

Blood Eosinophil Count Is a Useful Biomarker to Identify Patients with Severe Eosinophilic Asthma

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Abstract

Rationale: Measurement of sputum or blood eosinophils may allow identification of a severe eosinophilic asthma population responsive to mepolizumab.

Objectives: The primary objective was assessment of a single blood eosinophil measurement to predict future eosinophil measurements in the following year versus using multiple blood eosinophil measurements. In addition, we examined whether a single sputum or blood eosinophil measurement was a useful biomarker for predicting treatment response to mepolizumab.

Methods: Based on data from placebo subjects ($n = 155$), we determined whether a blood eosinophil count of $150/\mu\text{L}$ or greater at screening remained on average above this level during the following year. The rate of exacerbation reduction in the sputum substudy population based on the screening blood eosinophil count and sputum eosinophils was evaluated.

Measurements and Main Results: Of 115 patients with eosinophils $150/\mu\text{L}$ or greater at screening, 98 (85%) remained

above this level in their post-screening average. Using the average of two, three or four measurements $150/\mu\text{L}$ or greater, 97 (85%), 103 (90%), and 105 (92%) have postscreening averages above $150/\mu\text{L}$. Mepolizumab reduced exacerbations by 69% (95% confidence interval [CI] = 41–83%) in subjects with baseline sputum eosinophils of 3% or greater compared with 66% (95% CI = 7–87%) in subjects with baseline sputum eosinophils under 3%. The reduction was 72% (95% CI = 41–83%) in subjects with blood eosinophils of $150/\mu\text{L}$ or greater compared with 30% (95% CI = –134 to 79%) in subjects with blood eosinophils under $150/\mu\text{L}$.

Conclusions: A single measurement of $150/\mu\text{L}$ or greater predicted the average of subsequent measurements being $150/\mu\text{L}$ or greater in 85% of this population. Using an average of multiple measurements only marginally increases the sensitivity. Sputum eosinophils did not predict treatment response with mepolizumab.

Keywords: eosinophils; severe asthma; phenotype; anti-IL-5

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In recent years, there has been a marked increase in biologics to treat eosinophilic diseases with an emphasis on eosinophilic asthma (1–8). With this increased development, a need arises to have an easily obtainable and reliable biomarker to appropriately selected patients to

participate in clinical trials, and ultimately to select appropriate patients for treatment once the drug is brought to market. To date, the majority of asthma clinical trials with biologics have explored the use of a single blood eosinophil measurement as an inclusion criterion and potential

biomarker to identify patients with eosinophilic asthma and/or as a potential biomarker of response (1, 4, 5). Prior studies with mepolizumab in severe asthma have also used induced sputum as an entry criterion and as a potential biomarker (5, 9). Sputum eosinophils are considered the

method of choice for establishing eosinophilic lung inflammation, and have also been shown to be useful in adjusting inhaled or systemic corticosteroids in asthma (10–12). However, in the Dose Ranging Efficacy And safety with Mepolizumab (DREAM) study, it was reported that blood eosinophils were correlated with response to mepolizumab, but the presence of sputum eosinophilia did not correlate with response (5).

A *post hoc* analysis of data from the DREAM study demonstrated that a single peripheral blood eosinophil count at screening of 150 cells/ μ L or greater coupled with clinical characteristics, such as frequent exacerbations, successfully predicted treatment response (5). Furthermore, those subjects whose screening eosinophil count was under 150 cells/ μ L had a lower rate of reduction of asthma exacerbations (5). However, because blood eosinophil counts are inherently variable, and because there is limited literature that describes blood eosinophil counts over time, it is important to understand the utility of a single measurement to identify patients with eosinophilic asthma who will respond to mepolizumab, and whether another biomarker, such as sputum eosinophils, would be more predictive of treatment response.

We used the data from subjects who received placebo in the DREAM study to characterize the variability of blood eosinophil measurements over time. Peripheral blood eosinophil counts were obtained at screening (Week –2), Week 0, and then every 4 weeks up to Week 56. The details of this study are published elsewhere (5). The analysis described here evaluates the eosinophil measurements over a 58-week period in the placebo group ($n = 155$) to determine the utility of a single blood eosinophil count as a criterion to select responders to mepolizumab. In addition, we analyzed the sputum eosinophil substudy population to establish the usefulness of sputum eosinophilia as a biomarker of response to mepolizumab. We investigated the extent to which a single screening measurement under 150 cells/ μ L is predictive of an average measurement of less than 150 cells/ μ L in the following 56 weeks. Furthermore, we investigated whether blood eosinophils would serve as a more reliable biomarker than sputum eosinophils as a predictor of treatment response.

