

LETTER TO THE EDITOR



WILEY

Eosinophil-derived neurotoxin and clinical outcomes with mepolizumab in severe eosinophilic asthma

To the Editor,

Severe eosinophilic asthma is characterized by increased blood eosinophil levels and recurrent exacerbations, and often associated with nasal polyposis.¹ Eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), granule proteins released by eosinophils, are markers of eosinophil activation and have been identified as potential biomarkers of type 2 eosinophilic disease in patients with asthma.²⁻⁴

The anti-interleukin (IL)-5 monoclonal antibody mepolizumab has been shown to reduce peripheral blood eosinophil counts (PBEC) and asthma exacerbations versus placebo in clinical studies.⁵⁻⁷ Elevated PBEC and frequent exacerbations are key determinants for predicting patients with severe asthma who are most likely to respond to mepolizumab;^{6,8} however, identifying additional biomarkers may enhance patient selection. This post hoc analysis of data from the Phase III MENSA study (GSK ID: 115588; NCT01691521)⁷ investigated the relationship between baseline type 2 biomarkers and clinical outcomes in patients with severe asthma receiving mepolizumab.

MENSA was a randomized, double-blind trial in patients aged ≥ 12 years with severe eosinophilic asthma.⁷ Patients were randomized (1:1:1) to receive mepolizumab 75mg intravenously (IV) or 100 mg subcutaneously (SC), or placebo every 4 weeks for 32 weeks plus standard of care (see Appendix S1 for further details). Levels of the biomarkers EDN, ECP, chemokines (CCL-13, CCL-17, CCL-22 and eotaxin-1), periostin and IL-13 were determined using serum samples taken at Weeks 0 (randomization) and 32 (exit).

Endpoints included the following: annualized rate of clinically significant exacerbations (defined as worsening of asthma requiring systemic corticosteroids for ≥ 3 days and/or hospitalization/emergency department visit); ratio to baseline of PBEC; change from baseline in prebronchodilator forced expiratory volume in 1 second (FEV_1), asthma control questionnaire (ACQ)-5 score, St George's Respiratory Questionnaire (SGRQ) total score (all at Week 32). Post hoc analysis assessments included baseline biomarker levels by PBEC subgroups (<150 , $150-300$, $300-500$, ≥ 500 cells/ μ L) and presence of nasal polyposis at screening, correlation between biomarker levels and PBEC at baseline, and change from baseline in biomarker levels at Week 32. Additionally, the ratio of PBEC to baseline, changes from baseline in FEV_1 , ACQ-5 and SGRQ scores, and annualized exacerbations (all at Week 32) were assessed in high

($>$ median) and low ($\leq$ median) EDN (median = 57.6 μ g/L) or ECP (median = 21.06 μ g/L) subgroups at baseline (initial results determined which biomarkers were analysed further [EDN and ECP]). The annualized exacerbation rate was also assessed in high/low EDN and ECP subgroups further stratified by PBEC; baseline EDN level and baseline PBEC as predictors of response to mepolizumab treatment were also evaluated. Statistical analyses are described in Appendix S1. The MENSA trial was conducted in accordance with all applicable country-specific regulatory requirements, and all patients provided written informed consent. Ethical approval was not required for this post hoc analysis.

In MENSA, 194 patients received mepolizumab 100 mg SC, 191 received mepolizumab 75 mg IV and 191 received placebo. At baseline, levels of most biomarkers increased with increasing PBEC, and the biomarkers EDN, ECP and IL-13 showed a moderately positive correlation with PBEC (Table S1). Patients with nasal polyposis also had numerically higher baseline PBEC, EDN, ECP, CCL-13, CCL-17 and IL-13 levels than those without nasal polyposis (data not shown). EDN was reduced by 70% from baseline to Week 32 with mepolizumab versus placebo, with a similar trend noted for ECP (58% reduction) (Figure S1A). In contrast, there was a trend for an increase in the levels of CCL-13, CCL-17, CCL-22 and eotaxin-1 between baseline and Week 32 with mepolizumab versus placebo (Figure S1B).

