Control Questionnaire (ACQ-5), Mini-Asthma Quality of Life Questionnaire (Mini-AQLQ), St. George's Respiratory Questionnaire (SGRQ), VAS for asthma symptoms (scale 0-10 cm), and spirometry. We measured small-airway function using airwave oscillometry (Tremoflo, Thorasys, Montreal, Canada) in addition to standard spirometry. Inflammatory outcomes include FENO and blood and sputum eosinophil counts. Sputum samples were obtained by sputum induction using hypertonic saline (up to 5%). Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines while on usual asthma treatments. All study measurements were taken before and after prednisolone and placebo. Baseline measurements were taken at the randomization visit. Premepolizumab measurements were taken at the first study visit for the subgroup who entered the study at mepolizumab initiation and retrospectively from medical records for those who entered the study at randomization. Details of the study schedule (see Table E2 in this article's Online Repository at www.jaci-inpractice.org) and assessments (see Table E3 in this article's Online Repository at www.jaci-inpractice.org) are available in this article's Online Repository at www.jaci-inpractice.org.

End points

This was an exploratory study that aimed to determine the clinical and inflammatory effects of prednisolone in SEA after mepolizumab. Prespecified primary end points include change in asthma symptoms, quality of life, lung function, FENO, and blood and sputum eosinophils after prednisolone versus placebo in the overall study population. *Post hoc* exploratory analyses were performed to examine differences in prednisolone response according to baseline FENO (<25 parts per billion [ppb], 25-50 ppb, and >50 ppb), blood eosinophil count (<0.10 × 10^9/L and ≥0.10 × 10^9/L), and ACQ-5 score (<1.5 and ≥1.5).

Statistical analysis

No sample size calculation was indicated because of the exploratory nature of the study. All statistical analyses were conducted under an intention-to-treat basis, and 2-tailed *P* values were used throughout. We reported mean ± SD for normally distributed continuous outcomes, median (interquartile range) for skewed continuous outcomes, and proportions for categorical outcomes. We estimated differences in study outcomes between prednisolone and placebo using the analysis of covariance approach, which includes the within-subject difference in treatment responses as the dependent variable and the corresponding difference in baseline responses as a covariate.[18] We took the log of FENO, sputum eosinophils, and blood eosinophils to reduce their skew. We added a small amount to zero measurements for these logged variables (1 ppb for FENO, 0.25% for sputum eosinophils, and 0.01 cell/μL for blood eosinophils) because the log of zero is undefined. Results were presented as mean differences for normally distributed variables and ratios for skewed variable. We tested for carryover effects by calculating the sum of the posttreatment values for all patients and testing for differences by treatment sequence allocation using unpaired *t* tests and Mann-Whitney *U* tests.[19] Analyses were conducted under a complete case framework using STATA 16 SE (StataCorp LLC, College Station, TX).

RESULTS

A total of 33 patients were recruited into the study; 30 were randomized. Three patients did not complete the study; therefore, 27 provided outcome data for both arms of the study (intention-to-treat cohort) and were included in the primary analysis, of which 26 participants had good adherence to study treatment (per-protocol cohort) (Figure 1). Reasons for study withdrawals are listed in Table E4 in this article's Online Repository at www.jaci-inpractice.org. The mean age at study entry was 56.9 ± 12.1 years. Participants were nonsmokers and 66.7% were male. Baseline demographic and clinical characteristics are presented in Table I. Mean premepolizumab FEV_1 was 69.6% of predicted (±15.0%). Median (Q1, Q3) FENO, blood eosinophil count, and percentage of sputum eosinophils before mepolizumab treatment were 47 ppb (24, 69), 0.43 × 10^9/L (0.24, 0.73), and 9.2% (0.7, 33.0), respectively. Premepolizumab measurements are presented in Table II.

At study baseline (after ≥12 weeks of mepolizumab), median (Q1, Q3) ACQ-5 score was 0.6 (0.2, 2.0), mini-AQLQ score was 5.7 (4.0, 6.6), SGRQ score was 30.7 (13.1, 46.4), and overall asthma symptoms VAS was 2.0 cm (0.3, 4.5). Mean percentage of predicted FEV_1 was 77.0% ± 20.2%. Median (Q1, Q3) FENO was 37 ppb (22, 61). Blood eosinophil count (median, 0.06 × 10^9/L; interquartile range, 0.04-0.09) and percentage of total sputum eosinophils (median, 0.65%; interquartile range, 0.00-5.75) were within normal limits (Table III).

Prednisolone vs placebo

Prednisolone did not lead to clinically or statistically significant change in ACQ-5 score (mean difference in change for prednisolone vs placebo, −0.23; 95% CI, −0.58 to 0.11), mini-AQLQ score (0.03; 95% CI, −0.24 to 0.31), SGRQ score (0.24; 95% CI, −3.20 to 3.69), and VAS for overall asthma

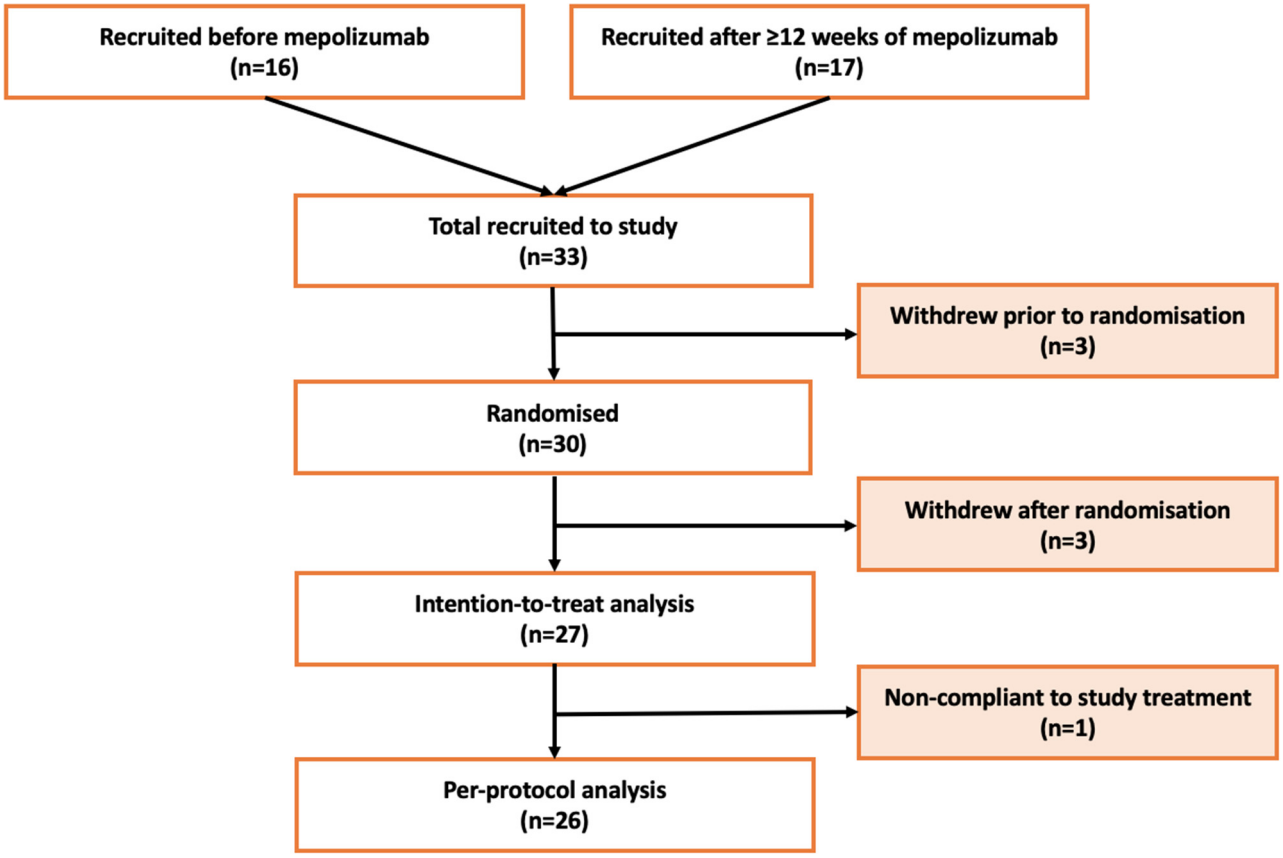

FIGURE 1. Study flow chart.

symptoms (0.11; 95% CI, −0.58 to 0.80), breathlessness (−0.19; 95% CI, −0.90 to 0.53), or cough (−0.16; 95% CI, −0.98 to 0.66) compared with placebo (Figure 2). Individual ACQ-5 questionnaire scores suggested that there was an improvement in wheeze following prednisolone (−0.41; 95% CI, −0.81 to −0.01); however, individual components of mini-AQLQ and SGRQ did not detect any improvements after prednisolone. There was a trend toward improvement in FEV_1 (mean difference in change, 105 mL; 95% CI, −4 to 213 mL). The improvement in forced expiratory flow at 25% and 75% (FEF_{25-75}) was 484 mL/s (95% CI, 151-816 mL/s). Airwave oscillometry did not detect any change in lung function. Prednisolone reduced FENO by 41% compared with placebo (95% CI, 25%-54%). Total white blood cell count (ratio, 4.12; 95% CI, 3.15-5.09) and blood neutrophil count (ratio, 3.91; 95% CI, 3.13-4.70) increased after prednisolone treatment. Blood eosinophil count and sputum eosinophils reduced by 49% (95% CI, 31%-62%) and 71% (95% CI, 26%-89%), respectively (see Table E5 in this article's Online Repository at www.jaci-inpractice.org).

Prednisolone response according to FENO, blood eosinophils, and ACQ-5

Change in FENO after prednisolone was not associated with changes in FEV_1 ($r = 0.05$; $P = .811$) or percentage of sputum eosinophils ($r = 0.32$; $P = .155$) (see Figures E2 and E3 in this article's Online Repository at www.jaci-inpractice.org). The number of participants with low (<25 ppb), intermediate (25-50 ppb), and high (>50 ppb) FENO at baseline was similar. In the low-FENO group (n = 8), FEF_{25-75} improved by 358 mL/s (95% CI, 82-633 mL/s). FEF_{25-75} did not improve in patients with intermediate (n = 10; 578 mL/s; 95% CI, −183-1339 mL/s) or high FENO (n = 9; 326 mL/s; 95% CI, −28-680 mL/s). However, patients with high FENO demonstrated a further reduction in blood eosinophil count (ratio, 0.44; 95% CI, 0.26-0.75) and percentage of sputum eosinophils (ratio, 0.14; 95% CI, 0.04-0.56), whereas patients with low FENO did not. ACQ-5, mini-AQLQ, and SGRQ scores did not improve after prednisolone treatment regardless of baseline FENO. In patients with low FENO, cough measured using VAS decreased by 2.37 cm (95% CI, 1.38-3.37) after prednisolone treatment, but no changes were observed in those with intermediate or high FENO (Table IV).

Change in clinical and inflammatory measurements for prednisolone versus placebo according to blood eosinophil count and ACQ-5 are presented in Table E6 and Table E7, respectively, in this article's Online Repository at www.jaci-inpractice.org. Interpretation of subgroup analyses according to blood eosinophil count and ACQ-5 was difficult due to significant differences in subgroup sample sizes.

