ORIGINAL ARTICLE

Weight-adjusted Intravenous Reslizumab in Severe Asthma with Inadequate Response to Fixed-Dose Subcutaneous Mepolizumab

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Abstract

Rationale: Clinical benefits of fixed-dose 100-mg subcutaneous (SC) mepolizumab in prednisone-dependent patients are modest when sputum eosinophilia is not adequately controlled.

Objectives: This study compared treatment response of weight-adjusted intravenous (IV) reslizumab in patients previously treated with 100-mg SC mepolizumab.

Methods: Ten prednisone-dependent patients with asthma (sputum eosinophils >3% and blood eosinophils >300 cells/μl), who had previously received mepolizumab (100 mg SC dosed every 4 wk [Q4W]) for at least 1 year, received two infusions of placebo (Q4W) followed by four infusions of 3.0 mg/kg reslizumab Q4W in a single-blind, placebo-controlled sequential trial. Primary outcomes were reduction of eosinophils in sputum and blood. Additional outcomes included FEV_1, asthma control questionnaire, eosinophil peroxidase, IL-5, sputum and blood innate lymphoid cells group 2, eosinophil progenitor cells, and autoimmune responses.

Measurements and Main Results: IV reslizumab attenuated sputum eosinophils by 91.2% ($P = 0.002$), blood eosinophil counts by 87.4% ($P = 0.004$), and sputum eosinophil peroxidase levels by 65.5% ($P = 0.03$) compared with placebo. Attenuation of both local and systemic eosinophilia was associated with statistically significant improvements in FEV_1 ($P = 0.004$) and asthma control questionnaire five-question instrument scores ($P = 0.006$). Decrease in percent sputum eosinophil was greater with reslizumab (by 42.7%) compared with mepolizumab (by 5.0%) and this was associated with greater improvement in asthma control questionnaire ($P = 0.01$; analysis of covariance of Δ between before and after treatment, mepolizumab vs. reslizumab, adjusted for baseline prednisone). Changes in sputum IL-5 and anti–eosinophil peroxidase IgG after anti–IL-5 therapy were predictors of response.

Conclusions: Weight-adjusted IV reslizumab was superior to fixed-dose SC mepolizumab in attenuating airway eosinophilia in prednisone-dependent patients with asthma, with associated improvement in asthma control.

Clinical trial registered with www.clinicaltrials.gov (NCT 02559791).

Keywords: anti–IL-5 monoclonal antibodies; mepolizumab; reslizumab; sputum; severe eosinophilic asthma

Monoclonal antibodies directed against IL-5 are very effective therapies for patients with asthma in whom eosinophils play a dominant pathobiologic role (1).

Mepolizumab is an IgG_1 monoclonal antibody (mAb) approved for clinical use as fixed dose of 100 mg administered subcutaneously (SC; dosing every 4 wk [Q4W]), whereas reslizumab is an IgG_4 mAb approved for clinical use in a weight-adjusted dosing regimen of 3 mg/kg administered intravenously (IV; Q4W).

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At a Glance Commentary

Scientific Knowledge on the Subject: Two anti–IL-5 monoclonal antibodies, mepolizumab and reslizumab, are effective therapies for eosinophilic asthma. Although mepolizumab is approved as a fixed dose of 100 mg subcutaneously, reslizumab is administered as a weight-adjusted dose of 3 mg/kg intravenously.

What This Study Adds to the Field: Although both drugs reduce blood eosinophils to normal levels, intravenous weight-adjusted dosing of reslizumab suppresses sputum eosinophils better, and this is associated with greater improvement in asthma control and FEV_1 in patients with severe prednisone-dependent asthma.

There has not been any head-to-head comparison of the two drugs, routes of administration, or the dosing regimens. The clinical benefits of high or low doses of mepolizumab (2) administered either IV or SC (3) is comparable in patients with asthma severe enough to require high doses of inhaled corticosteroids. In contrast, in patients who are severe enough to require daily prednisone, the clinical benefits seem to be better with the higher doses of mepolizumab administered IV (4) than the lower dose administered SC (5). This is likely to be caused by the inability of the lower doses to effectively neutralize airway IL-5 and to control airway eosinophilia despite normalizing blood eosinophil count (6, 7). There is also the rare possibility of low doses of anti–IL-5 mAb worsening of airway eosinophilia by immune-complex formation or complement consumption (8). There is no information on the clinical efficacy of IV reslizumab in patients with severe prednisone-dependent asthma.

In this study, we tested the hypothesis that reslizumab would be effective in severe prednisone-dependent asthma, and higher doses of anti–IL-5 mAb would be more effective than lower doses to control airway eosinophilia and that this would be associated with better clinical outcomes. Because mepolizumab was not available for clinical use by the IV route to treat asthma, we compared the cellular and clinical outcomes of patients with prednisone-dependent asthma who were treated with mepolizumab, 100 mg SC, with treatment with reslizumab, 3 mg/kg IV. Some of the results of this study have been accepted in the form of an abstract (9).

Methods

Trial Design and Patient Recruitment

Thirteen patients with prednisone-dependent eosinophilic asthma (sputum eosinophilia >3% and blood eosinophils >300/μl) from our clinic who had previously participated in an open label clinical trial of mepolizumab, 100 mg SC Q4W (MEA115661) for at least 1 year were invited to participate in this trial after a washout period of at least 1 year during which they were reestablished on their baseline treatment. Ten patients met the inclusion criteria (*see* Table E1 in the online supplement enlisting all criteria for patient recruitment) and they were recruited into a sequential clinical trial of 2 months of placebo followed by 4 months of weight-adjusted IV reslizumab. All patients had evidence of asthma confirmed by bronchodilator reversibility of 12% and Δ200 ml after 200–400 μg of short-acting β_2 agonist, and/or methacholine challenge test less than 8 mg/ml; and documented history of persistent eosinophilia (sputum eosinophils ⩾3% and/or blood eosinophils ⩾300/μl) despite maintenance treatment with systemic glucocorticoids (5–30 mg per day of prednisone or its equivalent) before entering the study.