Owing to the positive correlation of EDN and ECP with PBEC, and their use as markers of eosinophil activation, we investigated clinical outcomes following mepolizumab treatment in patients with differing baseline EDN and ECP levels. Baseline PBEC was higher in patients with high versus low EDN or ECP levels at baseline and mepolizumab significantly reduced PBEC between baseline and Week 32 to a similar extent in both high and low EDN and ECP subgroups versus placebo. Mepolizumab-induced improvements in other clinical outcomes were also numerically greater in high versus low baseline EDN or ECP subgroups (Table S2); as such, EDN and ECP may predict improvements in FEV_1 .

We also found mepolizumab reduced the exacerbation rate versus placebo in both baseline EDN subgroups, with a greater reduction in patients with high versus low EDN (Figure 1). To further investigate whether this effect could be explained in terms of confounding by baseline PBEC, subgroups were stratified by baseline PBEC. In patients with baseline PBEC ≥ 300 cells/ μ L, there was a larger reduction

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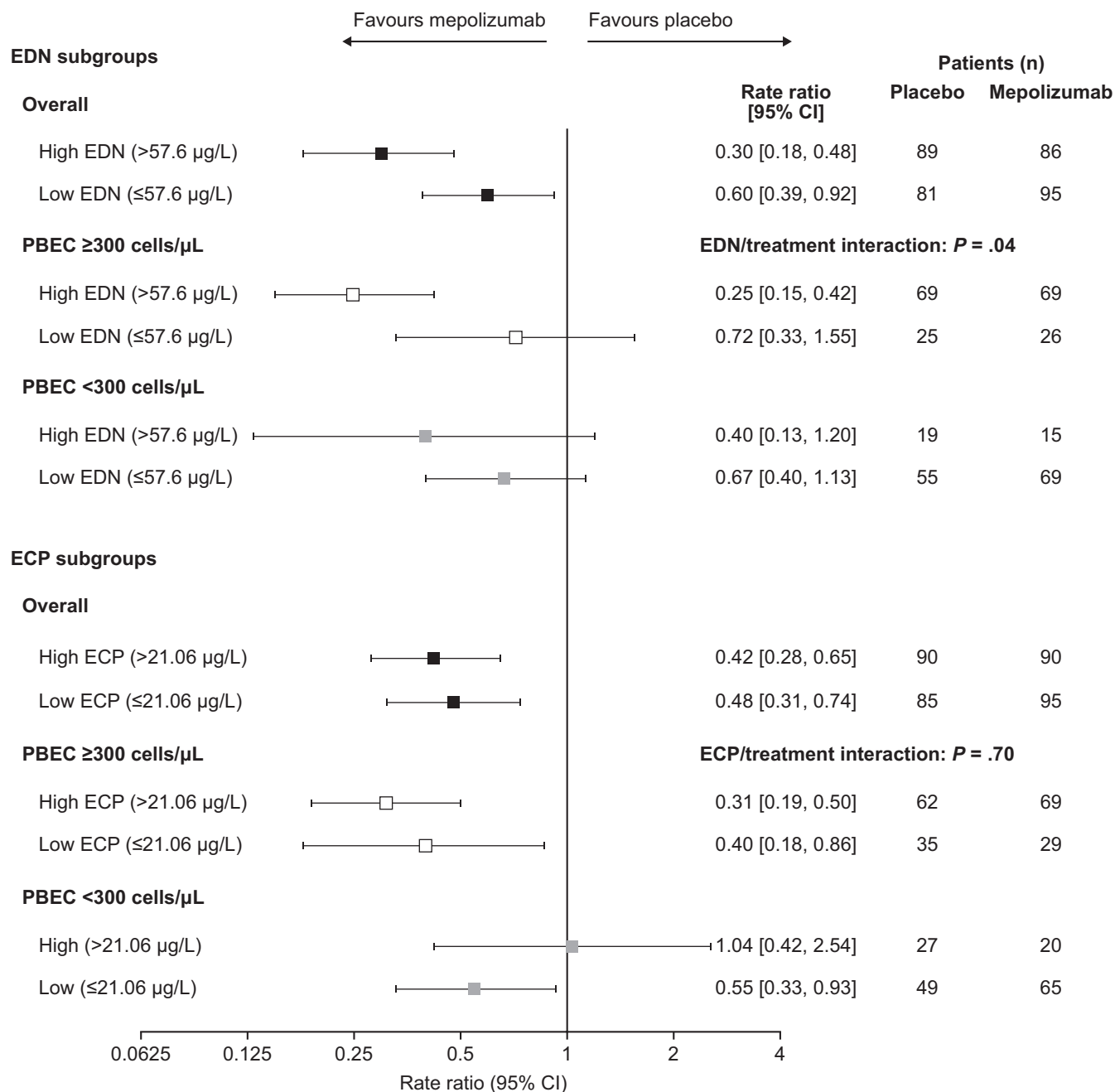