Prednisolone versus placebo in per-protocol cohort

Twenty-six patients were compliant to study treatment and included in the per-protocol analysis. Results were similar in the per-protocol cohort apart from the change in FEV_1 became statistically significant when only those compliant to

TABLE I. Baseline demographic characteristics (n = 27)

Characteristic	n	Count (%)
Sex	27	
Male		18 (66.7)
Female		9 (33.3)
Age at study entry (y)*	27	56.9 ± 12.1
Ethnicity		
African		1 (3.7)
White		26 (96.3)
Smoking status	27	
Never smoker		17 (63.0)
Ex-smoker		10 (37.0)
E-cigarette smoker	27	0 (0.0)
No. of pack years (y)†	10	8 (5-12)
Asthma history	27	
Age of asthma diagnosis (y)*		31.7 ± 22.1
No. of exacerbations during 12 mo before mepolizumab†		5 (4-7)
Any ED attendances during 12 mo before mepolizumab		4 (14.8)
Any hospital admissions during 12 mo before mepolizumab		4 (14.8)
Any ICU/HDU admission ever		3 (11.1)
Any mechanical ventilations ever		0 (0.0)
Asthma-related comorbidities	27	
Allergic rhinitis		5 (18.5)
Perennial rhinitis		14 (51.9)
Nasal polyps		9 (33.3)
Eczema		7 (25.9)
Depression/anxiety		5 (18.5)
Bronchiectasis		3 (11.1)
Reflux disease		7 (25.9)
Asthma treatments	27	
Inhaled corticosteroid dose (beclomethasone equivalent, μg)†		2000 (2000- 2000)
Long-acting β-agonist		27 (100.0)
Leukotriene receptor antagonist		18 (66.7)
Long-acting muscarinic antagonist		21 (77.8)
Theophylline		6 (22.2)
Home nebulizer		3 (11.1)
Long-term antibiotics		4 (14.8)
BMI	26	31.5 (5.2)

BMI, Body mass index; *ED*, emergency department; *ITU*, intensive care unit; *HDU*, high dependency unit.
*Results are presented as mean ± SD.
†Results are presented as median (range).

TABLE II. Clinical and inflammatory measurements before mepolizumab treatment (n = 27)

Measurement	N	Median (Q1, Q3)
ACQ-5 score	22	1.6 (1.4, 3.2)
Mini-AQLQ score	18	4.6 (3.5, 5.5)
SGRQ score	19	47.3 (36.9, 65.3)
VAS (cm)	10	
Overall symptoms		4.5 (1.0, 7.0)
Shortness of breath		4.5 (2.0, 6.1)
Cough		4.0 (2.0, 6.1)
SNOT-20 total score	11	37.0 (29.0, 48.0)
Airwave oscillometry	11	
R5 ($cmH_2O \cdot s/L$)		2.9 (2.8, 4.2)
R5-20 ($cmH_2O \cdot s/L$)		0.2 (0.0, 0.6)
AX (cmH_2O/L)		6.3 (4.7, 14.0)
X5 ($cmH_2O \cdot s/L$)		−0.5 (−0.7, −0.1)
Spirometry*	27	
FEV_1 % of predicted (%)		69.6 ± 15.0
FVC % of predicted (%)		86.3 ± 14.3
FEV_1/FVC (%)		63.8 ± 11.6
FEF_{25-75} % of predicted (%)	12	50.5 ± 12.3
PEF (L/min)*	16	468 ± 121
FENO (ppb)	27	47 (24, 69)
Blood cell counts	27	
White cell count ($\times 10^9$/L)		7.9 (6.6, 9.5)
Neutrophils ($\times 10^9$/L)		4.77 (3.70, 6.30)
Eosinophils ($\times 10^9$/L)		0.43 (0.24, 0.73)
Sputum cell counts	13	
Macrophages % of total (%)		25.00 (9.73, 32.21)
Neutrophils % of total (%)		54.73 (30.70, 70.62)
Eosinophils % of total (%)		9.20 (0.71, 33.00)
Lymphocytes % of total (%)		0.00 (0.00, 0.00)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.
*Results are presented as mean ± SD.

prednisolone were included (mean difference in change, 125 mL; 95% CI, 20-230 mL). The change in FEF_{25-75} remained clinically and statistically significant (484 mL/s; 95% CI, 151-816 mL/s). There were no improvements in asthma symptoms or quality of life. FENO (ratio, 0.58; 95% CI, 0.45-0.75) and blood (ratio, 0.51; 95% CI, 0.37-0.68) and sputum eosinophils (ratio, 0.21; 95% CI, 0.10-0.46) also reduced after prednisolone treatment in the per-protocol cohort as they did in the intention-to-treat group (see Table E8 in this article's Online Repository at www.jaci-inpractice.org).

Stratification according to baseline FENO showed that those with low FENO (n = 7) had an improvement in FEV_1 (mean difference in change, 171 mL; 95% CI, 2-340 mL) and FEF_{25-75} (358 mL/s; 95% CI, 82- 633 mL/s) after prednisolone treatment. Blood and sputum eosinophils further reduced by 56% (95% CI, 25%-74%) and 86% (95% CI, 44%-96%), respectively, in the high-FENO cohort (n = 9). Asthma symptoms and quality-of-life scores did not improve regardless of FENO (see Table E9 in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

Targeted biologic therapies have successfully replaced OCSs in many patients with severe asthma, but some remain dependent on OCS despite humanized mAb therapies. Understanding the role and efficacy of OCS after mepolizumab treatment will facilitate the appropriate use of prednisolone. We showed in stable patients with SEA treated with mepolizumab that OCS improved small-airway function and further reduced T2 inflammation (including FENO and blood and sputum eosinophils), but without corresponding improvements in overall

TABLE III. Clinical and inflammatory measurements after ≥12 wk of mepolizumab treatment (n = 27)

Measurement	n	Median (Q1, Q3)
ACQ-5 score	27	0.6 (0.2, 2.0)
Mini-AQLQ score	27	5.7 (4.0, 6.6)
SGRQ score	27	30.7 (13.1, 46.4)
VAS (cm)	25	
Overall symptoms		2.0 (0.3, 4.5)
Shortness of breath		2.0 (0.2, 5.0)
Cough		1.0 (0.0, 4.5)
Airwave oscillometry		
R5 ($cmH_2O \cdot s/L$)		3.5 (2.6, 5.0)
R5-20 ($cmH_2O \cdot s/L$)		0.7 (0.3, 1.9)
AX (cmH_2O/L)		7.9 (4.9, 31.8)
X5 ($cmH_2O \cdot s/L$)		−1.1 (−2.6 to −0.5)
Spirometry*	27	
FEV_1 % of predicted (%)		77.0 ± 20.2
FVC % of predicted (%)		91.8 ± 16.6
FEV_1/FVC (%)		65.9 ± 12.2
FEF_{25-75} % of predicted (%)	18	53.1 ± 24.5
PEF (L/min)*	27	446 ± 145
FENO (ppb)	27	37 (22, 61)
Blood cell counts	27	
White cell count ($\times 10^9$/L)		6.0 (5.5, 7.8)
Neutrophils ($\times 10^9$/L)		3.70 (3.01, 4.40)
Eosinophils ($\times 10^9$/L)		0.06 (0.04, 0.09)
Sputum cell counts	22	
Macrophages % of total (%)		19.49 (8.70, 48.26)
Neutrophils % of total (%)		69.78 (34.00, 82.70)
Eosinophils % of total (%)		0.65 (0.00, 5.75)
Lymphocytes % of total (%)		0.00 (0.00, 0.35)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.
*Results are presented as mean ± SD.

asthma symptoms or quality of life. Our findings suggest an ongoing role for OCS following mepolizumab treatment; however, the clinical benefits are small and of uncertain clinical significance.

This was the first placebo-controlled study of prednisolone response in SEA treated with anti−IL-5. Studies of biologic-naive patients with SEA showed that prednisolone improved asthma symptoms alongside FEV_1 increase and FENO decrease.[20-27] In contrast, the current study of mepolizumab-treated patients demonstrated that asthma symptoms did not improve despite improvement in small-airway obstruction and reduction in FENO. The lack of symptom improvement after prednisolone in patients on mepolizumab is in keeping with a previous study that showed that improvements in breathlessness, wheeze, and cough measured by VAS were less in those on mepolizumab compared with those without mepolizumab.[15]

There are various mechanisms by which prednisolone could further reduce inflammation and improve lung function after mepolizumab treatment; hypotheses include additional T2 suppression, effect on mast cell activity, reduction of airway hyperresponsiveness, and increased corticosteroid sensitivity. Mepolizumab reduces IL-5, blood eosinophils, and serum eosinophil cationic protein, but T-cell activation and production of other cytokines are relatively unaffected.[28] Prednisolone has wider- ranging effects on other proinflammatory cytokines, epithelial and submucosa mast cells, and T lymphocytes.[23,29] In the current study, FENO concentrations reduced following prednisolone treatment. This likely results from modulation of upstream cytokines such as IL-4 and IL-13. Dupilumab blocks both IL-4 and IL-13 signaling and reduces exacerbations in patients with elevated FENO or eosinophilia, but FEV_1 improved only in those with elevated FENO and eosinophilia.[30] Therefore, prednisolone may improve lung function after IL-5 suppression of other inflammatory pathways, including those mediated by IL-4 and IL-13, although notably, we did not find any improvement in FEV_1 in those with elevated FENO, suggesting that these are dissociated. We showed that prednisolone reduced blood and sputum eosinophils further despite normal baseline values. This is likely because corticosteroids are potent inducers of eosinophil apoptosis in blood and tissue.[31] Additional eosinophilic suppression is achievable with higher doses of mepolizumab[9] or benralizumab, an anti−IL-5Ra, but this does not translate into greater improvements in lung function or exacerbation reduction.[14] Therefore, it is more likely that mechanisms independent of T2 inflammation are responsible for prednisolone responsiveness after IL-5 inhibition.

Airway eosinophilia and hyperresponsiveness are well-established traits of severe asthma,[32] but previous literature has shown a dissociation between these 2 factors.[33] Mepolizumab treatment in patients with persistent sputum eosinophilia reduced sputum eosinophil concentrations but had no effect on airway hyperresponsiveness or lung function.[8] We demonstrated a large increase in FEF_{25-75} following prednisolone treatment, which suggests that lung function improvements were concentrated in the smaller airways. Interestingly, similar changes were not demonstrated by airwave oscillometry. One possible explanation is the involvement of mast cells in the lower airways, which communicate with airway smooth muscle to control airway hyperresponsiveness and small-airway inflammation.[34] Unlike mepolizumab, prednisolone is known to reduce mast cell numbers[23] and this effect may contribute to improvements in small-airway obstruction following IL-5 inhibition. Anti−mast cell treatments have been shown to be promising for T2-low asthma[35] and our *post hoc* analysis, albeit with small cohort numbers, showed that lung function predominantly improved in the low-FENO cohort. Our study population had well-characterized SEA with good evidence of T2 inflammation; therefore, it is unlikely that IL-5 inhibition led to complete reversal to T2-low disease, although it is possible that anti−IL-5 treatment in SEA unmasked those who had noneosinophilic inflammatory drivers alongside eosinophilic inflammation before biologic therapy. An Unbiased Biomarker for the Prediction of Respiratory Disease Outcomes study used proteomics to identify subgroups within SEA. One subgroup had comparably lower FEV_1 (52% of predicted) and higher prevalence of atopy (82%).[36] Our low-FENO group on mepolizumab may represent this atopic subphenotype and have untreated mast cell activity contributing to persistent airway obstruction, hence prednisolone responsiveness after eosinophil reduction.