The study was divided into two treatment phases separated by a wash-out period where the patients were not on any monoclonal therapy (Figure 1). The first phase involved monthly treatment with 100 mg SC mepolizumab for 1 year. In the wash-out phase (after discontinuation of mepolizumab), all patients were reviewed monthly and were re-established on their respective optimized maintenance dose of daily oral and inhaled corticosteroids and long-acting bronchodilators. For the second phase of the study, each patient received two monthly infusions of a placebo, followed by four infusions of weight-adjusted drug (reslizumab, 3 mg/kg, Q4W,

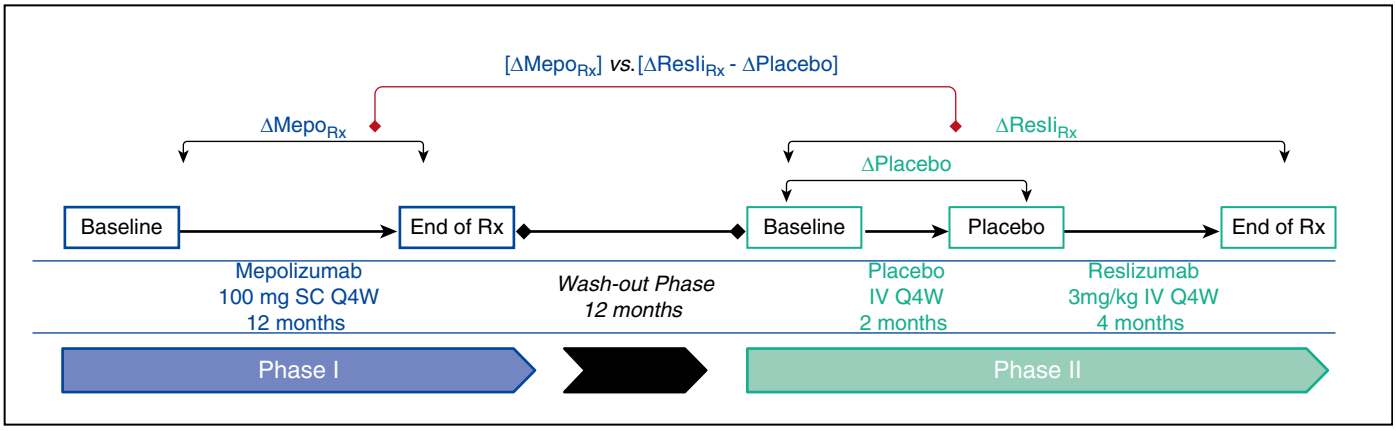

Figure 1. Schematic of study design. The study was conducted in two distinct phases. Phase I consisted of subcutaneous (SC) 100 mg mepolizumab treatment (Rx), dosed every 4 weeks (Q4W), followed by a wash-out phase, and then start of phase II with 2 months of placebo (Q4W, intravenous [IV]) and 4 months of active drug (reslizumab, 3 mg/kg, Q4W IV). Clinical and exploratory outcomes were evaluated at five time points: *1*) premepolizumab baseline, *2*) end of mepolizumab Rx, *3*) prereslizumab baseline, *4*) end of placebo, and *5*) end of reslizumab Rx. Mepo = mepolizumab; Resli = reslizumab.

Table 1. Patient Demographics

Patient Characteristics	Mepolizumab (*n* = 10)	Reslizumab (*n* = 10)	*P* Value
FEV_1 % predicted	53.7 ± 13.7	47.6 ± 14.8	0.11
VC % predicted	66.3 ± 10.1	65.1 ± 15.9	0.57
FEV_1/VC	57.4 ± 9.3	63.0 ± 9.7	0.002*
ACQ-5	1.7 ± 0.8	2.04 ± 1.4	0.37
Blood eosinophil, $\times 10^9$/L	0.3 ± 0.2	0.5 ± 0.2	0.06
Sputum eosinophil, %	14.9 ± 18	30.4 ± 15	0.07
Prednisone dose, mg/daily (median, min–max)	15 (7.5–30)	10 (5–25)	0.11
Inhaled corticosteroid, μg daily (median, min–max)	1,750 (1,000–2,500)	1,625 (1,000 –2,400)	0.19

Definition of abbreviations: ACQ-5 = asthma control questionnaire, five-question instrument; max = maximum; min = minimum.
Values are presented as mean ± SD; inhaled corticosteroid is given as equivalent of fluticasone propionate; values are those recorded at the time patients were evaluated for mepolizumab treatment and the start of the reslizumab trial.
*Statistically significant difference.

IV). The infusions were prepared by a research pharmacist who remained blinded to the clinical details, and were administered by a study coordinator. The patients and the clinical and immunologic assessors were also blinded to the sequence of allocation.

The study was conducted in accordance with Good Clinical Practice guidelines and Declaration of Helsinki, with approval from the Hamilton integrated Research Ethics Board (HiREB), St Joseph's Healthcare, Hamilton, Ontario. Each patient provided written informed consent prior to participation.

Primary and Secondary Endpoints: Clinical Parameters

All endpoint measurements were made at baseline (before mepolizumab), after mepolizumab (end of treatment), at the start of placebo (prereslizumab baseline), at the end of placebo, and at the end of treatment (reslizumab) (*see* Figure 1). The primary study endpoints were the reduction in sputum eosinophil % and blood eosinophil count (absolute). Sputum was induced and processed as described previously (10). Secondary efficacy measures included FEV_1 (American Thoracic Society recommendation) and asthma control questionnaire (five-question instrument [ACQ-5]) (11).