Methods

The primary analysis was conducted using data from the placebo subjects enrolled in the DREAM study (NCT01000506) between November 2009 and December 2011 (5). The DREAM study has been previously reported, but in brief, this was a multicenter, randomized clinical trial, which evaluated the effect of three different doses of the mepolizumab versus placebo added to standard of care on the rate of severe asthma exacerbations. Mepolizumab is an anti-IL-5 monoclonal antibody under investigation to reduce the frequency of exacerbations in patients with evidence of eosinophilic inflammation at step 5/6 of the Global Initiative for Asthma guidelines (13). To qualify for randomization, subjects had a history of severe, refractory asthma, as evidenced by a requirement for an inhaled corticosteroid dose of 880 μ g/d of fluticasone propionate or equivalent, and at least two asthma exacerbations in the prior 12 months despite this maximal therapy. In addition, subjects had evidence of an eosinophilic phenotype by one or more of the following criteria at the screening visit or in the prior 12 months: peripheral blood eosinophil count of 300 cells/ μ L or greater; sputum eosinophils of 3% or greater; an exhaled nitric oxide concentration of 50 ppb or more; or prompt loss of asthma control after a 25% or lower reduction in inhaled or systemic corticosteroids. Because we used historical levels of blood eosinophils, this provided the opportunity to identify a threshold for characterizing clinical response to mepolizumab. Peripheral blood eosinophils were measured unblinded at screening, and randomization (Week 0), and then measured blinded every 4 weeks through Week 56. The time of day for drawing the blood sample was not mandated; however, most samples were obtained during the morning. Blood eosinophils were measured as part of the complete blood count plus differential, and all samples were analyzed at the central laboratory of Quest Diagnostics (Valencia, CA and Middlesex, UK). In a subset of 94 subjects, sputum eosinophils were collected at screening (Week –2), Week 4, Week 16, and Week 52. Sputum cell count was conducted at the Glenfield Hospital (Leicester, UK). The protocol was approved by local or central research ethics committees, and all subjects signed informed consent.

Statistical Analysis

The baseline characteristics for the placebo population are presented using summary statistics. We modeled (on the log scale) the post-screening blood eosinophil measurements using a repeated measures model with fixed covariates of visit, screening blood eosinophil measurement, and an interaction term for visit by screening measurement. The geometric mean of the modeled post-screening measurements against the screening value was plotted for each subject to examine how well the screening measurement predicted the post-screening average. The rate of reduction of exacerbation in the sputum substudy population based on the screening blood eosinophil count being greater than or equal to or less than 150 cells/ μ L, or based on the presence or absence of sputum eosinophils of 3% or greater at screening was evaluated. Pavord and colleagues (5) reported the results of the whole study split by baseline blood eosinophil count; the results presented here are for the sputum substudy population. As there was not a dose-response effect across the three doses of mepolizumab in the DREAM study, all doses were combined for this analysis. All analyses were conducted with SAS (versions 9.1.3 and 9.2; SAS Institute, Cary, NC).

Results

The baseline characteristics of the 155 subjects randomized to placebo and of those who participated in the sputum substudy are shown in Table 1. In the placebo cohort, there were 28 subjects who withdrew early from the study and 127 who completed the study. The placebo group characteristics were well matched to those randomized to receive mepolizumab (5). The sputum substudy was conducted at specialized referral centers, which tend to evaluate and treat the most severe patients and that also had the necessary experience to conduct sputum induction and processing. Therefore, it is not unexpected to see that the sputum substudy population has a higher percent (48%) of subjects on maintenance oral corticosteroids when compared with those in the general population that were randomized to placebo (29%). In addition, approximately one-third of the sputum substudy subjects