Corticosteroid resistance is highly prevalent in patients with severe asthma.[37] Previous studies have shown that IL-5 and IL-13 can reduce the proapoptotic function of corticosteroids.[38,39] Thus, it is hypothesized that corticosteroid insensitivity may occur beyond a certain level of IL-5 and IL-13.[40] Inhibition of

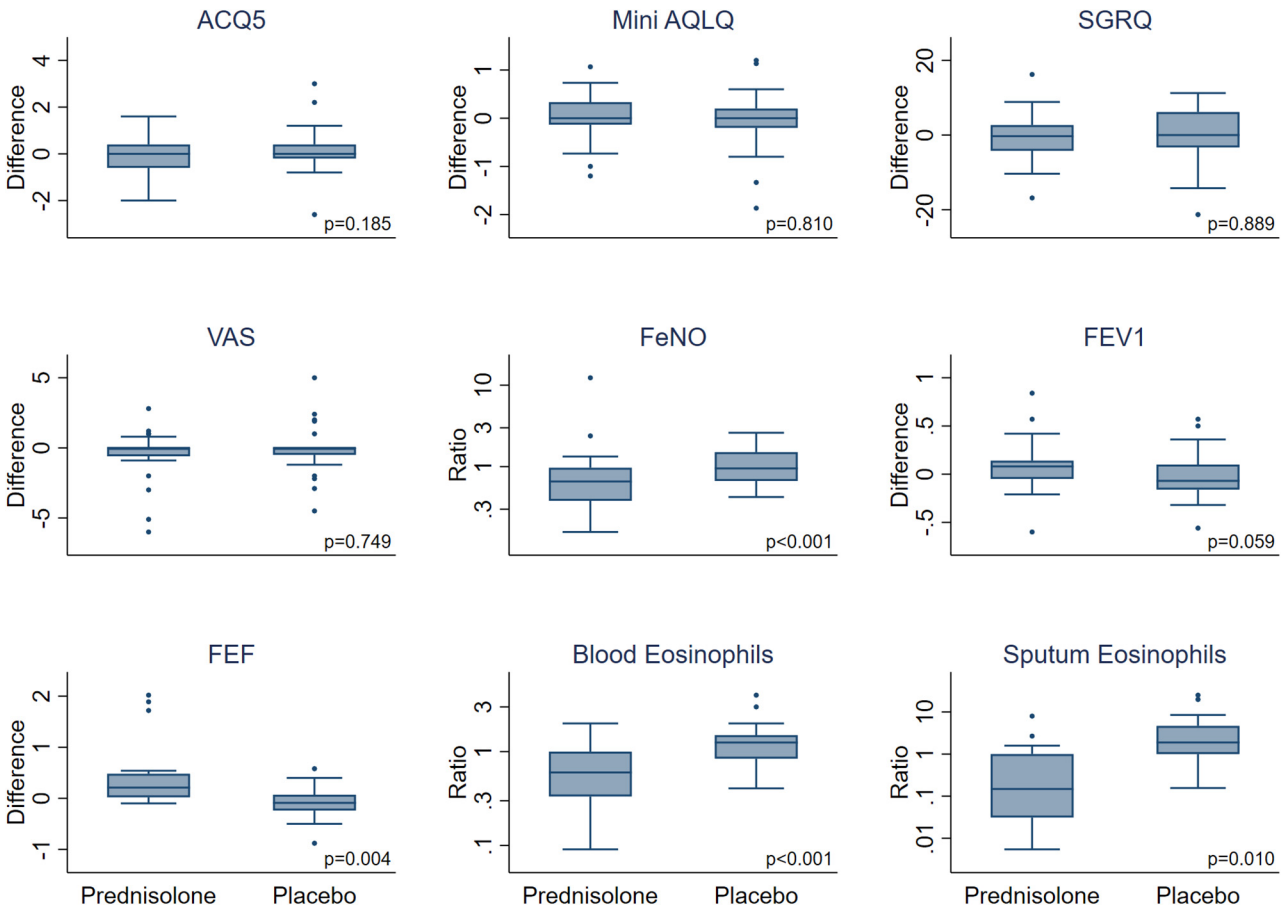

FIGURE 2. Effect of prednisolone vs placebo on asthma symptoms, quality of life, lung function, and T2-inflammation markers.

IL-5 with mepolizumab could overcome corticosteroid resistance to lead to improvements in lung function observed in the current study. This may also explain why our FENO-high subgroup was less corticosteroid-responsive because ongoing IL-13 over a certain threshold can also hinder the responsiveness of corticosteroids. Furthermore, systemic corticosteroids can lead to reversal of bronchodilator subsensitivity in patients with moderate to severe asthma via upregulation of beta2-adrenergic receptors on lymphocytes and smooth muscle cells,[41,42] a phenomenon not yet demonstrated with mepolizumab. Regardless of the mechanism of action for prednisolone efficacy in patients on mepolizumab, we clearly demonstrated T2 inflammation and small-airway obstruction in SEA that were treatable with prednisolone following IL-5 suppression. The question is how these findings guide the use of prednisolone after mepolizumab.

There are 2 scenarios whereby prednisolone use is considered after mepolizumab: an acute exacerbation or loss of asthma control related to downtitration of maintenance OCS dose. We showed that OCS response is nonhomogeneous after mepolizumab; thus, a biomarker of corticosteroid response is needed to guide prednisolone use in these scenarios. In prebiologic patients, FENO positively correlates with the risk of exacerbations and corticosteroid responsiveness.[43-45] To our knowledge, the same correlation has not been demonstrated in patients on mepolizumab. The MEX study showed that although FENO differentiated eosinophilic from noneosinophilic exacerbations on mepolizumab, it was not related to the annualized exacerbation rate.[46] Furthermore, the current study suggests that OCS responsiveness was not exclusive to those with high FENO while on mepolizumab. In fact, patients who had low FENO benefited the most from additional prednisolone in terms of improvements in small-airway obstruction. This is an exploratory study; therefore, our findings require confirmation with larger trials. It is, however, noteworthy that the relationship between FENO and corticosteroid responsiveness may differ before and after biologic therapy. Potential explanations for this change include previously described mechanisms related to corticosteroid insensitivity and subphenotypes within SEA. The lack of corticosteroid responsiveness in some patients following IL-5 suppression presents an opportunity for more selective use of OCS. FENO appears to be a good biomarker for differentiating OCS responsiveness on mepolizumab, but further studies are needed to confirm the relationship between FENO and corticosteroid responsiveness while on anti−IL-5 therapy.

Our findings were generated using a crossover study design, which provided a robust control and maximizes statistical power. Carryover effects were mitigated by a 4-week washout period and screened for as part of our analyses. There were however several limitations to the current study. First, selection bias may have

TABLE IV. Prednisolone vs placebo in patients with low (<25 ppb), intermediate (25-50 ppb), and high FENO (>50 ppb)—Intention-to-treat cohort (n = 27)

	Low FENO (n = 8)		Intermediate FENO (n = 10)		High FENO (n = 9)	
Measurement	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**
ACQ-5 score	8	0.31 (−0.27 to 0.89)	10	−0.39 (−1.10 to 0.31)	9	−0.31 (−0.95 to 0.33)
Mini-AQLQ score	8	−0.31 (−0.71 to 0.09)	10	0.25 (−0.41 to 0.91)	9	0.15 (−0.34 to 0.63)
SGRQ score	8	2.19 (−1.32 to 5.70)	10	0.78 (−5.78 to 7.34)	9	−1.59 (−9.43 to 6.26)
VAS (cm)	7		9		7	
Overall symptoms		0.45 (−0.23 to 1.12)		−0.19 (−1.95 to 1.57)		0.29 (−0.09 to 0.66)
Shortness of breath		0.51 (−0.11 to 1.12)		−0.07 (−1.63 to 1.49)		**−0.72 (−1.31 to −0.13)**
Cough		**2.37 (1.38 to 3.37)**		−0.69 (−1.72 to 0.34)		−1.12 (−2.70 to 0.47)
SNOT-20 total score*	8	−2.13 (−13.31 to 9.06)	10	−2.70 (−13.79 to 8.39)	8	−3.00 (−15.88 to 9.88)
Airwave oscillometry	7		10		9	
R5 ($cmH_2O \cdot s/L$)		−0.16 (−0.87 to 0.55)		0.25 (−0.40 to 0.90)		0.11 (−0.22 to 0.43)
R5-20 ($cmH_2O \cdot s/L$)		−1.27 (−4.14 to 1.60)		0.08 (−0.19 to 0.34)		0.12 (−0.05 to 0.30)
AX (cmH_2O/L)		0.32 (−14.91 to 15.54)		7.90 (−3.35 to 19.15)		1.81 (−1.38 to 5.00)
X5 ($cmH_2O \cdot s/L$)		−0.07 (−1.22 to 1.09)		−0.46 (−1.69 to 0.78)		−0.04 (−0.36 to 0.29)
Spirometry	8		10		9	
FEV_1 (L)		0.169 (−0.092 to 0.431)		0.047 (−0.161 to 0.255)		0.128 (−0.028 to 0.284)
FEV_1/FVC (%)		**2.27 (0.76 to 3.77)**		**2.87 (0.23 to 5.51)**		0.91 (−2.07 to 3.90)
FEF_{25-75} (L/s)	4	**0.358 (0.082 to 0.633)**	8	0.578 (−0.183 to 1.339)	6	0.326 (−0.028 to 0.680)
FEF_{25-75} % of predicted (%)	4	**10.54 (2.56 to 18.53)**	8	1.25 (−9.12 to 11.62)	6	12.10 (−5.73 to 29.93)
PEF (L/min)	7	−33.2 (−130.8 to 64.4)	10	18.4 (−26.9 to 63.6)	9	7.5 (−15.9 to 31.0)
Blood cell counts	8		10		9	
White cell count ($\times 10^9$/L)		**3.03 (1.64 to 4.42)**		**4.98 (3.52 to 6.45)**		**4.13 (2.28 to 5.97)**
Neutrophils ($\times 10^9$/L)		**2.851 (1.764 to 3.937)**		**4.520 (3.159 to 5.880)**		**4.283 (2.807 to 5.759)**
Lymphocytes ($\times 10^9$/L)		−0.016 (−0.670 to 0.639)		**0.432 (0.006 to 0.857)**		−0.066 (−0.531 to 0.399)
Eosinophils ($\times 10^9$/L)†		0.56 (0.28 to 1.12)		**0.62 (0.42 to 0.92)**		**0.44 (0.26 to 0.75)**
Basophils ($\times 10^9$/L)		−0.025 (−0.052 to 0.001)		0.013 (−0.030 to 0.057)		0.004 (−0.024 to 0.032)
Sputum cell counts	4		5		6	
Macrophages % of total (%)		−8.452 (−20.401 to 3.498)		10.547 (−23.640 to 44.734)		1.371 (−7.254 to 9.996)
Neutrophils % of total (%)		**16.837 (3.009 to 30.665)**		−18.792 (−72.261 to 34.676)		3.713 (−7.974 to 15.400)
Eosinophils % of total (%)†		8.06 (0.01 to 6326.42)		0.28 (0.06 to 1.23)		**0.14 (0.04 to 0.56)**
Lymphocytes % of total (%)		−0.478 (−2.115 to 1.158)		−0.108 (−0.390 to 0.174)		−0.199 (−0.920 to 0.522)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.

Results are presented as mean difference in change (95% CI) apart from FENO, blood eosinophil count, and sputum percentage of total eosinophils.

Statistically significant results are highlighted in bold.

*Difference between prednisolone and placebo are calculated using posttreatment measurements only.