FIGURE 1 Ratio of the annual rate of clinically significant exacerbations with mepolizumab 100 mg SC versus placebo by baseline biomarker and PBEC subgroup. CI, confidence interval; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; PBEC, peripheral blood eosinophil count; SC, subcutaneous

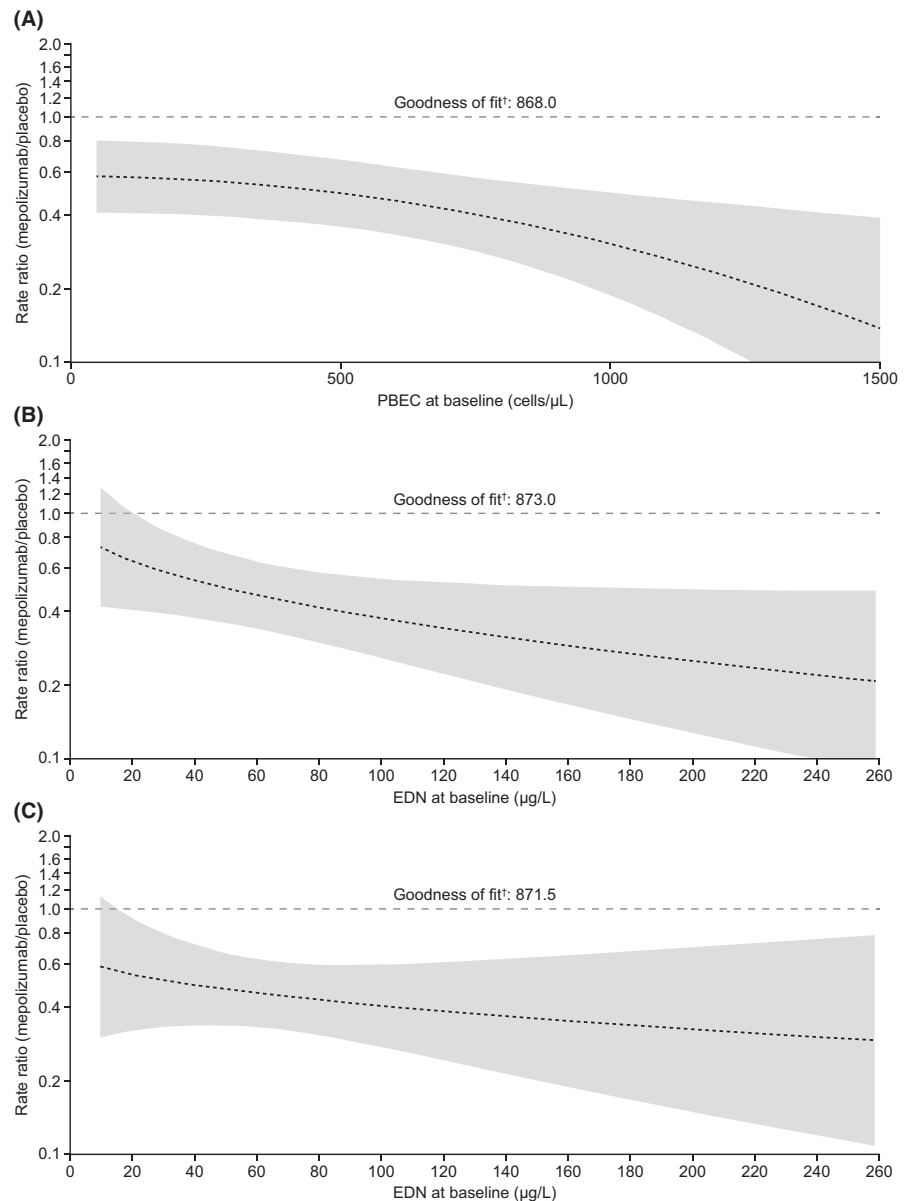
in exacerbations with mepolizumab in the high (75%) versus low (28%) baseline EDN subgroups. A similar trend was noted between the two < 300 cells/µL EDN subgroups. However, no difference was observed in the ECP subgroups. Furthermore, predicted rate ratio modelling of exacerbations demonstrated that a predictive model based on baseline PBEC was marginally better than that based on baseline EDN in terms of precision and model fit (Figure 2).