Table 1. Baseline characteristics of subjects

Characteristics	Placebo Patients in DREAM (n = 155)	Sputum Substudy: All Patients (n = 94)	Sputum Substudy: Placebo (n = 27)	Sputum Substudy: Mepolizumab (n = 67)
Female sex, n (%)	97 (63)	54 (57)	14 (52)	40 (60)
Age, yr	46.4 (11.3)	48.9 (9.6)	47.6 (8.8)	49.5 (9.9)
White, n (%)	140 (90)	92 (98)	27 (100)	65 (97)
Body mass index, kg/m ²	28.3 (6.1)	28.3 (6.0)	29.0 (6.2)	28.0 (6.0)
Maintenance oral corticosteroids, n (%)	45 (29)	45 (48)	10 (37)	35 (52)
Nasal polyps, n (%) [*]	16 (10)	15 (16)	3 (11)	12 (18)
Atopic, n (%) [†]	81 (52)	42 (45)	14 (52)	28 (42)
FEV ₁ % predicted (pre-BD)	59 (15)	60 (17)	62 (12)	59 (18)
Blood eosinophil count, cells/ μ l [‡]	280 (0–2,300)	240 (0–1,200)	280 (0–1,000)	220 (0–1,200)
Sputum eosinophil % [‡]	—	7.9 (0.1–83) n = 86	6.8 (0.1–75) n = 24	8.5 (0.1–83) n = 62
Exhaled nitric oxide, ppb [‡]	33.7 (5–224)	35.8 (5–211)	39.1 (9–211)	34.6 (5–142)
Former smoker, n (%)	34 (22)	31 (33)	11 (41)	20 (30)

Definition of abbreviations: BD = bronchodilator; DREAM = Dose Ranging Efficacy And safety with Mepolizumab study.

Data presented are means (SD) unless otherwise stated.

^{*}Self-reported.

[†]Atopy was defined as positive radioallergosorbent test for any of four prespecified allergens.

[‡]Data presented are geometric means (range).

were former smokers, whereas about one-fifth were former smokers in the general population randomized to placebo population.

Of the 155 subjects randomized to placebo, 115 (74%) had a screening eosinophil count of 150 cells/ μ l or greater. Based on the quadrants in Figure 1 of these 115 subjects (with a screening eosinophil count of ≥ 150 cells/ μ l), 98 (85%) on average remained at 150 cells/ μ l or greater in the following year. The remaining 17 subjects (15%) had a geometric mean eosinophil count that fell below the 150 mark over the following year, with the majority of these clustering between 100 and less than 150 cells/ μ l. There were 40 subjects that had under 150 cells/ μ l at screening. Of these, 27 (67.5%) remained, on average, below the proposed cut-off of 150 cells/ μ l or greater.

We examined whether using the average of repeated consecutive eosinophil measurements would produce a more accurate prediction of the future eosinophil count. The use of a single sample identifies 17 (15%) subjects who will, on average, be below the 150 cells/ μ l mark after screening. Using the average of two, three, or four consecutive measurements (screening, Week 0, Week 4, Week 8) of 150/ μ l or greater selects 120 (80%), 116 (81%), and 118 (81%) patients, respectively, whose average eosinophil count was 150/ μ l or

greater; of these, 18 (15%), 12 (10%), and 10 (8%) have postscreening averages below 150/ μ l. Figure 1 demonstrates that subjects with extreme values at screening tend to have less extreme values after screening. This observation of a regression to the

mean effect shows that extreme values seen at screening tended not to be repeated. The model (Figure 1) suggests that subjects with an eosinophil count of 150 cells/ μ l at screening would have a mean post-screening average in the following year of

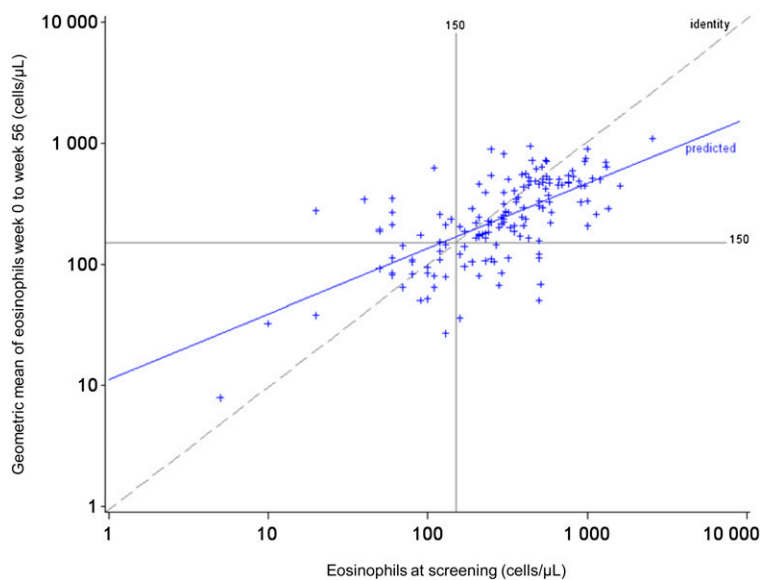


Figure 1. Screening eosinophils versus the geometric mean after screening. Screening eosinophils are predictive of the eosinophil count in the following year. The blue line represents the predicted geometric mean after screening. The dotted line represents the line of identity. The graph is divided into quadrants, with the perpendicular line representing the cell counts that are over 150 cells/ μ l or under 150 cells/ μ l at screening, and the horizontal line representing the geometric mean after screening of greater than 150 cells/ μ l or less than 150 cells/ μ l.