Exploratory Outcomes: Assessment of Airway Eosinophilic Inflammation

Eosinophil activity in sputum was assessed by measuring eosinophil peroxidase (EPX) in the cell-free sputum supernatants using an in-house ELISA (12, 13). Sputum IL-5 was detected using an ELISA platform (IL-5 Duo-set; R&D Systems) modified for alkaline phosphatase-based colorimetric detection instead of horseradish peroxidase reagents to evade interference from the endogenous peroxidases in sputum (14). Because the secondary detection antibodies supplied in the Duo-set are biotinylated, BluePhos Microwell Phosphatase Substrate System

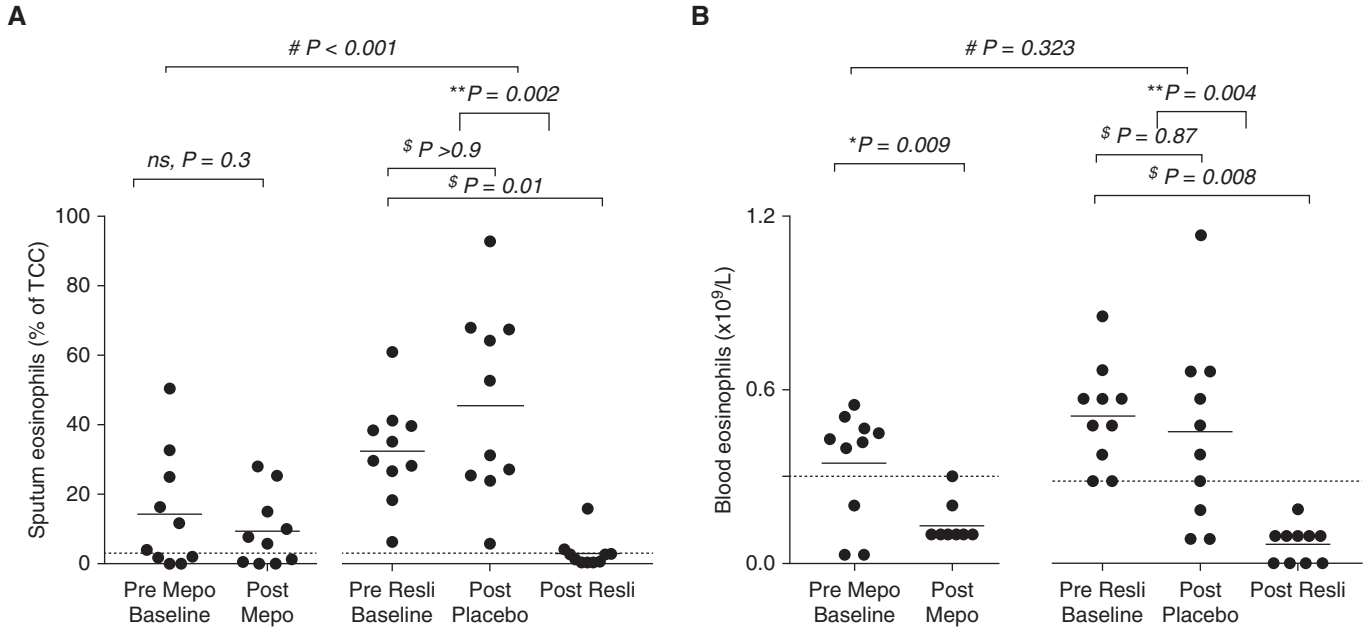

Figure 2. Changes in primary outcome over study period. Changes in primary outcome (*A*) sputum and (*B*) blood eosinophil levels for *n* = 10 patients for all five time-points measured for phase I (mepolizumab treatment) and phase II (reslizumab trial) are shown. The treatment effect of the individual drugs (i.e., $\Delta Mepo_{Rx}$ and $\Delta Resli_{Rx}$) is analyzed by Wilcoxon paired rank test, whereas difference of treatment effect between the drugs is compared by analysis of covariance (adjusted for baseline prednisone dose). The effect of prereslizumab baseline versus placebo versus postreslizumab time-points is analyzed by Friedman test followed by Dunn multiple comparison test. **P* values as per Wilcoxon test (** indicates significant difference). $^{\$}P$ values for Friedman. $^{\#}P$ values from analysis of covariance analysis. *P* value of <0.05 is considered significant. Mepo = mepolizumab; ns = nonsignificant; Resli = reslizumab; TCC = total cell count.

(KPL Inc.) was used for color development. IL-5 levels were measured in the immunoprecipitated fraction of the sputum supernatants (8) and in the whole supernatant. The total sputum IL-5 levels were presented as a sum of "immunoglobulin-bound" and "free IL-5," respectively.

Exploratory Outcomes: Molecular Endpoints for Assessing Persistence of Eosinophilic Inflammation

Eosinophil progenitors (EoPs; $CD34^+CD45^+CD125^+$) and hematopoietic progenitor cells (HPCs) in sputum and peripheral blood mononuclear cells were evaluated by flow cytometry (15). In addition, classical and alternative sources of IL-5 (i.e., $CD4^+$ lymphocytes and lineage negative innate lymphoid cells of group 2 [ILC2], $CD45^+CD127^+ST2^+$) respectively, were enumerated as described previously (6, 7). Furthermore, markers of localized autoimmune phenomenon recently reported to contribute to the persistence of eosinophilic inflammation in patients with severe eosinophilic asthma were assessed (15). Reduction in the levels of anti-EPX IgG and antinuclear antibodies was examined using an ELISA developed in-house and line immunoassay strips (Human Worldwide), respectively (8, 16).

