Biomarkers for severe eosinophilic asthma

Steven W. Yancey, MSc,[a] Oliver N. Keene, MSc,[b] Frank C. Albers, MD, PhD,[c] Hector Ortega, MD, ScD,[a] Stewart Bates, PhD,[d] Eugene R. Bleecker, MD,[e] and Ian Pavord, FMedSci, DM[f] *Research Triangle Park, NC; Uxbridge, Stevenage, and Oxford, United Kingdom; and Tucson, Ariz*

The last decade has seen the approval of several new biologics for the treatment of severe asthma-targeting specific endotypes and phenotypes. This review will examine how evidence generated from the mepolizumab clinical development program showed that blood eosinophil counts, rather than sputum or tissue eosinophil counts, evolved as a pharmacodynamic and predictive biomarker for the efficacy of treatment with mepolizumab in patients with severe eosinophilic asthma. Based on the available evidence and combined with clinical judgement, a baseline blood eosinophil threshold of 150 cells/μL or greater or a historical blood eosinophil threshold of 300 cells/μL or greater will allow selection of patients with severe eosinophilic asthma who are most likely to achieve clinically significant reductions in the rate of exacerbations with mepolizumab treatment. (J Allergy Clin Immunol 2017;140:1509-18.)

Key words: *Eosinophils, biomarkers, severe eosinophilic asthma*

Abbreviations used

DREAM: Dose Ranging Efficacy and safety With Mepolizumab in Severe Asthma
FENO: Fraction of exhaled nitric oxide
GINA: Global Initiative for Asthma
ICS: Inhaled corticosteroid
MENSA: Mepolizumab Adjunctive Therapy in Subjects with Severe Uncontrolled Refractory Asthma
OCS: Oral corticosteroid
RR: Rate ratio
SIRIUS: Steroid Reduction with Mepolizumab Study

Biological therapies, in addition to regular controller medication, for patients with severe asthma are becoming the new standard of care for patients who were previously without alternative treatment options.[1] Omalizumab, which neutralizes IgE, was the first biologic introduced for asthma in 2003 for patients who could not achieve asthma control with inhaled corticosteroids (ICSs), leukotriene inhibitors, and bronchodilators.[1-4] However, targeting treatment based on total or allergen-specific IgE levels was not completely effective in selecting patients likely to respond to therapy.[5,6] More recently, biologic therapies that disrupt IL-5 signaling and ultimately reduce eosinophil counts in blood and lung tissue (mepolizumab [GlaxoSmithKline, Research Triangle Park, NC] and reslizumab [Teva, Petach Tikva, Israel])[7-10] have been approved for treatment in patients with severe asthma and an eosinophilic phenotype (with baseline eosinophil counts at different threshold levels), a condition now termed severe eosinophilic asthma.[11-13] In addition, benralizumab (AstraZeneca, Cambridge, United Kingdom), which is currently in development, depletes eosinophil counts by antibody-dependent cell-mediated cytotoxicity through binding to the α chain of the IL-5 receptor on the eosinophil surface.[14-16] In clinical trials all 3 anti-targeted IL-5 pathway therapies (mepolizumab, reslizumab, and benralizumab) reduced rates of exacerbations in patients with severe eosinophilic asthma.[8,14,15,17-19] In addition, mepolizumab and benralizumab have been shown to reduce or eliminate the dependency for oral corticosteroids (OCSs) without a loss of asthma control.[20,21]

Some overlap in the asthma phenotypes, principally severe allergic asthma and severe eosinophilic asthma, can lead to an overlap in eligibility for the different biological therapies.[22] In circumstances in which the patient can be identified clearly as having a particular phenotype, the treatment options recommended in the Global Strategy for Asthma Management and Prevention (Global Initiative for Asthma [GINA]) guidelines are to prescribe anti-IgE and anti–IL-5 therapies, respectively.[1] Integral to the successful clinical development of these drugs has been identification and use of biomarkers to identify patients likely to respond to treatment.