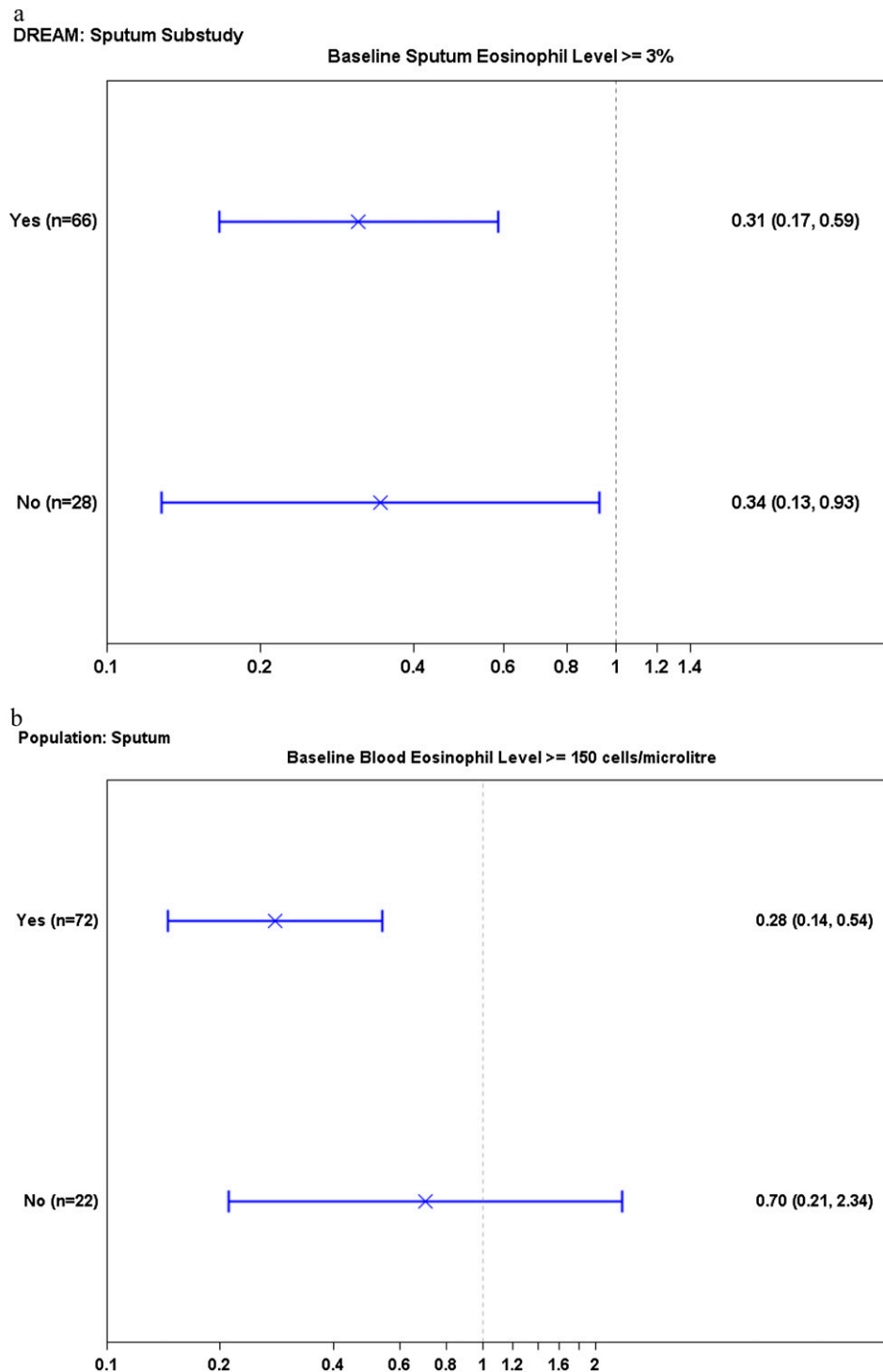


Figure 2. Effect of mepolizumab on the reduction of exacerbations compared with placebo. The annualized rate of exacerbations for subjects treated with mepolizumab is compared with the rate of exacerbations on placebo. To determine the percent reduction in exacerbations due to treatment with mepolizumab, the rate ratio is subtracted from 1. For a rate ratio that is equal to 1, there is no reduction compared with placebo response. (A) Effect of mepolizumab on the rate ratio of reduction of exacerbations by sputum eosinophils of 3% or greater. The mean rate of reduction in exacerbations (69 versus 66%) is essentially the same regardless of the level of sputum eosinophils at screening. (B) Effect of mepolizumab on the rate ratio of reduction of exacerbations by blood eosinophils of 150 cells/ μ l or greater. The mean rate of reduction in exacerbations is higher (72%) in those that have a screening eosinophil count of 150 cells/ μ l or greater compared with those who are under 150 cells/ μ l at screening (30%).

168 cells/ μ l 95% confidence interval (CI) for mean (149, 189).

We evaluated the effect of mepolizumab compared with placebo on the rate of exacerbations based on the presence or absence of sputum eosinophils of 3% or greater or blood eosinophils of 150 cells/ μ l or greater in the subpopulation of subjects who had both sputum and blood collected at baseline. As shown in Figure 2, the reduction in the exacerbation rate was 69% (95% CI = 41–83%) in subjects with baseline sputum eosinophils of 3% or greater compared with a 66% reduction (95% CI = 7–87%) in subjects with baseline sputum eosinophils of less than 3%. This indicates that the efficacy of mepolizumab is essentially the same, regardless of presence or absence of sputum eosinophils of 3% or greater (69 and 66% reduction, respectively). By contrast, the reduction in the rate of exacerbations was 72% (95% CI = 41–83%) in subjects with blood eosinophils of 150 cells/ μ l or greater compared with a 30% reduction (95% CI = –134 to 79%) in subjects with blood eosinophils under 150 cells/ μ l. Therefore, the use of blood eosinophils as biomarker using the threshold of 150 cells/ μ l or greater is helpful in identifying the responder population (72% reduction versus 30% reduction in those with blood eosinophils <150 cells/ μ l).

Discussion