Statistical Analysis

Statistical analysis was performed using Graphpad Prism version 7.0 and SPSS version 23. For individual drug effect, the change in the respective outcomes between placebo and drug was assessed by Wilcoxon paired analysis. For assessing significant difference in the measured outcomes between the two treatments, analysis of covariance (ANCOVA) was conducted, adjusting for baseline prednisone use. Although parametric in nature, ANCOVA has been recommended for estimating treatment response in clinical trials, which generally tend to have data-sets with nonnormal distribution (17).

Results

A total of 13 patients who received 100 mg SC mepolizumab were screened, out of which three were excluded from the study (one declined, one was noncompliant and potentially pregnant, the other developed significant comorbidities with complications of peripheral vascular disease and coronary artery disease). Finally, 10 patients (five males; mean age 50.9 ± 7.6 yr; mean body mass index 28.9 ± 4.9) with elevated blood and sputum eosinophilia (despite mepolizumab treatment) were recruited for the placebo-controlled single-blind reslizumab trial. The baseline demographic characteristics of the 10 recruited patients at the start of both interventions are tabulated in Table 1. The mean baseline ACQ scores, FEV_1% predicted, sputum and blood eosinophilia, and maintenance prednisone dose is comparable and well-balanced (Wilcoxon ranked test, $P > 0.05$). The mean ± SD dose of reslizumab IV administered at 3 mg/kg was 254.3 ± 57.7 mg ($n = 10$; average weight in kg, 84.8 ± 19.2), with maximum dose of 375 mg and minimum dose of 183.6 mg.

Primary Outcome: Reduction in Sputum Eosinophils

Reslizumab reduced sputum eosinophilia by 91.2% (absolute values by 29.3 ± 14) from baseline compared with placebo ($P = 0.002$; Wilcoxon paired test) (Figure 2A; *see* Figure E1 in the online supplement). Treatment effect of

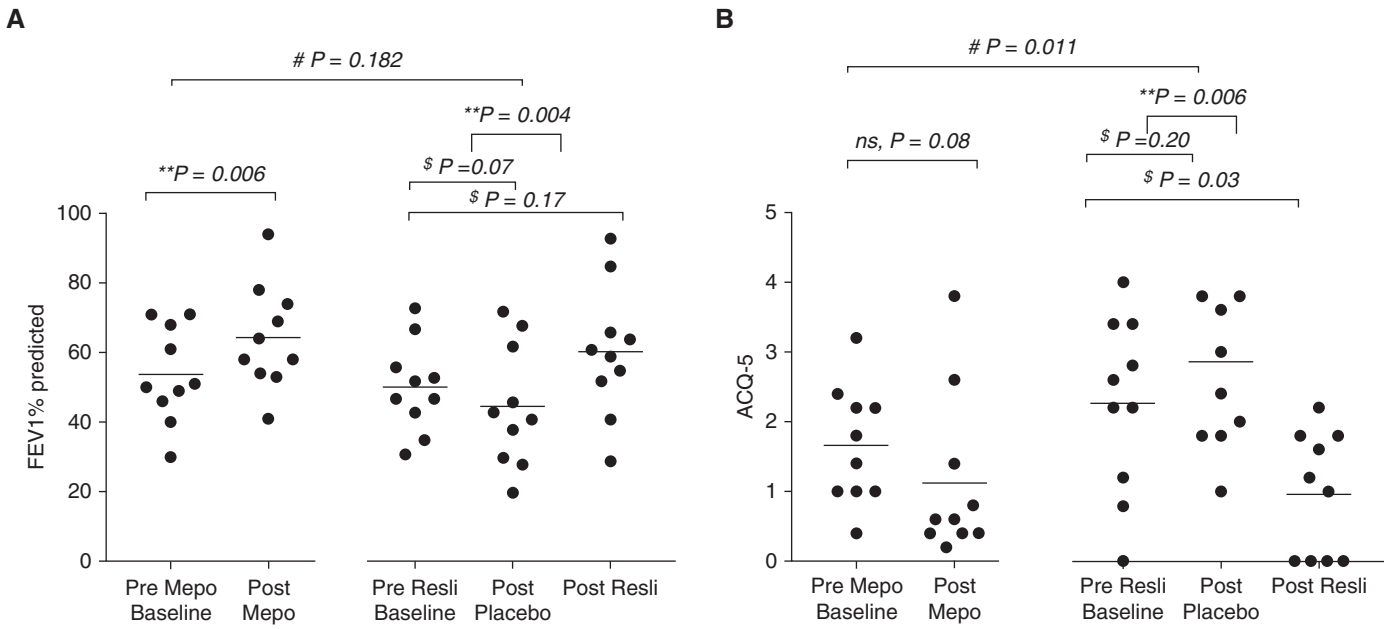

Figure 3. Changes in secondary outcomes over study period. Changes in secondary outcomes (*A*) FEV_1 % predicted and (*B*) asthma control questionnaire, five-question instrument symptom score at all five time-points measured for phase I (mepolizumab treatment) and phase II (reslizumab trial) are shown. The treatment effect of the individual drugs (i.e., $\Delta Mepo_{Rx}$ and $\Delta Resli_{Rx}$) is analyzed by Wilcoxon paired rank test, whereas difference of treatment effect between the drugs is compared by analysis of covariance (adjusted for baseline prednisone dose). The effect of prereslizumab baseline versus placebo versus postreslizumab time-points is analyzed by Friedman test followed by Dunn multiple comparison test. ***P* values as per Wilcoxon test. $^{\$}P$ values for Friedman. $^{\#}P$ values from analysis of covariance analysis. *P* value of <0.05 is considered significant. ACQ-5 = asthma control questionnaire, five-question instrument; Mepo = mepolizumab; ns = nonsignificant; Resli = reslizumab.

reslizumab ($\Delta Resli_{Rx} - \Delta Placebo$) (Figure 1) (42.7%, absolute values) was superior to that of mepolizumab ($\Delta Mepo_{Rx}$) (Figure 1) (5.01%, absolute values) ($P < 0.001$; ANCOVA, adjusted for baseline prednisone) (Figure 2A). Of the six (of the 10) patients who had persistent sputum eosinophils greater than 3% despite mepolizumab treatment, reslizumab reduced sputum eosinophil (≤3%) in all but one (*see* Figure 2A, dotted line represents 3% threshold).