A biomarker is any characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathologic processes, or responses associated with a therapeutic intervention.[23] There are several different types

From [a]the Respiratory Therapeutic Area and [c]the Respiratory Medical Franchise, GlaxoSmithKline, Research Triangle Park, NC; [b]Clinical Statistics, GlaxoSmithKline, Stockley Park, Uxbridge; [d]Respiratory Discovery Medicine, GlaxoSmithKline, Stevenage; [e]the Division for Genetics, Genomics and Personalized Medicine, University of Arizona College of Medicine, Tucson; and [f]the Respiratory Medicine Unit and Oxford Respiratory BRC, Nuffield Department of Medicine, University of Oxford.

This article reports data from studies funded by GlaxoSmithKline.

Disclosure of potential conflict of interest: S. W. Yancey, O. N. Keene, F. C. Albers, H. Ortega, and S. Bates are employees of GlaxoSmithKline and hold stock/shares in GlaxoSmithKline. E. R. Bleecker has performed clinical trials administered by his previous employer, Wake Forest School of Medicine, for AstraZeneca, MedImmune, Boehringer Ingelheim, Pfizer, Cephalon/Teva, Forest, Genentech, GlaxoSmithKline, Johnson & Johnson (Jansen), Novartis, and Sanofi and has also served as a paid consultant for AstraZeneca, MedImmune, Boehringer Ingelheim, Pfizer, GlaxoSmithKline, Forest, Novartis, Regeneron, and Sanofi. I. Pavord has received honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim, Aerocrine, Almirall, Novartis, and GlaxoSmithKline; received payment for organizing an educational event from AstraZeneca; received advisory board honoraria from Almirall, Genentech, Regeneron, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Merck, Schering-Plough, Novartis, Mylan Specialty (Dey Pharma), Napp Pharmaceuticals, and Respivert; and received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GlaxoSmithKline, AstraZeneca, and Napp Pharmaceuticals.

Received for publication August 9, 2017; revised October 13, 2017; accepted for publication October 17, 2017.

Corresponding author: Steven W. Yancey, MSc, 5 Moore Dr, PO Box 13398, Research Triangle Park, NC 27709. E-mail: steve.w.yancey@gsk.com.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2017.10.005

of biomarkers that include pharmacodynamic, predictive, diagnostic, and prognostic biomarkers.

Pharmacodynamic biomarkers inform on the biological response to pharmacologic intervention. For example, the effect of anticoagulation treatments (eg, warfarin) for thromboembolic disease can be assessed by measuring prothrombin time as a pharmacodynamic biomarker.[24]

A predictive biomarker identifies patients likely to derive benefit from treatment or identify patients who are unlikely to respond, which thus influences clinical decisions. Although there are no predictive biomarkers for heart failure currently in routine use,[25] predictive biomarkers are used routinely in oncology. For example, for patients with invasive breast cancer, overexpression or gene amplification of human epidermal growth factor receptor 2 is predictive of response to drugs targeted at the human epidermal growth factor receptor 2, such as trastuzumab, pertuzumab, and lapatinib.[26] Conversely, in patients with rheumatoid arthritis, a characteristic interferon type I genetic signature was found to be predictive of nonresponse to rituximab.[27]

A diagnostic biomarker identifies patients with a specific condition or disease, whereas a prognostic biomarker categories the risk of disease progression in the absence of treatment. In the case of heart failure, the presence of increased concentrations of B-type natriuretic peptide, which is produced in response to myocardial stress, has both positive and negative diagnostic value.[28] B-type natriuretic peptide concentration on admission to the hospital also has a linear relationship with morbidity and mortality outcomes and is the gold standard prognostic biomarker for heart failure.[28]

In the case of asthma, early observations on the association between eosinophil overexpression and asthma severity were made in 1990 by Bousquet et al.[29] Multiple studies have since confirmed that blood, tissue, or sputum eosinophil counts can be used to characterize patients with severe asthma and eosinophilic inflammation.[30-37] Through the drug development process for mepolizumab, investigators identified blood eosinophils, rather than sputum eosinophils, as the treatment target for mepolizumab, providing an accessible and multipurpose biomarker for severe eosinophilic asthma.[18,19,38,39] This review will examine how evidence generated during the mepolizumab clinical development program showed that the blood eosinophil count can serve as a pharmacodynamic and predictive biomarker in patients with severe eosinophilic asthma.