Overall, we found baseline PBEC correlated with baseline EDN levels, as reported elsewhere,⁹ with a more pronounced reduction in the placebo-adjusted annualized exacerbation rate with mepolizumab in patients with high versus low baseline EDN levels (trend

not seen with ECP). Modelling analysis results demonstrated EDN levels and the combination of EDN levels, PBEC and treatment interaction did not show improved predictive power versus that of PBEC alone for treatment response to mepolizumab regarding exacerbation reduction. This result indicates that the predictive power of EDN is largely due to its correlation with baseline PBEC and highlights that PBEC remains an important and clinically relevant biomarker for identifying patients with severe asthma who are likely to respond to mepolizumab treatment.

In conclusion, our results demonstrate that in general, patients with higher versus lower EDN levels are likely to have improved

FIGURE 2 Predicted rate ratio (95% CI) of mepolizumab 100 mg SC versus placebo for clinically significant exacerbations per year versus (A) baseline PBEC, (B) baseline EDN concentration and (C) baseline EDN concentration adjusted for baseline PBEC and treatment interaction. [†]Akaike information criterion; lower values indicate a better model fit. Shading indicates 95% CI; wider bands indicate lower precision in predicting exacerbation rate. All analyses performed using a negative binomial regression model with covariates: treatment, maintenance corticosteroid use, exacerbation number in previous year. Additional covariates: (A) baseline PBEC (square transformation) including additional term for treatment interaction; (B) baseline EDN concentration (square-root transformation) with term for treatment interaction; (C) baseline EDN concentration (square-root transformation) with term for treatment interaction and baseline PBEC (log transformation) with treatment interaction. CI, confidence interval; EDN, eosinophil-derived neurotoxin; PBEC, peripheral blood eosinophil count; SC, subcutaneous



responses to mepolizumab, although further research is needed on the role of EDN as a potential biomarker for treatment response to mepolizumab. However, baseline PBEC has greater precision as a predictive biomarker of treatment response to mepolizumab than EDN and is more widely assessed in clinical practice. Our data provide further evidence that PBEC is the most suitable biomarker identified to date for identifying patients likely to respond to mepolizumab, although the identification of additional biomarkers may further aid patient selection in the future.