Primary Outcome: Reduction in Blood Eosinophils

Compared with their respective placebo-control subjects, treatment with both anti–IL-5 mAbs allowed significant reduction in blood eosinophil levels (Figure 2B; *see* Figures E1C and E1D). Both reslizumab and mepolizumab showed comparable efficiency in depleting blood eosinophils ($P > 0.05$, ANCOVA). Indeed, blood eosinophil count documented for all patients at the end of each treatment regime was less than 300 cells/μl.

Secondary Outcomes: Reduction in Clinical Indices of Asthma Severity

Attenuation of sputum and blood eosinophilia by reslizumab treatment was associated with significant improvement in lung function (20% increase in FEV_1% predicted from baseline, $P = 0.004$, Wilcoxon paired test) and ACQ-5

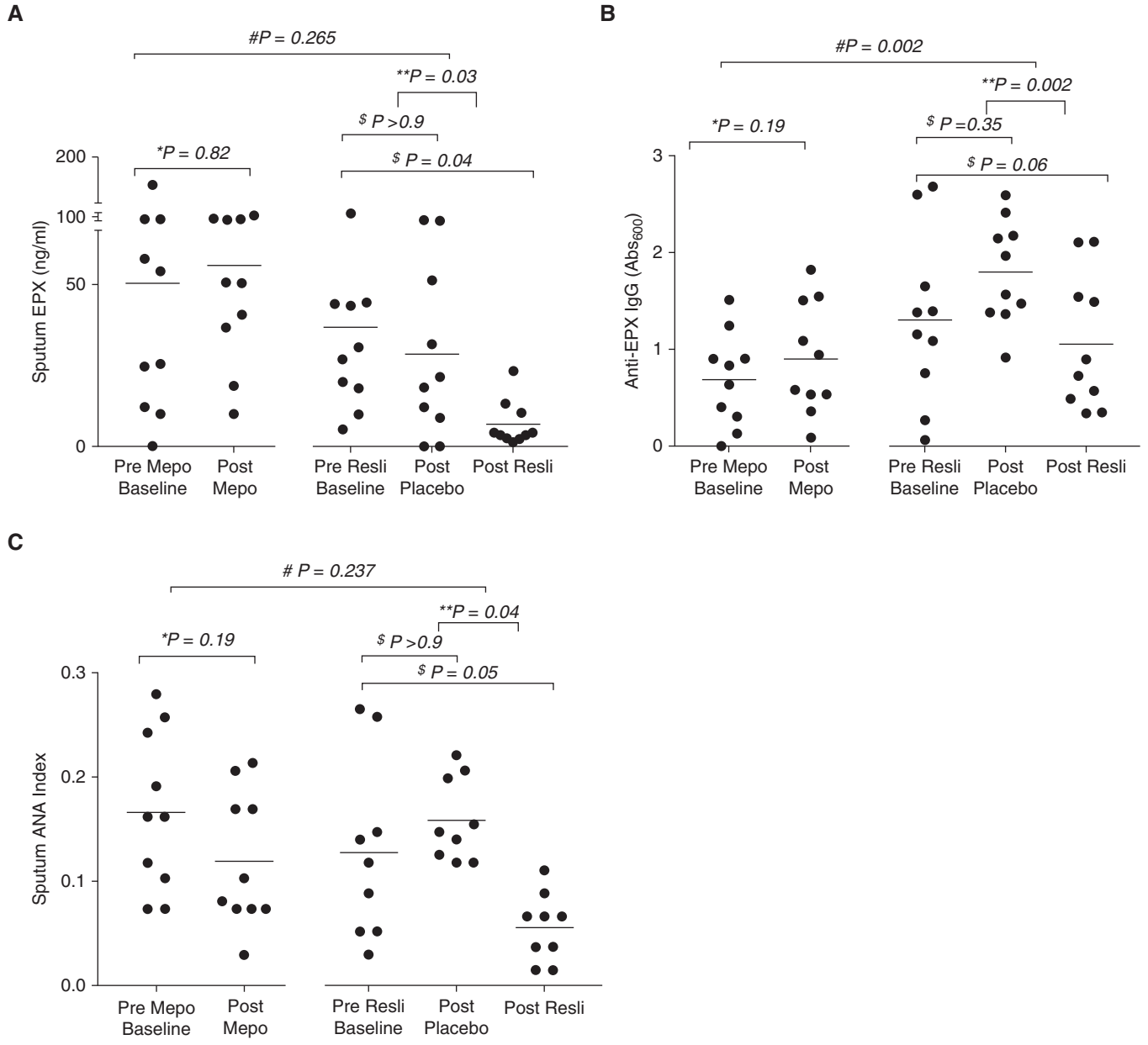

Figure 4. Comparative treatment effect on eosinophilic inflammation. (*A*) Variation in luminal eosinophilic activity based on eosinophil peroxidase levels (ng/ml) in sputum supernatants. (*B* and *C*) Treatment effect on localized autoimmune responses with respect to anti–eosinophil peroxidase IgG (*B*) and antinuclear antibody index (*C*). *P values as per Wilcoxon test (** indicates significant difference). $P values for Friedman. #P values from analysis of covariance analysis. ANA = antinuclear antibody; EPX = eosinophil peroxidase; Mepo = mepolizumab; Resli = reslizumab.