PHARMACODYNAMIC BIOMARKERS FOR MEPOLIZUMAB TREATMENT RESPONSE

Mepolizumab binds with high specificity and affinity to human IL-5,[40] the key T2 cytokine responsible for regulation of blood and tissue eosinophils.[41] Mepolizumab prevents IL-5 from binding to the α chain of the IL-5 receptor complex expressed on the eosinophil cell surface and thus inhibits IL-5 signaling, blocking eosinophil survival and proliferation. Although the exact mechanism of action of IL-5 inhibitors is not fully elucidated, the desired physiologic goal is to neutralize the effect of activated eosinophils in blood and tissues, such as the lung.

During the development of mepolizumab, the pharmacodynamic response was assessed in blood, sputum, and tissue eosinophils.[18-20,42,43] Early studies showed that mepolizumab produced modest reductions in airway tissue and bone marrow eosinophil counts, suggesting limited pharmacodynamic effects in these compartments.[9] Haldar et al[43] then showed that treatment with 750 mg of intravenous mepolizumab in patients with severe asthma and sputum eosinophil counts of greater than 3% at least once in the previous 2 years reduced blood and sputum eosinophil counts during treatment, although sputum eosinophilia was present in 36% of exacerbations despite mepolizumab therapy.

After this, the Dose Ranging Efficacy and safety With Mepolizumab in Severe Asthma (DREAM) study (NCT01000506) was conducted, which was a phase 2b/3 clinical trial of mepolizumab in patients with severe asthma and eosinophilic inflammation and included assessments of the pharmacodynamic response of mepolizumab.[19] The inclusion criteria for the DREAM study are shown in Fig 1. Patients in the DREAM study received either placebo or 75, 250, or 750 mg of intravenous mepolizumab, representing a 10-fold dose range. At the 750-mg intravenous dose, there were comparable reductions of 88% in blood and sputum eosinophil counts; however, for the 250-mg intravenous dose, the reduction in blood eosinophil counts was 86% compared with 65% for sputum, and for the 75-mg intravenous dose, the reduction in blood eosinophil counts was 78% compared with 32% for sputum (Fig 2).[19] All doses of mepolizumab had similar beneficial effects on the primary outcome measure, the rate of clinical significant asthma exacerbations.

A second study[42] assessing a range of subcutaneous doses (12.5, 125, or 250 mg administered subcutaneously vs 75 mg administered intravenously) showed that this reduction in blood eosinophil count occurred 2 days after the first dose of mepolizumab, was dose dependent, and was not affected by administration route (intravenous vs subcutaneous) after adjusting for bioavailability.

Overall, results from the DREAM study and Pouliquen et al[42] suggest that blood eosinophil but not sputum or tissue eosinophil counts are the key pharmacodynamic biomarker response to mepolizumab treatment in patients with severe eosinophilic asthma. This is reflected by the reduction in exacerbation rates with mepolizumab versus placebo in these patients. Since then, studies of mepolizumab in patients with hypereosinophilic syndrome, eosinophilic granulomatosis with polyangiitis, and eosinophilic esophagitis all show a consistent pharmacodynamic effect of mepolizumab on eosinophils that can be measured easily and reproducibly by using blood eosinophil counts.[18,19,42,44-47]

In addition to blood eosinophil counts, other potential pharmacodynamic biomarkers, such as fraction of exhaled nitric oxide (FENO), were assessed during the development of mepolizumab.[19,43] Nitric oxide is released by several pulmonary cells, including epithelial cells, eosinophils, and macrophages, and nitric oxide levels have been shown to be increased in patients with conditions associated with inflammation, such as asthma and viral infections. In the DREAM study across the 10-fold dose range of 75 to 750 mg administered intravenously, there was no pharmacodynamic response with FENO (Fig 2).[19] This lack of response to FENO was also shown in the earlier mepolizumab study by Haldar et al.[43] This suggests that FENO is not responsive to modulation through the IL-5 pathway and is potentially more relevant to different aspects of the T2 inflammatory response (eg, IL-13).