KEYWORDS

asthma, asthma treatment, biomarkers, eosinophils

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assembling tables and figures, collating authors comments, grammatical editing and referencing) was provided by Roisin McCorkell, MSc, and Laura Gardner, PhD, of Fishawack Indicia Ltd, UK, and was funded by GSK.

CONFLICTS OF INTEREST

PH, SM, SB, SY and NK are employees of GlaxoSmithKline (GSK) and hold stocks/shares. FCA is a former employee of GSK and holds stocks/shares. SQ has served as a consultant to AstraZeneca, Novartis, Sanofi, Genentech, TEVA, ALK, Mundipharma and GSK; and has received lecture fees from Chiesi, Novartis, GSK, Leti, AstraZeneca and Mundipharma. AP reports grants, personal fees, nonfinancial support and other from Chiesi, AstraZeneca, Boehringer Ingelheim, Mundipharma and TEVA; personal fees and nonfinancial support from Menarini, Novartis and Zamboni; and personal fees from Sanofi, all outside the submitted work. EI has served as a consultant to and received personal

fees from AstraZeneca, Equillium, GSK, Novartis, 4D Pharma, Pneuma Respiratory, Regeneron Pharmaceuticals, Sanofi, Sienna Biopharmaceuticals and TEVA; reports nonfinancial support from TEVA, and other from Vorso Corp; and has received clinical research grants from AstraZeneca, Genentech, Novartis and Sanofi.

TRIAL REGISTRATION

Data from this post hoc analysis are from the MENSA trial, which is registered on ClinicalTrials.gov (GSK ID: MEA115588; NCT01691521).

DATA AVAILABILITY STATEMENT

Anonymized individual participant data from the study listed within this publication and its associated documents can be requested for further research from www.clinicalstudydatarequest.com.

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SUPPORTING INFORMATION

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

Supplementary materials

Eosinophil-derived neurotoxin and clinical outcomes with mepolizumab in severe eosinophilic asthma

Howarth *et al.*

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Supplementary information

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Supplementary Table 2. Mean change from baseline at Week 32 in FEV₁, ACQ-5 score and SGRQ total score.

Supplementary information

MENSA study details

The MENSA study included patients ≥ 12 years of age, with ≥ 2 exacerbations in the previous year despite receiving regular treatment with high-dose inhaled corticosteroids in the 12 months prior to screening, plus additional controller medication(s) for ≥ 3 months prior to screening. Patients also had a blood eosinophil count of ≥ 150 cells/ μL at screening or ≥ 300 cells/ μL in the previous year.¹

Measurement of biomarker levels

ECP levels were measured using the immunoCAP platform (Thermo Fisher Scientific; Waltham, MA, USA), EDN levels were measured by ELISA (Immundiagnostik AG; Bensheim, Germany), while eotaxin-1, CCL 13, CCL-17 and CCL-22 levels were measured using a multiplex immunoassay (V-PLEX, Meso Scale Discovery; Rockville, MD, USA). Assays for these biomarkers were analysed by Q² Solutions (Morrisville, North Carolina, USA). Periostin levels were assessed using an immunocapture mass-spectrometry assay, while IL-13 levels were measured using a custom immunoassay (Singulex; Alameda, CA, USA); both periostin and IL-13 assays were analysed by the GSK Bioanalysis, Biomarkers and Immunogenicity group (GSK, Ware, Hertfordshire, UK). All biomarker assays were validated for standard analytical parameters prior to their use and only data meeting pre-defined analytical acceptance criteria were reported.

Statistical analysis

Biomarker analyses were conducted using data from patients in the mepolizumab 100 mg SC and placebo subgroups; periostin and IL-13 analyses that were conducted using data from patients in the mepolizumab 75 mg IV subgroup only owing to sample availability. Analyses of mepolizumab response were based on data from patients who received placebo or mepolizumab 100 mg SC.