†Modeled on a log-scale. Results are presented as ratios (95% CI).

been introduced during the recruitment process because of poor patient acceptance for additional systemic corticosteroid exposure. Exclusion of patients on maintenance OCS may lead to further selection bias toward those with milder disease or certain subphenotypes. The male predominance in our study population may reflect sex differences in study uptake, albeit patients with noneosinophilic asthma are much more likely to be female than those with eosinophilic asthma.[47] Second, patients had very few residual symptoms after mepolizumab and mild airway obstruction. This could limit the capacity to detect effects from prednisolone. Third, we did not test for prednisolone responsiveness before mepolizumab treatment, which makes any differences after mepolizumab treatment difficult to interpret. Fourth, the number of patients with complete sputum results was low because of difficulty obtaining samples in a highly treated and stable asthma population. Results may be difficult to interpret due to small numbers, particularly after stratifications. In addition, our analysis is subject to multiple testing, which increases the chance of a type 1 error within our study. Consequently, our findings, which reached the threshold for statistical significance, should be interpreted with appropriate caution. Fourteen patients had paired sputum samples to calculate change after prednisolone and placebo treatment. The percentage of sputum eosinophils increased after placebo treatment. This is unlikely from poor inhaled corticosteroid adherence because patients were recruited from asthma centers with vigorous prebiologic adherence testing, such as INhaler Compliance Assessment monitoring and FENO suppression testing.[48] In addition, FENO is extremely sensitive to inhaled corticosteroid treatment and is expected to rise in the presence of nonadherence. Mean FENO values before (37 ppb) and after mepolizumab treatment (36 ppb and 40 ppb at pretreatment visits) were similar and did not rise after 2 weeks of placebo. This suggests that the increase in percentage of sputum eosinophils is more likely from natural variations in sputum eosinophil count over time.[49] Lastly, a major limitation is that prednisolone was given during stable rather than exacerbation state. As such, patients had well-controlled asthma symptoms, which may not be amenable to further improvements. A placebo-controlled study of prednisolone during exacerbations on mepolizumab is needed. However, this presents obvious ethical challenges because of the risk of fatal attacks.

CONCLUSIONS

SEA remains OCS-responsive following IL-5 inhibition with mepolizumab, although the clinical benefit of additional OCS is less clear. In patients on mepolizumab, improvements in small-airway obstruction were dissociated from additional reduction in T2 inflammation. Our findings suggest that the clinical efficacy of prednisolone and phenotype for corticosteroid responsiveness should not be assumed after biologic therapy. Although OCSs will inevitably have a continued role in severe asthma management in years to come, we should aim to differentiate patients who are corticosteroid-responsive from those who are not after biologic therapy to reduce unnecessary corticosteroid exposure. We should also be cautious with previous interpretations of biomarkers for corticosteroid response following biologic treatment. Larger studies are needed to clarify the clinical benefits of prednisolone in SEA after IL-5 inhibition and during exacerbation states. This will become essential as we move toward increased use of targeted biologic therapies for moderate to severe asthma.

Acknowledgments

We acknowledge the work of the laboratory staff and clinical staff across the 4 study sites for their contribution to this work: Vanessa Brown, Martha McIlvenny, Tracyanne Grandison, Steven Smith, Sarah Edwards, Clare Conolly, Hattie Ellis, Linda Cheyne, Marc Jones, Samantha Thulborn, Tilly Downs, William Monteiro, Hannah Arnold, Claudia Micieli, and Stephen Drain and thank the patients for their participation.

REFERENCES

1. Kerkhof M, Tran TN, Soriano JB, Golam S, Gibson D, Hillyer EV, et al. Healthcare resource use and costs of severe, uncontrolled eosinophilic asthma in the UK general population. Thorax 2018;73:116-24.
2. Bahadori K, Doyle-Waters MM, Marra C, Lynd L, Alasaly K, Swiston J, et al. Economic burden of asthma: a systematic review. BMC Pulmon Med 2009;9:24.
3. O'Neill S, Sweeney J, Patterson CC, Menzies-Gow A, Niven R, Mansur AH, et al. The cost of treating severe refractory asthma in the UK: an economic analysis from the British Thoracic Society Difficult Asthma Registry. Thorax 2015;70:376-8.
4. Sweeney J, Patterson CC, Menzies-Gow A, Niven RM, Mansur AH, Bucknall C, et al. Comorbidity in severe asthma requiring systemic corticosteroid therapy: cross-sectional data from the Optimum Patient Care Research Database and the British Thoracic Difficult Asthma Registry. Thorax 2016;71:339-46.
5. Bleecker ER, Menzies-Gow AN, Price DB, Bourdin A, Sweet S, Martin AL, et al. Systematic literature review of systemic corticosteroid use for asthma management. Am J Respir Crit Care Med 2020;201:276-93.
6. Bourdin A, Adcock I, Berger P, Bonniaud P, Chanson P, Chenivesse C, et al. How can we minimise the use of regular oral corticosteroids in asthma? Eur Respir Rev 2020;29:190085.
7. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. J Allergy Clin Immunol 2015;135:299-310. quiz 1.
8. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009;360:973-84.
9. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
10. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. N Engl J Med 2014;371:1189-97.
11. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med 2014;371:1198-207.
12. Chupp GL, Bradford ES, Albers FC, Bratton DJ, Wang-Jairaj J, Nelsen LM, et al. Efficacy of mepolizumab add-on therapy on health-related quality of life and markers of asthma control in severe eosinophilic asthma (MUSCA): a randomised, double-blind, placebo-controlled, parallel-group, multicentre, phase 3b trial. Lancet Respir Med 2017;5:390-400.
13. Pavord ID. Mepolizumab, quality of life, and severe eosinophilic asthma. Lancet Respir Med 2017;5:362-3.
14. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti-IL5 therapies for asthma. Cochrane Database Syst Rev 2017;9:CD010834.
15. Shrimanker R. Mepolizumab and the response to oral prednisolone in patients with severe eosinophilic asthma. Eur Respir J 2017;PA3967:50.
16. National Institute for Health and Care Excellence. National Institute for Heathcare and Care Excellence Guidelines for mepolizumab for treating severe eosinophilic asthma. Accessed February 3, 2021. https://www.nice.org.uk/guidance/ta671

17. Scottish Medicines Consortium. Scottish Medicines Consortium Guidelines for mepolizumab (Nucala). Accessed April 8, 2019. https://www.scottishmedicines.org.uk/medicines-advice/mepolizumab-nucala-abbreviated-smc2139/
18. Mehrotra DV. A recommended analysis for 2 × 2 crossover trials with baseline measurements. Pharm Stat 2014;13:376-87.
19. Wellek S, Blettner M. On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications. Dtsch Arztebl Int 2012;109:276-81.
20. Dente FL, Bacci E, Bartoli ML, Cianchetti S, Costa F, Di Franco A, et al. Effects of oral prednisone on sputum eosinophils and cytokines in patients with severe refractory asthma. Ann Allergy Asthma Immunol 2010;104:464-70.
21. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. "Refractory" eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. Am J Respir Crit Care Med 2004;170:601-5.
22. Bentley AM, Hamid Q, Robinson DS, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 1996;153:551-6.
23. Djukanović R, Homeyard S, Gratziou C, Madden J, Walls A, Montefort S, et al. The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. Am J Respir Crit Care Med 1997;155:826-32.
24. Fukakusa M, Bergeron C, Tulic MK, Fiset PO, Al Dewachi O, Laviolette M, et al. Oral corticosteroids decrease eosinophil and CC chemokine expression but increase neutrophil, IL-8, and IFN-gamma-inducible protein 10 expression in asthmatic airway mucosa. J Allergy Clin Immunol 2005;115:280-6.
25. Sousa AR, Marshall RP, Warnock LC, Bolton S, Hastie A, Symon F, et al. Responsiveness to oral prednisolone in severe asthma is related to the degree of eosinophilic airway inflammation. Clin Exp Allergy 2017;47:890-9.
26. Kupczyk M, Haque S, Middelveld RJ, Dahlen B, Dahlen SE, BIOAIR Investigators. Phenotypic predictors of response to oral glucocorticosteroids in severe asthma. Respir Med 2013;107:1521-30.
27. Busby J, Khoo E, Pfeffer PE, Mansur AH, Heaney LG. The effects of oral corticosteroids on lung function, type-2 biomarkers and patient-reported outcomes in stable asthma: a systematic review and meta-analysis. Respir Med 2020;173:106156.
28. Büttner C, Lun A, Splettstoesser T, Kunkel G, Renz H. Monoclonal anti-interleukin-5 treatment suppresses eosinophil but not T-cell functions. Eur Respir J 2003;21:799-803.
29. Robinson D, Hamid Q, Ying S, Bentley A, Assoufi B, Durham S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. Am Rev Respir Dis 1993;148:401-6.
30. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med 2018;378:2486-96.
31. Walsh GM. Eosinophil apoptosis and clearance in asthma. J Cell Death 2013;6:17-25.
32. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: eosinophilic airway inflammation in nonallergic asthma. Nat Med 2013;19:977-9.
33. Birrell MA, Battram CH, Woodman P, McCluskie K, Belvisi MG. Dissociation by steroids of eosinophilic inflammation from airway hyperresponsiveness in murine airways. Respir Res 2003;4:3.
34. Murphy RC, Hallstrand TS. Exploring the origin and regulatory role of mast cells in asthma. Curr Opin Allergy Clin Immunol 2021;21:71-8.
35. Cahill KN, Katz HR, Cui J, Lai J, Kazani S, Crosby-Thompson A, et al. KIT inhibition by imatinib in patients with severe refractory asthma. N Engl J Med 2017;376:1911-20.
36. Schofield JPR, Burg D, Nicholas B, Strazzeri F, Brandsma J, Staykova D, et al. Stratification of asthma phenotypes by airway proteomic signatures. J Allergy Clin Immunol 2019;144:70-82.
37. Phipatanakul W, Mauger DT, Sorkness RL, Gaffin JM, Holguin F, Woodruff PG, et al. Effects of age and disease severity on systemic corticosteroid responses in asthma. Am J Respir Crit Care Med 2017;195:1439-48.
38. Spahn JD, Szefler SJ, Surs W, Doherty DE, Nimmagadda SR, Leung DY. A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity. J Immunol 1996;157:2654-9.
39. Pazdrak K, Straub C, Maroto R, Stafford S, White WI, Calhoun WJ, et al. Cytokine-induced glucocorticoid resistance from eosinophil activation: protein phosphatase 5 modulation of glucocorticoid receptor phosphorylation and signaling. J Immunol 2016;197:3782-91.
40. Dunican EM, Fahy JV. Asthma and corticosteroids: time for a more precise approach to treatment. Eur Respir J 2017;49:1701167.
41. Tan KS, Grove A, McLean A, Gnosspelius Y, Hall IP, Lipworth BJ. Systemic corticosteriod rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. Am J Respir Crit Care Med 1997;156:28-35.
42. Ellul-Micallef R, Fenech FF. Effect of intravenous prednisolone in asthmatics with diminished adrenergic responsiveness. Lancet 1975;2:1269-71.
43. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011;184:602-15.
44. Busse WW, Wenzel SE, Casale TB, FitzGerald JM, Rice MS, Daizadeh N, et al. Baseline FeNO as a prognostic biomarker for subsequent severe asthma exacerbations in patients with uncontrolled, moderate-to-severe asthma receiving placebo in the LIBERTY ASTHMA QUEST study: a post-hoc analysis. Lancet Respir Med 2021;9:1165-73.
45. Donohue JF, Jain N. Exhaled nitric oxide to predict corticosteroid responsiveness and reduce asthma exacerbation rates. Respir Med 2013;107:943-52.
46. McDowell PJ, Diver S, Yang F, Borg C, Busby J, Brown V, et al. The inflammatory profile of exacerbations in patients with severe refractory eosinophilic asthma receiving mepolizumab (the MEX study): a prospective observational study. Lancet Respir Med 2021;9:1174-84.
47. Heaney LG, Perez de Llano L, Al-Ahmad M, Backer V, Busby J, Canonica GW, et al. Eosinophilic and noneosinophilic asthma: an expert consensus framework to characterize phenotypes in a global real-life severe asthma cohort. Chest 2021;160:814-30.
48. Butler CA, Heaney LG. Fractional exhaled nitric oxide and asthma treatment adherence. Curr Opin Allergy Clin Immunol 2021;21:59-64.
49. Li H, Zhang Q, Wang J, Gao S, Li C, Wang J, et al. Variability of type 2 inflammatory markers guiding biologic therapy of severe asthma: a 5-year retrospective study from a single tertiary hospital. World Allergy Organ J 2021;14:100547.