symptom scores by 57.2% from baseline ($P = 0.006$, Wilcoxon paired test) compared with its placebo control (Figure 3; *see* Figure E2). Mepolizumab also showed significant improvement of 21% increase in FEV_1 after therapy ($P = 0.006$, Wilcoxon paired test) but without significant improvement in ACQ scores ($P = 0.08$; Figure 3B). The improvement in ACQ-5 score with reslizumab (−1.92) was significantly greater than the improvement with mepolizumab (−0.54) (Figure 3B) (ANCOVA adjusted for baseline prednisone, $P = 0.011$). Both drugs had comparable effects on FEV_1 (Figure 3A) (ANCOVA, $P = 0.2$).

Exploratory Outcomes: Attenuation of Airway Eosinophil Activity

Reslizumab reduced sputum EPX levels by 65.4% compared with baseline (Figure 4A) ($P = 0.03$, Wilcoxon paired test), whereas no significant reduction in EPX levels was evident after mepolizumab treatment (Figure 4A) ($P = 0.82$, Wilcoxon paired test). Although there is no significant difference in the baseline prednisone dose between the two studies (Table 1), it is interesting to note that when adjusted for the baseline prednisone, there was no difference in the treatment effects between the two interventions ($P = 0.26$, ANCOVA) (Figure 4A). This is likely because EPX, a direct measure of airway eosinophil activity (degranulation), is responsive to small changes in prednisone dose, and depletion of IL-5 itself is not adequate in curbing the local mechanisms underlying eosinophil degranulation.

Exploratory Outcomes: Attenuation of Airway Autoimmune Markers

A significant reduction in both anti-EPX IgG ($P = 0.002$) and antinuclear antibodies signals in sputum ($P = 0.04$) compared with the respective placebo was demonstrable with reslizumab, unlike mepolizumab (Figures 4B and 4C). Increase in anti-EPX IgG after mepolizumab treatment was seen in four of six patients who were clinically assessed to have inadequate response. There was a significant increase in the anti-EPX IgG levels in the nonresponders compared with those who showed adequate treatment response to mepolizumab (*see* Figure E3C) ($P = 0.04$, Mann Whitney). When the treatment response with both the drugs was compared after adjusting for the baseline prednisone dose, reslizumab was superior to mepolizumab in attenuating anti-EPX levels in the sputum of all patients (Figure 4B) (ANCOVA, $P = 0.002$). Importantly, in those patients who showed an increase in anti-EPX IgG with mepolizumab, reslizumab was effective in reducing the autoantibody titers. The effect on antinuclear antibodies showed a similar trend, but was not statistically significant (ANCOVA, $P = 0.2$).

Exploratory Outcomes: Attenuation of Inflammatory Cells that Promote T2 Inflammation

Reslizumab significantly reduced the levels of blood HPCs ($P = 0.004$), sputum HPCs ($P = 0.006$), blood $CD34^+$ EoPs ($P = 0.004$), and sputum $CD4^+$ lymphocytes ($P = 0.02$) compared with placebo (Table 2), but not blood or sputum ILC2. In comparison, mepolizumab treatment also caused a significant but modest decrease in blood ILC2 ($P = 0.04$) but not on any of the other measurements. After adjusting for the baseline prednisone dose, reslizumab was superior to mepolizumab in suppressing peripheral blood HPCs ($P = 0.02$, ANCOVA) and EoPs ($P = 0.03$, ANCOVA), thereby suppressing lung recruitment of these progenitor cells that aid *in situ* eosinophilopoesis.

Assessment of Responders to Anti–IL-5 mAb Therapy

Response to anti–IL-5 therapy was considered not to be of clinical relevance if there was less than 50% reduction in sputum and blood eosinophils (absolute values) and/or reaching normal values (i.e., <3% sputum eosinophils, <300 cells/μl in blood), improvement in ACQ score less than 1.5, and/or exacerbations requiring additional prednisone dose (burst) or IV solumedrol. Based on these criteria, 6 out of 10 patients showed inadequate response to mepolizumab, and one for reslizumab ($P = 0.02$, chi-square test). As expected, the responders at the end of both drug treatments had sputum eosinophils less than 3%, except one. It is important to note that this patient at the end of reslizumab study fulfilled the criteria of a responder:

Table 2. Exploratory Outcome: Comparative Treatment Effect on Inflammatory T2 Cells

Cell Type	Phase I: Mepolizumab Rx		Phase II: Single-Blind Reslizumab Trial			Treatment Response
	$\Delta Mepo_{Rx}$	*P* Value	ΔPlacebo	$\Delta Resli_{Rx}$	*P* Value	*P* Value (ANCOVA)
PB HPC	−68.5 ± 77	0.44	25.5 ± 67	−43.1 ± 48	0.004*	0.02*
Sputum HPC	5.3 ± 246	0.82	4.3 ± 72	−43.4 ± 71	0.006*	0.65
PB EoP	30.9 ± 65	0.37	0.3 ± 40	−24.4 ± 27	0.004*	0.03*
Sputum EoP	−5.6 ± 112	0.16	−1.6 ± 9.2	−6.1 ± 6.3	0.13	0.84
Blood ILC2	−76.7 ± 107	0.04*	4.0 ± 10	−0.7 ± 1.7	0.16	0.09
Sputum ILC2	n/d	n/d	−2.6 ± 27	−8.6 ± 21	0.69	—
Sputum $IL\text{-}5^+$ ILC2	n/d	n/d	81.2 ± 1478	−877.7 ± 791	0.25	—
PB $CD4^+$ lymphocytes	n/d	n/d	33.8 ± 111	−35.9 ± 106	0.32	—
Sputum $CD4^+$ lymphocytes	n/d	n/d	−61 ± 159	−188 ± 154	0.02*	—