Analysis of clinically significant exacerbations was performed using a negative binomial regression model with the covariates treatment, use of maintenance corticosteroids and number of exacerbations in the previous year. Additional covariates for individual predictive models are indicated in the relevant figure footnote. Analyses of ratio to baseline in PBEC, and of change from baseline in FEV₁, ACQ-5 score and SGRQ total score were performed

using mixed model repeated measures for each subgroup, with the covariates mentioned previously plus baseline value, visit, and terms for the interaction of visit with baseline value and of visit with treatment group.

Reference:

1. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371(13):1198-1207.

Supplementary Table 1. PBEC and biomarker levels at baseline.

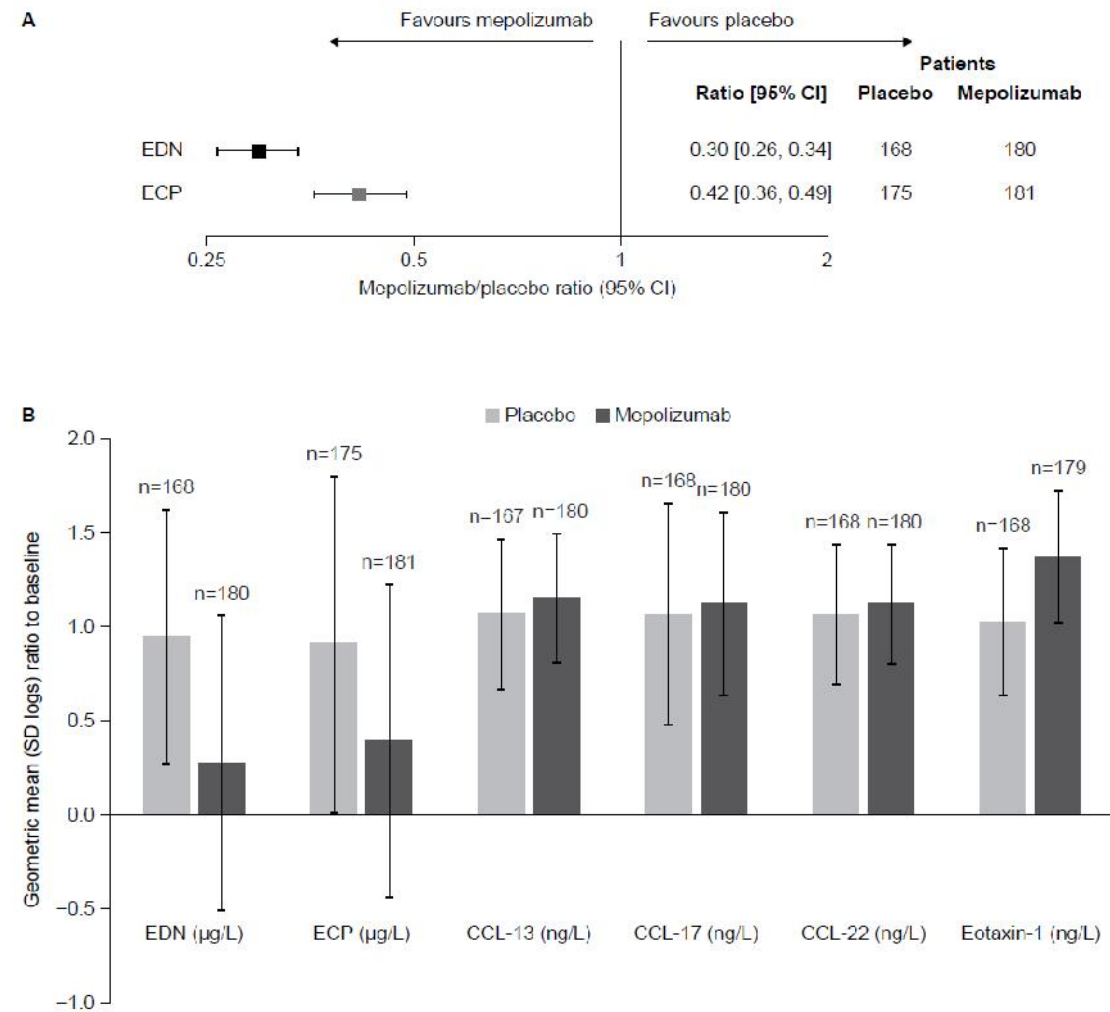
	EDN (µg/L)	ECP (µg/L)	CCL-13 (MCP-4) (ng/L)	CCL-17 (TARC) (ng/L)	CCL-22 (MDC) (ng/L)	Eotaxin-1 (ng/L)	Periostin (µg/L)	IL-13 (ng/L)
Biomarker levels by PBEC at baseline, geometric mean (SD logs)								
<150 cells/µL	30.1 (0.49) (n=63)	9.6 (0.70) (n=65)	118.7 (0.52) (n=62)	261.0 (0.57) (n=63)	855.7 (0.40) (n=63)	202.0 (0.59) (n=62)	186.3 (0.37) (n=41)	0.04 (0.89) (n=39)
150–<300 cells/µL	44.2 (0.59) (n=94)	15.8 (0.75) (n=94)	134.8 (0.47) (n=94)	294.5 (0.64) (n=94)	1155.6 (0.41) (n=94)	181.5 (0.50) (n=94)	194.5 (0.45) (n=34)	0.1 (1.18) (n=36)
300–<500 cells/µL	64.7 (0.55) (n=68)	21.6 (0.68) (n=69)	151.2 (0.41) (n=68)	321.3 (0.66) (n=68)	1153.1 (0.37) (n=68)	174.7 (0.42) (n=68)	202.6 (0.36) (n=39)	0.1 (1.13) (n=36)
≥500 cells/µL	113.1 (0.74) (n=121)	35.6 (0.84) (n=124)	162.9 (0.49) (n=121)	429.1 (0.68) (n=121)	1225.2 (0.36) (n=121)	168.6 (0.49) (n=121)	210.0 (0.35) (n=51)	0.4 (1.12) (n=50)
Correlation between biomarker levels and PBEC at baseline[†]								
Number of patients included in correlation analysis	347	356	345	346	346	345	165	161
Correlation coefficient	0.649	0.568	0.257	0.267	0.307	-0.084	0.119	0.608
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.119	0.127	<0.001