Although more than 100 years have passed since the discovery of the eosinophil, limited information has been published describing the inherent variability in peripheral blood eosinophil counts over time. Eosinophils are primarily tissue dwellers, and are only present in the intravascular compartment for a short period of time. The reported half-life of blood eosinophils ranges from 7 hours up to 25 hours, with an 18 hour half-life being noted most frequently (14–18). Furthermore, eosinophils are highly sensitive to multiple intrinsic and extrinsic factors, including allergen exposure, treatment with glucocorticoids, and exposure to infections or stress (14). Due to this variable and short half-life, coupled with the sensitivity to multiple factors, one can anticipate daily fluctuations in the blood eosinophil count. The magnitude of this intraday variance has been reported, in two small studies, to range from 54 to 186%

of the mean count for a given subject ($n = 20$) or 17 to 396% for absolute variability ($n = 12$) (14, 19). Our study is the first to report on eosinophil counts in the population with severe eosinophilic asthma over a 58-week timeframe. The data in our study show that, for subjects who had a screening blood eosinophil count of 150 cells/ μ l or greater, 85% remained, on average, at or above 150 cells/ μ l in the following 56 weeks (based on monthly eosinophil measurements). It is important to recognize that the protocol required the dose and regimen of inhaled and maintenance oral glucocorticoids to be constant over this period. Although the threshold we report here may appear “low” when compared with a threshold of 300 or 400 cells/ μ l, the threshold represents the minimum requirement to show a response to mepolizumab. The geometric mean of blood eosinophils at baseline was 280 cells/ μ l in the placebo subjects. Furthermore, the population studied was treated with an inhaled corticosteroid dose of at least 880 μ g/d of fluticasone propionate or equivalent. In fact, 30% of these subjects were also taking approximately 10 mg of prednisone daily. Consequently, the blood eosinophil count is already partially suppressed by the use of steroids.

In our study, using two consecutive samples (screening and Week 0) with an average of 150 cells/ μ l or greater provided no additional benefit in identifying subjects who would remain at or above the 150-cell cutoff. Selecting subjects that had three or four consecutive 4-weekly samples of an average of 150 cells/ μ l or greater would have resulted in marginal increases (5 and 7%, respectively) in identifying subjects who remain at or above this level. Although the gain from multiple consecutive samples is minimal, for those patients who have a history of severe eosinophilic asthma and a lower than expected eosinophil level coincidentally at the initial visit, a repeat blood test could be recommended a few weeks later to confirm the phenotype for any patients within a narrow bound of the 150-cells/ μ l cutoff.