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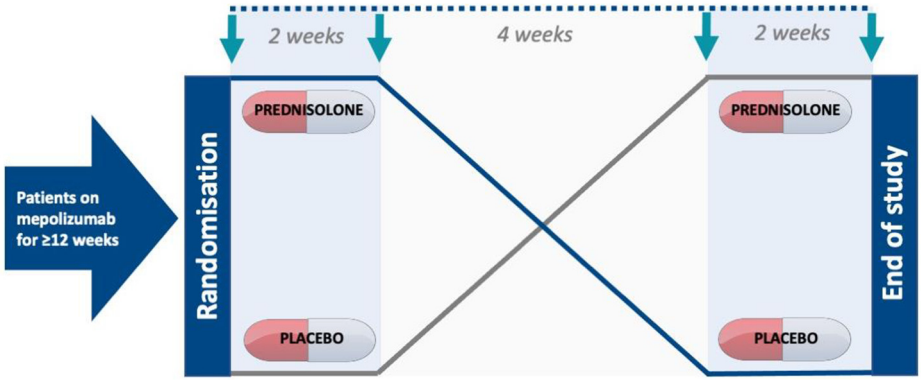

FIGURE E1. Study design.

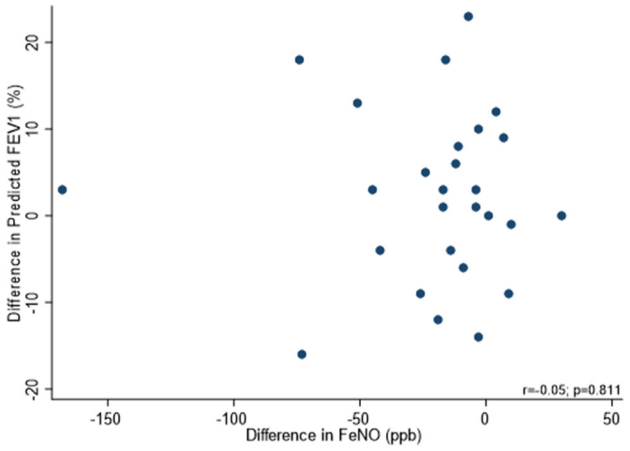

FIGURE E2. Relationship between FENO change and percentage of FEV_1 predicted change after prednisolone treatment.

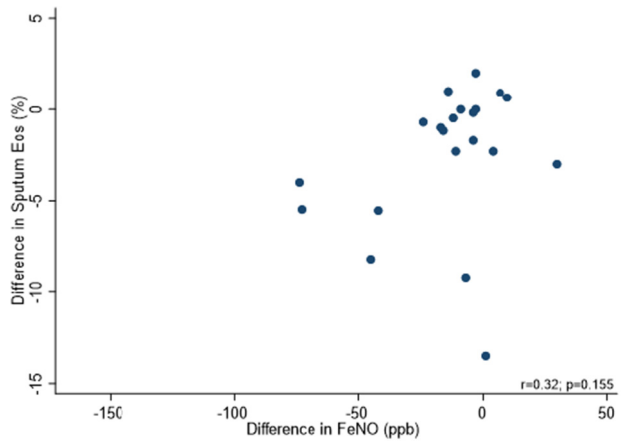

FIGURE E3. Relationship between FENO change and percentage of sputum eosinophils change after prednisolone treatment.

TABLE E1. Inclusion and exclusion criteria*

Inclusion criteria
1. Age ≥18 and ≤80 y at consent
2. Able and willing to provide written informed consent and to comply with the study protocol
3. Severe asthma diagnosis confirmed after assessment by an asthma specialist
4. Suitable for mepolizumab as per the National Institute for Health and Care Excellence/Scottish Medicines Consortium clinical guidelines
Exclusion criteria:
1. Maintenance OCSs treatment within the past 4 wk
2. Acute exacerbation requiring OCSs in the 4 wk before consent
3. Other clinically significant medical disease or uncontrolled concomitant disease despite treatment that is likely, in the opinion of the investigator, to require a change in therapy or impact the ability to participate in the study or be significantly worsened by OCSs
4. History of current alcohol, drug, or chemical abuse or past abuse that would impair or risk the subject's full participation in the study, in the opinion of the investigator
5. Treatment with an investigational agent within 30 d of visit 1 (or 5 half-lives of the investigational agent, whichever is longer)
6. Women of child-bearing potential who are pregnant, lactating, planning pregnancy during the study period, or are unwilling to use a highly effective form of contraception
7. Known hypersensitivity to prednisolone or its excipients
8. Previous psychiatric adverse reactions to steroid therapy in the past
9. Concomitant medication with systemic antifungals such as ketoconazole, retinoids, tetracycline, other systemic immunosuppressants, eg, ciclosporin, azathioprine, mycophenolate, and live vaccines during the crossover trial
10. Received another biologic for asthma within the last 4 mo
11. For the subgroup of patients established on mepolizumab at study entry, received the first mepolizumab injection <12 wk before consent

*Patients from 4 specialist UK asthma centers (Glasgow, Oxford, Leicester, and Belfast) were assessed and enrolled between December 2018 and October 2019 if they fulfilled the criteria mentioned in the table.

TABLE E2. Study schedule of procedures for new mepolizumab patients

Procedures	Visit 1: Baseline Premepolizumab (−7 to 0 d)	Visit 2 (V2): Pre−Rx 1 Minimum 12 wk postmepolizumab	Visit 3 (V3): Post−Rx 1 14 d after V2 (−2 to +1 d)	Visit 4 (V4): Pre−Rx 2 Minimum 4 wk after V3	Visit 5: Post−Rx 2 14 d after V4 (−2 to +1 d)
Written informed consent	x				
Check inclusion/exclusion criteria	x				
Medical check pretreatment		x		x	
Medical history	x				
Vital signs (BP, heart rate, oxygen saturation, respiratory rate, temperature)	x				
Weight & height	x	x*			
Brief physical examination	x				
FENO	x	x	x	x	x
Oscillometry	x	x	x	x	x
Blood test for full blood cell count and biobanking	x	x	x	x	x
Questionnaires (ACQ-5, Mini-AQLQ, SGRQ, Symptoms VAS, SNOT-20)	x	x	x	x	x
Serum prednisolone & cortisol			x		x
Urine pregnancy test (if applicable)	x	x		x	
Induced sputum	x	x	x	x	x
Spirometry	x	x	x	x	x
Randomization to prednisolone/placebo first		x			
Supply of prednisolone or placebo		x		x	
Count of tablets returned			x		x
Exacerbation recording		x	x	x	x
Review/reporting of side effects		x	x	x	x
Concomitant medication check, review of inhaler technique, and medication adherence	x	x	x	x	x

Procedures	Visit 2 Pre−Rx 1 Minimum 12 wk postmepolizumab	Visit 3 Post−Rx 1 14 d after V2 (−2 to +1 d)	Visit 4 Pre−Rx 2 Minimum 4 wk after V3	Visit 5 Post−Rx 2 14 d after V4 (−2 to +1 d)
Written informed consent	x			
Check inclusion/exclusion criteria	x			
Medical check pretreatment	x		x	
Medical history including clinical information before initiation of mepolizumab	x			

(continued)

TABLE E2. *(Continued)*

	Visit 2 Pre−Rx 1	Visit 3 Post−Rx 1	Visit 4 Pre−Rx 2	Visit 5 Post−Rx 2
Procedures	**Minimum 12 wk postmepolizumab**	**14 d after V2 (−2 to +1 d)**	**Minimum 4 wk after V3**	**14 d after V4 (−2 to +1 d)**
Vital signs (BP, heart rate, oxygen saturation, respiratory rate, temperature)	x			
Weight & height	x			
Brief physical examination	x			
FENO	x	x	x	x
Oscillometry	x	x	x	x
Blood test for full blood cell count and biobanking	x	x	x	x
Questionnaires (ACQ-5, Mini-AQLQ, SGRQ, Symptoms VAS, SNOT-20)	x	x	x	x
Serum prednisolone & cortisol		x		x
Urine pregnancy test (if applicable)	x		x	
Induced sputum	x	x	x	x
Spirometry	x	x	x	x
Randomization to prednisolone/placebo first	x			
Supply of prednisolone or placebo	x		x	
Count of tablets returned		x		x
Exacerbation recording	x	x	x	x
Review/reporting of side effects	x	x	x	x
Concomitant medication check, review of inhaler technique, and medication adherence	x	x	x	x

BP, Blood pressure; *Rx*, treatment; *SNOT*, Sino-Nasal Outcome Test.
*Weight only.

TABLE E3. Study assessments

Symptoms and quality Cof life

Asthma symptom control and quality of life were measured using ACQ-5, Mini-AQLQ, SGRQ, and VAS for asthma symptoms.

ACQ-5 is a short questionnaire that measures asthma control over the past week. It is made up of 5 questions, and the final score ranges from 0 to 6, with higher scores indicating poorer asthma control. A score of 0-0.75 suggests well-controlled asthma and a score of >1.5 suggests poorly controlled asthma. The MCID is 0.5.

Mini-AQLQ measures asthma-related quality of life using 15 questions and 2-wk recall. The score ranges from 1 to 7, with higher scores indicating better quality of life. The MCID is 0.5. There are 4 domains, which are symptoms, activity limitation, emotional function, and environmental exposure. Each domain is also scored from 1 to 7.

SGRQ measures the impact of obstructive airways disease on overall health and quality of life over the past 3 mo. It comprises 50 questions in total and measures 3 components: symptoms, activities, and impact. The total score ranges from 0 to 100, with higher scores indicating poor quality of life and more limitations. The MCID is 4; however, a change of 8 and 12 suggests a significant and very significant treatment effect, respectively.

A VAS was used to measure overall asthma control, breathlessness, and cough, each measured on a scale ranging from 0 to 10. The lower end of the scale represented no symptoms, and the higher end of the scale represented worst possible symptoms.

Nasal symptoms were measured using the SNOT-20 questionnaire at posttreatment visits (3 & 5) for all patients. New mepolizumab patients also had premepolizumab baseline measurements taken at visit 1. SNOT-20 assesses the severity of chronic rhinosinusitis using 20 questions. The total score ranges from 0 to 100. Higher scores are indicative of severe symptoms.