[†] A correlation coefficient >0.5 was considered a moderately positive correlation.

CCL, CC chemokine ligand; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; IL, interleukin; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; PBEC, peripheral blood eosinophil count; SD, standard deviation; TARC, thymus- and activation-regulated chemokine.

PBEC and type 2 biomarkers were log-transformed.

Supplementary Figure 1. (A) Placebo-adjusted ratio to baseline of EDN and ECP at Week 32 and (B) ratio to baseline of assessed biomarkers at Week 32 for mepolizumab 100 mg SC and placebo.



Levels of the biomarkers periostin and IL-13 were not assessed in patients who received placebo or mepolizumab 100 mg SC owing to serum sample availability.

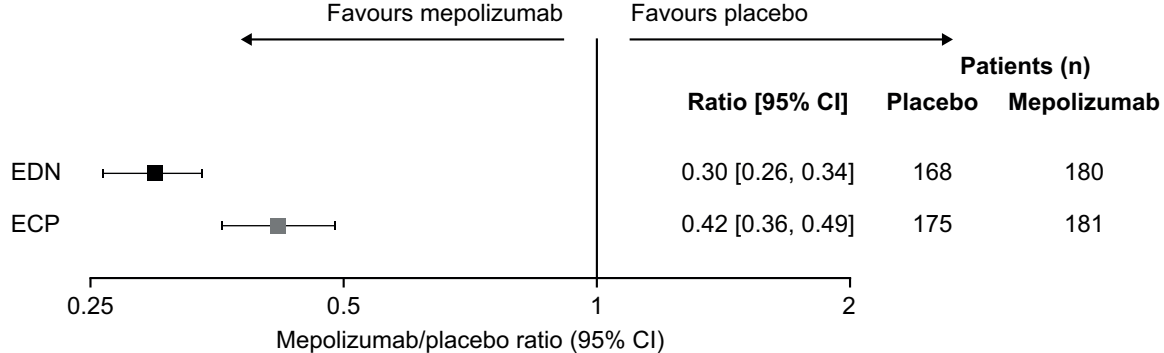
CCL, CC chemokine ligand; CI, confidence interval; ECP, eosinophil cationic protein; EDN, eosinophil derived neurotoxin; IL, interleukin; SC, subcutaneous; SD, standard deviation.