Biomarkers are of interest in asthma to help guide the identification of specific phenotypes, to aid clinicians in the choice of asthma therapy, or to provide a tool for monitoring of asthma therapy (16). Several biomarkers have been investigated to characterize inflammatory phenotypes of asthma without having to conduct invasive

procedures, such as bronchoalveolar lavage or endobronchial biopsy (16, 19–21). Surrogate markers, such as sputum eosinophils, exhaled nitric oxide, serum periostin, and blood eosinophils, have all been investigated to varying degrees. Measurement of eosinophils in induced sputum has been shown to be a reliable biomarker of airway inflammation, and to also be useful as a predictor of response to treatment with anti-inflammatory medications, such as corticosteroids (12, 16). However, measurement of sputum eosinophils is not widely used in the clinical setting, as it requires technical expertise and may cause some discomfort to patients. Serum periostin has also been shown to be a marker of airway eosinophilia, but remains experimental and not useful in the clinical setting at the present time (21). Exhaled nitric oxide was measured at baseline and serially in the DREAM study; although a threshold of 50 ppb was useful in identifying a population with features of eosinophilic inflammation, it was not helpful as a biomarker of treatment response (5).

Although sputum eosinophil measurement has been shown to be a noninvasive biomarker that correlates with airway eosinophilia, in this study, sputum eosinophils did not predict treatment response. An approximately 70% reduction in exacerbations occurred in both the high sputum eosinophilia group ($\geq 3\%$) and the low sputum eosinophilia group ($< 3\%$). Conversely, the presence or absence of blood eosinophils of 150 cells/ μ l or greater in the same subset of patients showed a 72% reduction in exacerbations for those at or above the 150-cell cutoff, whereas those below that level had a reduction of 30%. This reflects results observed in the DREAM study total population (5), where only blood eosinophils and number of exacerbations in the prior year were correlated with mepolizumab-induced reduction in exacerbations (5). Therefore, it appears that blood eosinophil count is the biomarker of choice for predicting treatment outcome with mepolizumab in severe asthma (5, 22). This may be due, in part, to IL-5-mediated eosinophilic inflammation being both local and systemic. Consistent with our findings, Malinovschi and colleagues (22) recently reported that increased blood eosinophils are associated with a higher risk for exacerbations, and hypothesized that this is due to greater involvement of IL-5.

The current study has some limitations. First, the population studied may not be reflective of the population of individuals with severe eosinophilic asthma as a whole. Subjects were included in the current trial based on four potential markers of eosinophilic inflammation (peripheral blood eosinophil count of ≥ 300 cells/ μ l, sputum eosinophils $\geq 3\%$, an exhaled nitric oxide concentration of 50 ppb or more, or prompt loss of asthma control after a 25% or lower reduction in inhaled or systemic corticosteroids), so the population does not represent a random sample of patients with severe asthma. In addition, the majority of subjects were white, so the results may not be generalizable to other races. Second, 68% of the placebo subjects had at least one exacerbation treated with systemic corticosteroids. If systemic corticosteroids were administered close to the time of blood eosinophil measurement, this could have suppressed the eosinophil count.

Third, we evaluated sputum response at a threshold of 3% or greater, but it is possible that using a different threshold could provide a different output. In addition, we did not require the samples to be measured at a set time of day. Most subjects underwent clinical procedures, including sputum induction, in the morning. As such, there is the potential that afternoon or evening measurements may have provided a different predictive result.

Conclusions

An ideal biomarker is reliable, minimally invasive, easy to collect and measure, inexpensive, and can be used to identify either a clinical phenotype or a treatment response phenotype, measure changes in disease activity, or confirm a diagnosis. When considering use of a biomarker, it is important to identify the question at hand and select the biomarker that is most fit for the purpose. A biomarker that is the best

predictor of airway inflammation may not be the best biomarker for treatment response or prediction of treatment response. In the DREAM study, sputum eosinophils were not predictive of treatment outcome, whereas blood eosinophils were. Despite inherent variability of blood eosinophil measurements, subjects on high-dose inhaled corticosteroid and with a history of exacerbations tend to remain either above or below the cutoff value, demonstrating that it is both acceptable and practical to use a single blood eosinophil measurement as an inclusion criterion for mepolizumab severe asthma trials. ■

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