FENO

FENO was measured using Niox VERO (Circassia, Northbrook House, Robert Robinson Avenue, The Oxford Science Park, Oxford, OX4 4GA, United Kingdom) across all sites. FENO is a biomarker of type 2 airway inflammation and used in monitoring of airway inflammation in patients with asthma. Cutoff points vary between guidelines (ATS/ERS, GINA, and NICE). We used ATS/ERS cutoff points for interpretation. FENO <25 ppb indicates low eosinophilic inflammation and >50 ppb indicated high eosinophilic inflammation. For patients with FENO >50 ppb, a change of 20% from baseline is significant. For patients with FENO <50 ppb, a change of 10 ppb from baseline is significant. FENO measurements were taken before airway oscillometry and spirometry.

AOS

AOS can detect small-airway dysfunction and measure treatment response to inhaled corticosteroids and bronchodilators. It measures patients' airflow and oscillation response, which is used to calculate the various components of resistance to breathing. We used TremoFlo C-100 (Thorasys, Montreal, Canada) across all sites for AOS measurements. A minimum of 3 measurements were taken. A coefficient of variation of ≤15% is required for valid measurements, which include R5, R5-20, X5, and AX. R5 is a measure of low-frequency resistance at 5 Hz and indicates the overall resistance of the respiratory system. R20 is a measure of midfrequency resistance and indicates the resistance of the conducting airways. R5-20 is the difference between R5 and R20. This is an important measurement in asthma because it reflects the contribution of small-airway resistance to the total respiratory resistance. This is the most sensitive measure of small-airway constriction compared with other frequencies. X5 represents low-frequency reactance, which indicates loss of compliance (ie, lung stiffening) and small-airways obstruction. AX is area of reactance, which is the area under the curve between the reactance values for 5 Hz and the resonance frequency. It is a useful indicator of small-airway patency and strongly correlates with R5-20. Patients with asthma have higher R5, R5-20, and AX values and more negative X5 measurements.

Spirometry

Lung function was measured by spirometry according to ATS/ERS guidelines while the patient was on usual asthma treatments. Acceptable results required the largest 2 measurements of FVC within 0.15 L and FEV_1 within 0.15 L. A minimum of 3 maneuvers and a maximum of 8 maneuvers were performed. Spirometry was performed before sputum induction.

Sputum induction

Sputum induction was performed using 3%, 4%, and 5% hypertonic saline solutions delivered by an ultrasonic nebulizer. Baseline FEV_1 was measured and repeated after each cycle of sputum induction before escalating to more concentrated hypertonic saline. The procedure was stopped if significant symptoms occurred, a 20% or 400 mL drop in FEV_1 was demonstrated, or adequate sputum sample has been obtained. Samples were processed locally within 2 h of expectoration for cell differential counts.

Study treatment adherence

Study treatments started 1 d after pretreatment visits (2 & 4). Participants were asked to keep a daily written record of the dose of study medication taken. This was reviewed by study staff along with a tablet count at posttreatment visits. Adherence is defined as ≥75% consumption of the prescribed duration. Serum cortisol and prednisolone levels were taken at posttreatment visits, but results were not accessed until end of the study once patients had been unblinded. Cortisol suppression and measurable prednisolone levels provided supportive evidence of adherence to study medications.

AOS, Airwave oscillometry; *ATS*, American Thoracic Society; *AX*, reactance area; *ERS*, European Respiratory Society; *FVC*, forced vital capacity; *GINA*, Global Initiative for Asthma; *MCID*, minimal clinically important change; *NICE*, National Institute for Health and Care Excellence; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.

TABLE E4. Reasons for study withdrawal

Participant	Reason for exclusion or withdrawal
Before randomization	
1 (GL05)	Mepolizumab stopped
2 (GL09)	Mepolizumab stopped
3 (GL13)	Recurrent exacerbation on mepolizumab
After randomization	
4 (BE03)	Did not complete study treatment
5 (GL06)	Mepolizumab stopped after diagnosis of malignancy
6 (GL15)	Unable to take study treatment because capsules contained gelatin

TABLE E5. Prednisolone vs placebo for all subjects (n = 27)

	Prednisolone		Placebo		Prednisolone vs placebo	
Measurement	**Preprednisolone, difference (95% CI)**	**Change after prednisolone, difference (95% CI)**	**Preplacebo (95% CI)**	**Change after placebo (95% CI)**	**n**	**Difference (95% CI)**
ACQ-5 score	0.6 (0.2 to 2.0)	−0.10 (−0.38 to 0.19)	0.6 (0.0 to 1.8)	0.21 (−0.19 to 0.60)	27	−0.23 (−0.58 to 0.11)
Woken at night	0.0 (0.0 to 2.0)	−0.15 (−0.39 to 0.09)	0.0 (0.0 to 1.0)	0.26 (−0.25 to 0.77)		−0.27 (−0.72 to 0.18)
Symptoms in morning	0.0 (0.0 to 2.0)	0.00 (−0.29 to 0.29)	0.0 (0.0 to 2.0)	0.15 (−0.35 to 0.65)		−0.15 (−0.57 to 0.27)
Limited in activities	1.0 (0.0 to 2.0)	−0.11 (−0.45 to 0.22)	1.0 (0.0 to 2.0)	0.19 (−0.23 to 0.60)		−0.24 (−0.67 to 0.20)
Shortness of breath	1.0 (0.0 to 2.0)	0.04 (−0.35 to 0.42)	1.0 (0.0 to 2.0)	0.33 (−0.13 to 0.80)		−0.18 (−0.57 to 0.21)
Wheezing	1.0 (0.0 to 2.0)	−0.26 (−0.81 to 0.30)	1.0 (0.0 to 3.0)	0.11 (−0.29 to 0.51)		**−0.41 (−0.81 to −0.01)**
Mini-AQLQ score	6.0 (5.0 to 6.6)	0.02 (−0.17 to 0.22)	6.1 (4.0 to 6.9)	−0.03 (−0.28 to 0.22)	27	0.03 (−0.24 to 0.31)
Symptoms score	6.0 (4.8 to 6.8)	0.01 (−0.34 to 0.37)	6.2 (4.8, 6.8)	−0.13 (−0.37, 0.11)		0.08 (−0.26 to 0.42)
Activity limitation score	6.0 (4.8 to 6.8)	−0.01 (−0.26 to 0.24)	6.5 (4.3 to 7.0)	−0.06 (−0.51 to 0.38)		0.02 (−0.40 to 0.45)
Emotional function score	6.0 (4.7 to 7.0)	0.01 (−0.18 to 0.20)	6.7 (3.7 to 7.0)	−0.02 (−0.35 to 0.30)		0.10 (−0.25 to 0.45)
Environmental stimuli score	5.7 (4.0 to 7.0)	0.09 (−0.13 to 0.30)	6.3 (3.7 to 7.0)	0.17 (−0.06 to 0.41)		−0.13 (−0.42 to 0.16)
SGRQ score	22.9 (13.1 to 46.2)	−0.18 (−2.90 to 2.53)	21.1 (9.6 to 46.4)	−0.08 (−3.12 to 2.96)	27	0.24 (−3.20 to 3.69)
Symptoms score	30.5 (16.0 to 53.6)	0.12 (−4.48 to 4.73)	27.8 (9.6 to 60.9)	3.07 (−1.45 to 7.59)		−2.79 (−7.60 to 2.02)
Activity score	36.5 (17.1 to 61.1)	0.50 (−4.65 to 5.65)	41.4 (17.4 to 59.5)	0.07 (−4.54 to 4.68)		1.02 (−3.68 to 5.71)
Impact score	13.2 (5.3 to 36.9)	−0.45 (−3.72 to 2.83)	11.7 (3.6 to 30.1)	−1.33 (−4.84 to 2.17)		0.23 (−2.95 to 3.41)
VAS (cm)					23	
Overall symptoms	1.0 (0.0 to .0)	−0.53 (−1.29 to 0.24)	1.1 (0.1 to 3.0)	−0.14 (−0.90 to 0.61)		0.11 (−0.58 to 0.80)
Shortness of breath	1.0 (0.3 to 5.0)	−0.58 (−1.28 to 0.12)	1.0 (0.2 to 2.5)	−0.06 (−0.88 to 0.76)		−0.19 (−0.90 to 0.53)
Cough	1.8 (0.3 to 3.6)	−0.58 (−1.28 to 0.11)	1.0 (0.2 to 3.1)	−0.04 (−0.98 to 0.89)		−0.16 (−0.98 to 0.66)
SNOT-20 score*		12.0 (1.0 to 34.0)		10.0 (2.0 to 32.0)	26	−2.62 (−8.37 to 3.14)
Airwave oscillometry					26	
R5 ($cmH_2O \cdot s/L$)	3.8 (2.8 to 4.8)	0.27 (−0.12 to 0.66)	3.5 (2.8 to 5.1)	−0.07 (−0.47 to 0.33)		0.27 (−0.16 to 0.70)
R5-20 ($cmH_2O \cdot s/L$)	0.7 (0.3 to 1.4)	0.24 (−0.02 to 0.50)	0.9 (0.3 to 1.8)	0.20 (−0.51 to 0.92)		−0.25 (−0.99 to 0.49)
AX (cmH_2O/L)	10.3 (4.9 to 27.7)	**7.34 (1.80 to 12.88)**	10.6 (5.0 to 38.7)	−0.54 (−6.27 to 5.19)		5.33 (−0.76 to 11.41)
X5 ($cmH_2O \cdot s/L$)	−1.0 (−1.8 to 0.3)	**−0.61 (−1.06 to −0.15)**	−1.1 (−2.6 to −0.4)	0.20 (−0.26 to 0.67)		−0.26 (−0.74 to 0.21)
Spirometry†					27	
FEV_1 (L)	2.48 ± 0.76	0.086 (−0.021 to 0.193)	2.48 ± 0.77	−0.023 (−0.118 to 0.073)		0.105 (−0.004 to 0.213)
FEV_1 % of predicted (%)	77.7 ± 18.9	2.11 (−1.18 to 5.41)	77.5 ± 19.7	−0.02 (−3.22 to 3.18)		2.28 (−1.48 to 6.04)
FVC (L)	3.73 ± 1.00	0.085 (−0.099 to 0.269)	3.77 ± 0.98	−0.048 (−0.175 to 0.079)		0.083 (−0.028 to 0.194)
FVC % of predicted (%)	91.4 ± 14.8	2.26 (−2.61 to 7.12)	93.0 ± 15.5	−0.78 (−4.03 to 2.48)		1.31 (−2.04 to 4.66)
FEV_1/FVC (%)	67.1 ± 12.7	0.24 (−1.82 to 2.30)	66.0 ± 12.1	0.30 (−1.81 to 2.40)		1.20 (−0.31 to 2.71)
FEF_{25-75} (L/s)	1.75 ± 0.98	**0.449 (0.127 to 0.771)**	1.88 ± 0.97	−0.089 (−0.249 to 0.071)	18	**0.484 (0.151 to 0.816)**
FEF_{25-75} % of predicted (%)	52.1 ± 18.3	**4.95 (1.09 to 8.81)**	54.9 ± 24.2	−1.06 (−6.19 to 4.08)	18	5.40 (−1.23 to 12.04)
PEF (L/min)†	462 ± 127	−6.0 (−35.8 to 23.8)	449 ± 152	−1.9 (−23.3 to 19.6)	26	−1.1 (−30.4 to 28.2)
F_{ENO} (ppb)‡	35 ± 0.85	**0.67 (0.48 to 0.94)**	39 ± 0.58	1.00 (0.82 to 1.20)	27	**0.59 (0.46 to 0.75)**
Blood cell counts					27	
White cell count ($\times 10^9$/L)	6.4 (5.5, 7.1)	**4.39 (3.42 to 5.36)**	6.2 (5.5 to 7.2)	0.30 (−0.13, 0.74)		**4.12 (3.15 to 5.09)**
Neutrophils ($\times 10^9$/L)	3.80 (2.87 to 4.57)	**4.276 (3.459 to 5.092)**	3.70 (3.20 to 4.23)	**0.374 (0.020 to 0.729)**		**3.914 (3.132 to 4.696)**