Definition of abbreviations: ANCOVA = analysis of covariance; EoP = eosinophil progenitor; HPC = hematopoetic progenitor cell; ILC2 = innate lymphoid cells group 2; Mepo = mepolizumab; n/d = protocols to assess in sputum were not established at this point; PB = peripheral blood; Resli = reslizumab; Rx = treatment; T2 = type 2. All values are represented as mean ± SD (10^3 cells/ml). $\Delta Mepo_{Rx}$ is the difference in absolute value between before and after mepolizumab Rx and *P* value is computed between absolute values of before and after treatment, Wilcoxon paired analysis; ΔPlacebo is the difference between baseline and end of placebo, Wilcoxon paired analysis; $\Delta Resli_{Rx}$ is the difference between baseline and end of reslizumab treatment, Wilcoxon paired analysis.
*Statistical significant difference.

had an improvement in ACQ with 2.4 score and 59% reduction in sputum eosinophils from baseline (blood eosinophils <300 cells/μl). However, the said patient documented an absolute sputum eosinophil count of 15.5% with many free granules, and has remained symptomatic. We termed him as a "partial nonresponder" and therefore, his data-point is plotted in the nonresponder subgroup, indicated by a different symbol (Figure 5B). At the end of both reslizumab and mepolizumab treatments, a delta increase in total IL-5 levels ("free" and "bound" form) from baseline was demonstrable in the sputum of the nonresponders (Figures 5A and 5B). Immunoglobulin-bound IL-5 was significantly more detectable in the sputum of patients receiving mepolizumab compared with reslizumab (Figure 5C) ($P = 0.004$, Wilcoxon), suggesting the possibility of immune-complex formation caused by inadequate concentration of mepolizumab in the airways.

Discussion

This is the first clinical trial that has evaluated the efficacy of two classes of anti–IL-5 mAbs in the same subset of severe prednisone-dependent patients treated sequentially. We report four novel observations. First, we demonstrate that four doses of IV reslizumab are effective in suppressing both sputum and blood eosinophilia in severe prednisone-dependent asthma. Second, the magnitude of this treatment effect is greater than that observed with 1 year (12 doses) of treatment with 100 mg SC mepolizumab. Third, suppression of airway eosinophilia was associated with a clinically meaningful improvement in asthma control and FEV_1 and this could be demonstrated in a small study of 10 patients. Finally, airway eosinophilopoietic factors, such as sputum IL-5 and EoPs, and airway autoimmune responses seem to be the determinant of response to anti–IL-5 therapy rather than blood eosinophil count.

This study supports our previous observations that unlike in patients with mild-to-moderate asthma who require inhaled corticosteroids to control their asthma, the dose of anti–IL-5 mAb may be relevant in patients with severe prednisone-dependent asthma. Indeed, the dose response of mepolizumab on sputum eosinophils has been previously observed (2), but this was considered to be not clinically relevant because of lack of apparent correlation with clinical outcomes of exacerbation reductions. However, the effect on the prednisone-dependent subpopulation, who are likely to have higher eosinophil-mediated disease, was not examined. These patients are likely to have both local and systemically derived IL-5 from both classical (e.g., $CD4^+$ lymphocytes) and nonclassical (e.g., ILC2) sources (6). The locally derived IL-5, which may not be effectively neutralized by low-dose SC anti–IL-5 mAb, could promote the local differentiation of EoPs to mature eosinophils and contribute to ongoing symptoms (7). Indeed, those patients with asthma who have both airway and blood eosinophilia are more symptomatic than those with eosinophilia in either compartment alone (18).

Consistent with this hypothesis, we demonstrated that both airway eosinophils and EoP numbers were suppressed in all patients particularly those in whom they were not suppressed with mepolizumab, 100 mg SC, administered for 1 year when higher doses of an anti–IL-5 mAb were administered IV for just four doses. As expected, we did not observe any effects of higher doses of the drug on the total or IL-5–positive ILC2 numbers (Table 2), but it would have neutralized the local IL-5 derived from these cells. Indeed, in univariate analyses, sputum IL-5 level was a significant predictor of response to reslizumab; higher the sputum IL-5 levels,