Supplementary Table 2. Mean change from baseline at Week 32 in FEV₁, ACQ-5 score and SGRQ total score.

	High EDN (>57.6 µg/L)	Low EDN (≤57.6 µg/L)	High ECP (>21.06 µg/L)	Low ECP (≤21.06 µg/L)
FEV₁				
Placebo				
LS mean change from baseline, mL (SE)	93 (44.4)	54 (40.7)	124 (45.2)	40 (40.7)
Responders [†] , % (n/N)	38 (34/89)	38 (31/81)	40 (36/90)	38 (32/85)
Mepolizumab 100 mg SC				
LS mean change from baseline, mL (SE)	255 (45.1)	136 (37.5)	248 (45.2)	136 (38.4)
LS mean difference (mepo-placebo) (95% CI)	162 (36, 288)	82 (-28, 193)	124 (-4, 252)	96 (-15, 207)
Responders [†] , % (n/N)	59 (51/86)	44 (42/95)	58 (52/90)	44 (42/95)
ACQ-5				
Placebo				
LS mean change from baseline (SE)	-0.61 (0.098)	-0.46 (0.107)	-0.53 (0.108)	-0.52 (0.093)
Responders [‡] , % (n/N)	52 (44/85)	49 (38/77)	49 (42/86)	51 (41/80)
Mepolizumab 100 mg SC				
LS mean change from baseline (SE)	-1.11 (0.099)	-0.85 (0.099)	-1.06 (0.108)	-0.88 (0.088)
LS mean difference (mepo-placebo) (95% CI)	-0.50 (-0.78, -0.23)	-0.39 (-0.68, -0.10)	-0.52 (-0.83, -0.22)	-0.36 (-0.61, -0.11)
Responders [‡] , % (n/N)	67 (56/83)	62 (53/86)	69 (59/86)	60 (52/87)
SGRQ				
Placebo				
LS mean change from baseline (SE)	-10.5 (1.62)	-8.1 (1.83)	-10.1 (1.72)	-8.4 (1.68)
Responders [§] , % (n/N)	64 (57/89)	56 (44/79)	61 (55/90)	58 (48/83)
Mepolizumab 100 mg SC				
LS mean change from baseline (SE)	-19.4 (1.66)	-13.7 (1.66)	-19.0 (1.73)	-13.9 (1.57)
LS mean difference (mepo-placebo) (95% CI)	-8.9 (-13.5, -4.3)	-5.5 (-10.5, -0.6)	-8.9 (-13.7, -4.0)	-5.6 (-10.2, -1.0)
Responders [§] , % (n/N)	78 (66/85)	72 (68/95)	76 (68/89)	73 (69/95)

[†]Responders defined as patients with a change from baseline in pre-bronchodilator FEV₁ of ≥10% and ≥80 mL at Week 32; [‡]responders defined as patients with an improvement from baseline in ACQ ≥0.5 at Week 32; [§]responders defined as patients with an improvement from baseline in SGRQ total score ≥4 at Week 32.

ACQ, Asthma Control Questionnaire; CI, confidence interval; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; FEV₁, forced expiratory volume in 1 second; LS, least squares; SC, subcutaneous; SE, standard error; SGRQ, St George's Respiratory Questionnaire.

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