(continued)

TABLE E5. *(Continued)*

	Prednisolone		Placebo		Prednisolone vs placebo	
Measurement	**Preprednisolone, difference (95% CI)**	**Change after prednisolone, difference (95% CI)**	**Preplacebo (95% CI)**	**Change after placebo (95% CI)**	**n**	**Difference (95% CI)**
Lymphocytes ($\times 10^9$/L)	1.80 (1.40 to 2.40)	0.111 (−0.261 to 0.482)	1.89 (1.60 to 2.10)	−0.080 (−0.200 to 0.041)		0.138 (−0.168 to 0.445)
Eosinophils ($\times 10^9$/L)‡	0.06 ± 0.65	**0.52 (0.38 to 0.71)**	0.05 ± 0.67	**1.21 (1.01 to 1.45)**		**0.51 (0.38 to 0.69)**
Basophils ($\times 10^9$/L)	0.04 (0.00 to 0.10)	−0.026 (−0.058 to 0.006)	0.03 (0.00 to 0.04)	0.004 (−0.007 to 0.016)		−0.002 (−0.023 to 0.018)
Sputum cell counts					15	
Macrophages % of total (%)	28.95 (16.78 to 55.75)	−6.815 (−21.717 to 8.088)	24.25 (8.70 to 38.00)	−5.676 (−22.695 to 11.343)		1.450 (−6.037 to 8.937)
Neutrophils % of total (%)	60.16 (29.75 to 76.02)	11.807 (−1.477 to 25.091)	69.00 (46.20 to 88.30)	7.034 (−12.560 to 26.628)		1.654 (−8.685 to 11.993)
Eosinophils % of total (%)‡	1.48 ± 1.77	**0.20 (0.07 to 0.54)**	0.93 ± 1.51	**2.12 (1.06 to 4.25)**		**0.29 (0.11 to 0.74)**
Lymphocytes % of total (%)	0.22 (0.00 to 1.50)	**−0.829 (−1.602 to 0.056)**	0.00 (0.00 to 0.20)	0.064 (−0.242 to 0.370)		−0.217 (−0.616 to 0.183)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.
Pretreatment results are presented as median (Q1, Q3) and changes are presented as mean change (95% CI).
Statistically significant results are highlighted in bold.
*Measurements were taken after treatment only; hence, difference was calculated using posttreatment values only. Results are presented as posttreatment median (Q1, Q3) and mean difference in change (95% CI).
†Normally distributed. Pretreatment results are presented as mean ± SD and changes are presented as mean change (95% CI).
‡Modeled on a log-scale. Pretreatment results are shown as geometric means ± geo SD and changes are presented as ratios (95% CI).

TABLE E6. Prednisolone vs placebo in patients with low (<0.10 $\times 10^9$/L) and high blood eosinophil count (≥0.10 $\times$ 10^9/L) (n = 27)

	Low blood eosinophil count (n = 22)		High blood eosinophil count (n = 5)	
Measurement	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**
ACQ-5 score	22	−0.28 (−0.71 to 0.14)	5	−0.26 (−0.66 to 0.14)
Mini-AQLQ score	22	0.09 (−0.24 to 0.41)	5	0.05 (−0.29 to 0.39)
SGRQ score	22	0.54 (−3.53 to 4.60)	5	−2.06 (−9.37 to 5.26)
VAS (cm)	20		3	
Overall symptoms		0.12 (−0.68 to 0.93)		0.04 (−0.02 to 0.10)
Shortness of breath		−0.23 (−1.05 to 0.59)		0.25 (−0.24 to 0.74)
Cough		−0.21 (−1.14 to 0.72)		−0.07 (−0.92 to 0.79)
SNOT-20 score*	21	−3.48 (−10.59 to 3.64)	5	1.00 (−5.51 to 7.51)
Airwave oscillometry	22		4	
R5 ($cmH_2O \cdot s/L$)		0.20 (−0.30 to 0.69)		**0.86 (0.78 to 0.95)**
R5-20 ($cmH_2O \cdot s/L$)		0.13 (−0.19 to 0.44)		−3.95 (−8.74 to 0.84)
AX (cmH_2O/L)		5.66 (−1.50 to 12.81)		3.31 (−1.38 to 8.01)
X5 ($cmH_2O \cdot s/L$)		−0.23 (−0.78 to 0.31)		−0.47 (−1.24 to 0.29)
FENO (ppb)†	22	**0.57 (0.44 to 0.74)**	5	0.65 (0.26 to 1.64)
Spirometry	22		5	
FEV_1 (L)		0.086 (−0.038 to 0.211)		0.192 (−0.056 to 0.440)
FEV_1/FVC (%)		1.21 (−0.33 to 2.74)		8.80 (−6.66 to 24.26)
FEF_{25-75} (L/s)	15	**0.523 (0.128 to 0.919)**	3	0.251 (−0.060 to 0.561)
FEF_{25-75} % of predicted (%)	15	4.64 (−3.16 to 12.44)	3	**7.87 (1.73 to 14.01)**
PEF (L/min)	21	10.1 (−16.7 to 36.9)		−36.7 (−159.9 to 86.6)
Blood cell counts	22		5	
White cell count ($\times$ 10^9/L)		**3.94 (2.92 to 4.97)**		**5.89 (2.60 to 9.19)**
Neutrophils ($\times$ 10^9/L)		−1.9 (−3.9 to 0.2)		**5.305 (2.043 to 8.568)**
Lymphocytes ($\times$ 10^9/L)		0.062 (−0.307 to 0.431)		**0.520 (0.143 to 0.897)**
Basophils ($\times$ 10^9/L)		−0.005 (−0.027 to 0.017)		0.023 (−0.031 to 0.077)
Sputum cell counts	12		3	
Macrophages % of total (%)		5.275 (−7.846 to 18.396)		**−6.168 (−7.413 to −4.923)**
Neutrophils % of total (%)		−7.842 (−26.681 to 10.997)		**17.182 (12.989 to 21.374)**
Eosinophils % of total (%)†		0.42 (0.17 to 1.03)		**0.02 (0.01 to 0.05)**
Lymphocytes % of total (%)		−0.291 (−0.785 to 0.202)		0.055 (−0.764 to 0.873)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.

Results are presented as mean difference in change (95% CI) apart from FENO, blood eosinophil count, and sputum percentage of total eosinophils.

Statistically significant results are highlighted in bold.

*Difference between prednisolone and placebo is calculated using posttreatment measurements only.

†Modeled on a log-scale. Results are presented as ratios (95% CI).

TABLE E7. Prednisolone vs placebo in patients with low (<1.5) and high ACQ-5 score (≥1.5) (n = 27)

	Low ACQ score (n = 18)		High ACQ score (n = 9)	
Measurement	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**
Mini-AQLQ score	18	0.11 (−0.17 to 0.40)	9	−0.10 (−0.76 to 0.56)
SGRQ score	18	−0.78 (−4.71 to 3.15)	9	2.26 (−5.03 to 9.55)
VAS (cm)	15		8	
Overall symptoms		0.32 (−0.06 to 0.70)		−0.38 (−2.34 to 1.57)
Shortness of breath		−0.26 (−0.93 to 0.42)		−0.03 (−1.76 to 1.71)
Cough		−0.09 (−1.01 to 0.83)		−0.20 (−1.99 to 1.59)
SNOT-20 total score*	18	−2.06 (−8.40 to 4.29)	8	−3.88 (−19.01 to 11.26)
Airwave oscillometry	18		8	
R5 ($cmH_2O \cdot s/L$)		0.14 (−0.22 to 0.51)		−0.36 (−1.10 to 0.38)
R5-20 ($cmH_2O \cdot s/L$)		−0.36 (−1.40 to 0.68)		−0.17 (−0.46 to 0.12)
AX (cmH_2O/L)		**4.97 (0.19 to 9.75)**		4.85 (−8.64 to 18.34)
X5 ($cmH_2O \cdot s/L$)		−0.24 (−0.66 to 0.19)		−0.31 (−1.78 to 1.16)
FENO (ppb)†	18	**0.66 (0.48 to 0.90)**	9	**0.50 (0.39 to 0.66)**
Spirometry	18		9	
FEV_1 (L)		**0.118 (0.005 to 0.231)**		0.193 (−0.031 to 0.417)
FEV_1/FVC (%)		**1.51 (0.32 to 2.69)**		1.70 (−1.91 to 5.31)
FEF_{25-75} (L/s)	12	0.112 (−0.096 to 0.321)	6	**1.247 (0.675 to 1.818)**
FEF_{25-75} % of predicted (%)	12	4.41 (−3.92 to 12.75)	6	6.65 (−5.53 to 18.83)
PEF (L/min)	18	5.7 (−16.4 to 27.7)	8	−21.2 (−118.3 to 75.9)
Blood cell counts	18		9	
White cell count ($\times 10^9$/L)		**3.93 (2.82 to 5.03)**		**4.51 (2.39 to 6.63)**
Neutrophils ($\times 10^9$/L)		**3.886 (2.954 to 4.819)**		**3.965 (2.368 to 5.563)**
Lymphocytes ($\times 10^9$/L)		0.019 (−0.402 to 0.440)		0.379 (−0.007 to 0.766)
Eosinophils ($\times 10^9$/L)†		**0.58 (0.37 to 0.92)**		**0.50 (0.27 to 0.91)**
Basophils ($\times 10^9$/L)		−0.013 (−0.038 to 0.012)		0.017 (−0.006 to 0.041)
Sputum cell counts	10		5	
Macrophages % of total (%)		2.109 (−3.690 to 7.908)		14.010 (−11.138 to 39.158)
Neutrophils % of total (%)		1.193 (−7.650 to 10.036)		−5.995 (−54.425 to 42.435)
Eosinophils % of total (%)†		0.34 (0.10 to 1.20)		0.22 (0.03 to 1.48)
Lymphocytes % of total (%)		−0.200 (−0.804 to 0.403)		**−0.226 (−0.445 to −0.007)**

ACQ, Asthma Control Questionnaire; *AX*, reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.

Results are presented as mean difference in change (95% CI) apart from FENO, blood eosinophil count, and sputum percentage of total eosinophils.

Statistically significant results are highlighted in bold.

*Difference between prednisolone and placebo is calculated using posttreatment measurements only.

†Modeled on a log-scale. Results are presented as ratios (95% CI).