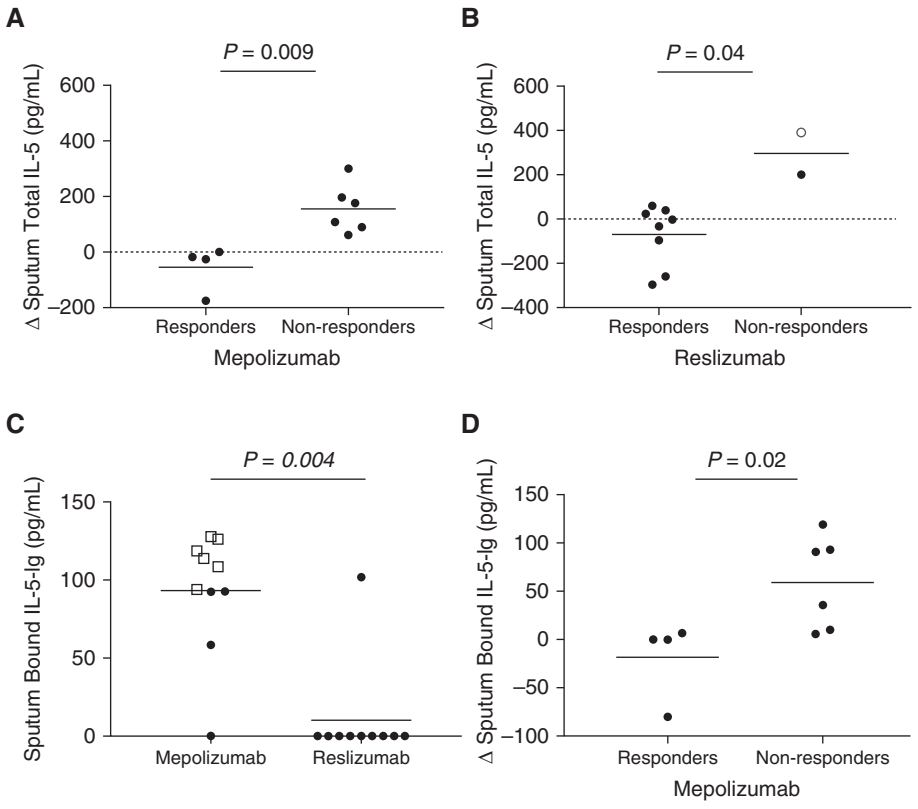

Figure 5. Sputum IL-5 as a predictor for response to anti–IL-5 therapy. (*A* and *B*) Comparison of total (free and bound IL-5 to immunoglobulin) IL-5 levels detectable in sputum between the responders and nonresponders of (*A*) mepolizumab, 100 mg subcutaneous therapy and (*B*) reslizumab, 3 mg/kg intravenously. Data are plotted as change between baseline and end-of-treatment absolute values. Comparisons were made by Mann-Whitney test. Partial nonresponder indicated by open circle. (*C*) Absolute values of immunoglobulin-bound IL-5 detected in immunoprecipitated immunoglobulin from sputum in all 10 patients at the end of mepolizumab treatment and reslizumab end-of-trial visit. Each open square symbol represents patients who had inadequate response to mepolizumab. Comparisons by Wilcoxon paired test. (*D*) Change in immunoglobulin-bound IL-5 in nonresponders versus responders, before and after mepolizumab therapy. Mann-Whitney test was used.

greater the chances that the patients would respond better to higher doses of an anti–IL-5 (adjusted $R^2 = 0.3$, $P = 0.002$). Furthermore, we show that the nonresponders to anti–IL-5 mAb treatment have higher sputum IL-5 levels (Figures 5A and 5B). Taken together, the data support our hypothesis that greater concentration of the anti–IL-5 mAb in the airway is required (by higher dose or route of administration or both) to neutralize the local IL-5 levels (derived from multiple sources including ILC2).

Increase in sputum IL-5 despite an anti–IL-5 therapy can be indicative of immune complexes formed when the administered mAbs are not in excess to the target antigen (i.e., inadequate mAb dosing). These immune complexes can act as "cytokine depots," which lead to increase *in vivo* potency/biologic activity of the bound IL-5, and thereby worsen symptoms (8). Detectable levels of immunoglobulin-bound IL-5 in the sputum of mepolizumab nonresponders (Figure 5D) coupled with an associated increase in sputum IL-5 (Figure 5A), and sputum eosinophil % can be argued to be an indirect proof. In weight-adjusted dosing with an IV delivery route, this phenomenon is possibly averted. In fact, immunoglobulin-bound IL-5 was detectable in only 1 out of 10 patients receiving reslizumab (Figure 5C).

We do not imply that reslizumab has higher potency or bioavailability than mepolizumab but simply that more drug may have reached the airway when administered by the IV route. It is remarkable that although two anti–IL-5 monoclonal antibodies have been approved for clinical use, there are no pharmacokinetic data on airway bioavailability of these molecules in patients with asthma. The currently approved doses have been established based on clinical pharmacodynamics data or from pharmacokinetic data of blood eosinophil count changes in normal volunteers or on subjects with mild asthma (19). We believe that it is very important to establish this in patients with severe asthma because there is a potential for worsening of disease with inadequate dosing in some patients (8) who may have an airway autoimmune component associated with intense airway eosinophil activity (16). Indeed, we observed that IV reslizumab, and not SC mepolizumab, could reduce this in those patients who had higher EPX and anti-EPX antibodies in their sputum ($P = 0.002$, ANCOVA) (Figure 4B). In fact, four out of six nonresponders to mepolizumab treatment had increased anti-EPX IgGs (*see* Figure E3).

The interpretations of our data are limited by a few factors. First, this is not a direct head-to-head randomized comparison of mepolizumab with reslizumab. Although this would have been ideal, it is logistically very difficult to organize such a clinical trial. Second, we also did not randomize patients who had previously been on mepolizumab to placebo or to reslizumab in a parallel group study because we believed that it was unethical to provide these severe prednisone-dependent patients placebo for 6 months when anti–IL-5 therapies were available for clinical use. Third, baseline prednisone dose had changed between the time interval of commencing mepolizumab and reslizumab. However, this difference was not statistically significant and we accounted for this as a baseline covariate in all our comparative analyses. Finally, we do not have either mepolizumab or reslizumab drug levels or antidrug antibody levels in sputum because these assays are not commercially available. We do know that none of our patients, including those who had inadequate clinical response to mepolizumab, had antimepolizumab neutralizing antibodies in their serum.

In summary, we demonstrate that fixed-dose mepolizumab administered SC may not be effective in reducing airway eosinophilia in some patients with severe prednisone-dependent asthma. In these patients, higher doses of anti–IL-5 mAb administered in the form of reslizumab IV controls the airway eosinophilia and leads to better improvement in asthma control. It remains to be seen if targeting the IL-5 receptor (20), which is an effective therapy for these severe patients, may be a better strategy than targeting the IL-5 ligand. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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