TABLE E8. Prednisolone vs placebo—Per-protocol cohort (n = 26)

	Prednisolone		Placebo		Prednisolone vs placebo	
Measurement	**Preprednisolone, difference (95% CI)**	**Change after prednisolone, difference (95% CI)**	**Preplacebo, difference (95% CI)**	**Change after placebo, difference (95% CI)**	**n**	**Difference (95% CI)**
ACQ-5 score	0.6 (0.2 to 2.0)	−0.10 (−0.40 to 0.20)	0.6 (0.2 to 1.8)	0.22 (−0.19 to 0.62)	26	−0.24 (−0.60 to 0.12)
Mini-AQLQ score	5.9 (5.0 to 6.6)	0.03 (−0.18 to 0.23)	6.1 (4.0 to 6.8)	−0.04 (−0.30 to 0.22)	26	0.04 (−0.25 to 0.33)
SGRQ score	25.0 (14.7 to 46.2)	−0.22 (−3.04 to 2.60)	24.8 (9.7 to 46.4)	−0.08 (−3.25 to 3.08)	26	0.22 (−3.35 to 3.80)
VAS (cm)					22	
Overall symptoms	1.0 (0.3 to 4.0)	−0.55 (−1.35, 0.25)	1.2 (0.5 to 3.0)	−0.15 (−0.94 to 0.64)		0.12 (−0.61 to 0.84)
Shortness of breath	1.0 (0.3 to 5.0)	−0.60 (−1.34, 0.13)	1.0 (0.5 to 2.5)	−0.06 (−0.91 to 0.80)		−0.20 (−0.94 to 0.55)
Cough	2.0 (0.5 to 3.6)	**−0.69 (−1.38 to −0.01)**	1.0 (0.3 to 3.1)	−0.05 (−1.02 to 0.93)		−0.27 (−1.10 to 0.57)
SNOT-20 total score*	—	13.5 (2.0 to 34.0)	—	10.0 (3.0 to 32.0)	25	−2.68 (−8.69 to 3.33)
Airwave oscillometry					25	
R5 ($cmH_2O \cdot s/L$)	3.8 (2.8 to 4.8)	0.24 (−0.16 to 0.63)	3.7 (2.9 to 5.1)	−0.09 (−0.50 to 0.33)		0.09 (−0.26 to 0.44)
R5-20 ($cmH_2O \cdot s/L$)	0.7 (0.3, 1.4)	0.19 (−0.06 to 0.44)	0.9 (0.3 to 1.8)	0.20 (−0.54 to 0.94)		−0.44 (−1.17 to 0.30)
AX (cmH_2O/L)	9.5 (4.9 to 21.7)	**6.43 (0.99 to 11.86)**	10.7 (5.4 to 38.7)	−0.88 (−6.80 to 5.04)		2.79 (−2.27 to 7.85)
X5 ($cmH_2O \cdot s/L$)	−1.0 (−1.6, −0.3)	**−0.55 (−1.01 to −0.09)**	−1.0 (−2.6 to −0.4)	0.23 (−0.25 to 0.71)		−0.14 (−0.61 to 0.33)
Spirometry†					26	
FEV_1 (L)	2.50 ± 0.77	0.093 (−0.017 to 0.204)	2.49 ± 0.79	−0.021 (−0.120 to 0.079)		**0.125 (0.020 to 0.230)**
FEV_1 % of predicted (%)	77.5 ± 19.3	2.35 (−1.05 to 5.74)	76.9 ± 19.8	0.09 (−3.23 to 3.42)		3.12 (−0.48 to 6.72)
FVC (L)	3.75 ± 1.01	0.093 (−0.098 to 0.283)	3.79 ± 0.99	−0.059 (−0.189 to 0.071)		0.104 (−0.002 to 0.210)
FVC % of predicted (%)	91.2 ± 15.1	2.50 (−2.54 to 7.54)	92.7 ± 15.8	−1.11 (−4.42 to 2.20)		1.92 (−1.28 to 5.13)
FEV_1/FVC (%)	67.1 ± 12.9	0.25 (−1.90 to 2.39)	65.7 ± 12.2	0.58 (−1.53 to 2.68)		1.58 (0.00 to 3.15)
FEF_{25-75} (L/s)	1.75 ± 0.98	**0.449 (0.127 to 0.771)**	1.88 ± 0.97	−0.089 (−0.249 to 0.071)	18	**0.484 (0.151 to 0.816)**
FEF_{25-75} % of predicted (%)	52.1 ± 18.3	**4.95 (1.09 to 8.81)**	54.9 ± 24.2	−1.06 (−6.19 to 4.08)	18	5.40 (−1.23 to 12.04)
PEF (L/min)†	464 ± 129	−4.8 (−35.7 to 26.2)	450 ± 155	−3.3 (−25.4 to 18.9)	25	1.9 (−27.9 to 31.8)
FENO (ppb)‡	36 ± 0.86	**0.66 (0.46 to 0.93)**	40 ± 0.58	0.99 (0.81 to 1.21)	26	**0.58 (0.45 to 0.75)**
Blood cell counts					26	
White cell count ($\times 10^9$/L)	6.6 (5.5, 7.1)	**4.47 (3.47 to 5.47)**	6.3 (5.6, 7.2)	0.27 (−0.18 to 0.72)		**4.22 (3.22 to 5.21)**
Neutrophils ($\times 10^9$/L)	3.83 (2.87, 4.57)	**4.357 (3.525, 5.190)**	3.70 (3.40 to 4.23)	0.335 (−0.025 to 0.695)		**4.025 (3.249 to 4.801)**
Eosinophils ($\times 10^9$/L)‡	0.06 ± 0.65	**0.52 (0.37 to 0.72)**	0.05 ± 0.68	1.21 (1.00 to 1.46)		**0.51 (0.37 to 0.68)**
Sputum cell counts					14	
Macrophages % of total (%)	27.89 (15.77 to 50.50)	−3.193 (−16.797 to 10.411)	22.73 (7.61 to 34.85)	−3.322 (−20.669 to 14.026)		1.027 (−6.606 to 8.660)
Neutrophils % of total (%)	63.06 (36.00 to 77.97)	8.352 (−3.459 to 20.164)	69.88 (41.72 to 90.89)	4.636 (−15.538 to 24.810)		2.078 (−8.378 to 12.533)
Eosinophils % of total (%)‡	1.62 ± 1.77	**0.16 (0.06 to 0.42)**	1.00 ± 1.51	**2.19 (1.04 to 4.57)**		**0.21 (0.10 to 0.46)**
Lymphocytes % of total (%)	0.19 (0.00 to 1.00)	**−0.542 (−1.054 to −0.029)**	0.00 (0.00 to 0.10)	0.126 (−0.168 to 0.421)		−0.211 (−0.635 to 0.213)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.
Pretreatment results are shown as median (Q1, Q3) and changes are shown as mean change (95% CI).
Statistically significant results are highlighted in bold.
*Measurements were taken after treatment only; hence, difference was calculated using posttreatment values only. Results are presented as posttreatment median (Q1, Q3) and mean difference in change (95% CI).
†Normally distributed. Pretreatment results are presented as mean ± SD and changes are presented as mean change (95% CI).
‡Modeled on a log-scale. Pretreatment results are presented as geometric means ± log SD and changes are presented as ratios (95% CI).

TABLE E9. Prednisolone vs placebo in patients with low (<25 ppb), intermediate (25-50 ppb), and high FENO (>50 ppb)—Per-protocol cohort (n = 26)

	Low FENO (n = 7)		Intermediate FENO (n = 10)		High FENO (n = 9)	
Measurement	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**	**n**	**Difference in change for prednisolone vs placebo**
ACQ-5 score	**7**	0.38 (−0.31 to 1.08)	10	−0.39 (−1.10 to 0.31)	9	−0.31 (−0.95 to 0.33)
Mini-AQLQ score	7	−0.34 (−0.80 to 0.12)	10	0.25 (−0.41 to 0.91)	9	0.15 (−0.34 to 0.63)
SGRQ score	7	2.43 (−1.69 to 6.55)	10	0.78 (−5.78 to 7.34)	9	−1.59 (−9.43 to 6.26)
VAS (cm)						
Overall symptoms	6	0.54 (−0.26 to 1.34)	9	−0.19 (−1.95 to 1.57)	7	0.29 (−0.09 to 0.66)
Shortness of breath	6	0.62 (−0.09 to 1.34)	9	−0.07 (−1.63 to 1.49)	7	**−0.72 (−1.31 to −0.13)**
Cough	6	**2.52 (1.24 to 3.80)**	9	−0.69 (−1.72 to 0.34)	7	−1.12 (−2.70 to 0.47)
SNOT-20 total score*	7	−2.29 (−15.64 to 11.07)	10	−2.70 (−13.79 to 8.39)	8	−3.00 (−15.88 to 9.88)
Airwave oscillometry						
R5 ($cmH_2O \cdot s/L$)	6	−0.15 (−0.92 to 0.62)	10	0.25 (−0.40 to 0.90)	9	0.11 (−0.22 to 0.43)
R5-20 ($cmH_2O \cdot s/L$)	6	−2.12 (−4.80 to 0.57)	10	0.08 (−0.19 to 0.34)	9	0.12 (−0.05 to 0.30)
AX (cmH_2O/L)	6	−2.00 (−9.35 to 5.34)	10	7.90 (−3.35 to 19.15)	9	1.81 (−1.38 to 5.00)
X5 ($cmH_2O \cdot s/L$)	6	0.05 (−0.70 to 0.80)	10	−0.46 (−1.69 to 0.78)	9	−0.04 (−0.36 to 0.29)
Spirometry						
FEV_1 (L)	7	**0.171 (0.002 to 0.340)**	10	0.047 (−0.161 to 0.255)	9	0.128 (−0.028 to 0.284)
FEV_1/FVC (%)	7	**2.67 (1.11 to 4.22)**	10	**2.87 (0.23 to 5.51)**	9	0.91 (−2.07 to 3.90)
FEF_{25-75} (L/s)	4	**0.358 (0.082 to 0.633)**	8	0.578 (−0.183 to 1.339)	6	0.326 (−0.028 to 0.680)
FEF_{25-75} % of predicted (%)	4	**10.54 (2.56 to 18.53)**	8	1.25 (−9.12 to 11.62)	6	12.10 (−5.73 to 29.93)
PEF (L/min)	6	−24.4 (−140.9 to 92.2)	10	18.4 (−26.9 to 63.6)	9	7.5 (−15.9 to 31.0)
Blood cell counts						
White cell count ($\times 10^9$/L)	7	**3.31 (1.81 to 4.80)**	10	**4.98 (3.52 to 6.45)**	9	**4.13 (2.28 to 5.97)**
Neutrophils ($\times 10^9$/L)	7	**3.148 (2.201 to 4.094)**	10	**4.520 (3.159 to 5.880)**	9	**4.283 (2.807 to 5.759)**
Eosinophils ($\times 10^9$/L)†	7	0.54 (0.28 to 1.06)	10	**0.62 (0.42 to 0.92)**	9	**0.44 (0.26 to 0.75)**
Sputum cell counts						
Macrophages % of total (%)	3	−7.583 (−24.982 to 9.817)	5	10.547 (−23.640 to 44.734)	6	1.371 (−7.254 to 9.996)
Neutrophils % of total (%)	3	16.866 (−4.019 to 37.751)	5	−18.792 (−72.261 to 34.676)	6	3.713 (−7.974 to 15.400)
Eosinophils % of total (%)†	3	0.01 (0.00 to 37.58)	5	0.28 (0.06 to 1.23)	6	**0.14 (0.04 to 0.56)**
Lymphocytes % of total (%)	3	0.465 (−1.081 to 2.011)	5	−0.108 (−0.390 to 0.174)	6	−0.199 (−0.920 to 0.522)

AX, Reactance area; *FVC*, forced vital capacity; *PEF*, peak expiratory flow; *R5*, resistance at 5 Hz; *R5-20*, difference between resistance at 5 Hz and 20 Hz; *SNOT*, Sino-Nasal Outcome Test; *X5*, reactance at 5 Hz.
Results are presented as mean difference in change (95% CI) apart from FENO, blood eosinophil count, and sputum percentage of total eosinophils.
Statistically significant results are highlighted in bold.
*Difference between prednisolone and placebo is calculated using posttreatment measurements only.
†Modeled on a log-scale. Results are presented as ratios (95% CI).