Because the clinical efficacy of mepolizumab was similar across all doses of mepolizumab (75, 250, and 750 mg), it was decided to progress the lowest dose (75 mg administered intravenously) of mepolizumab in further phase 3 studies. The

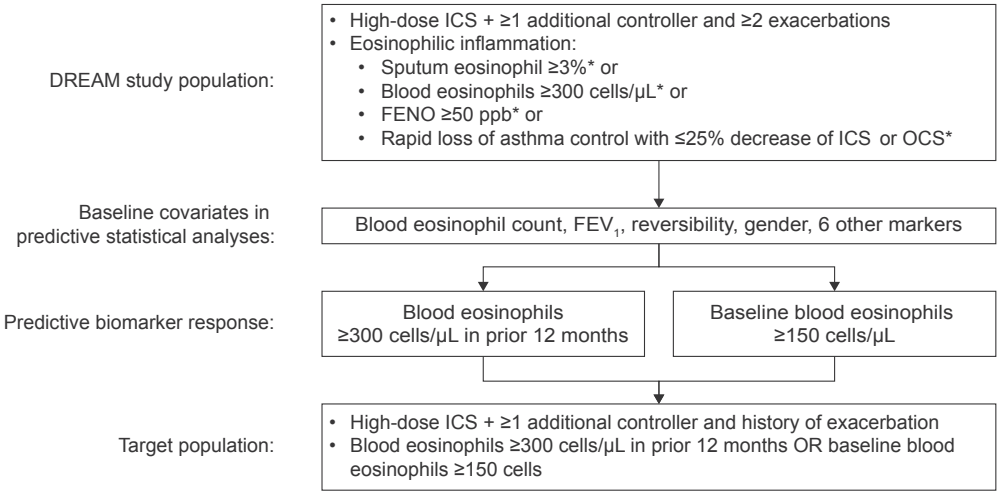

FIG 1. Biomarker identification from the DREAM study. *In prior 12 months or at screening.

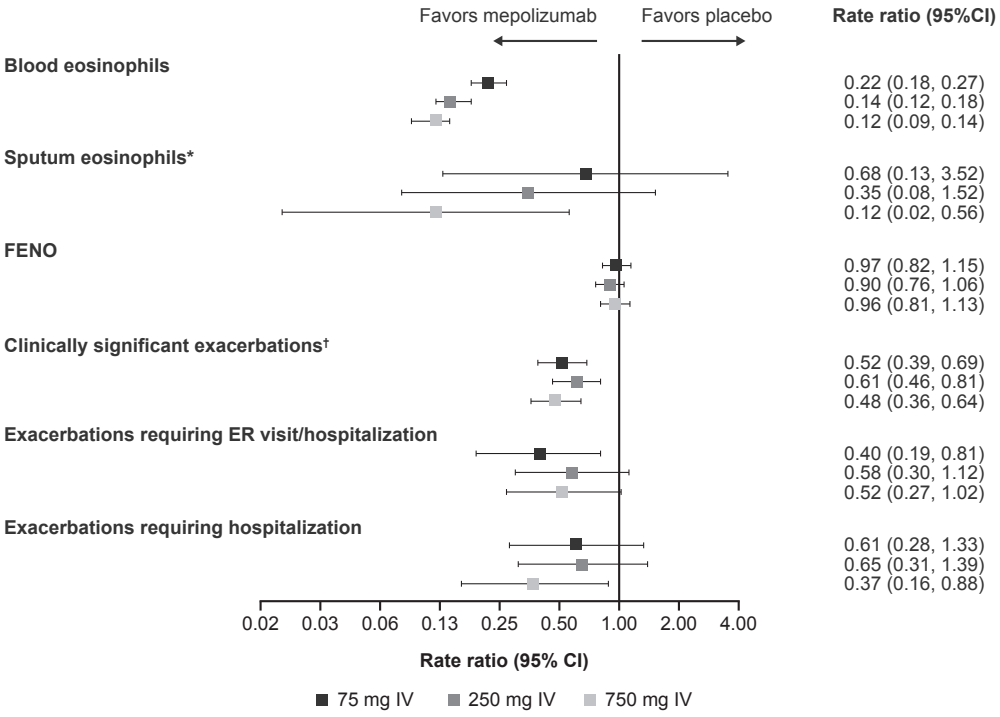

FIG 2. Eosinophil, FENO, and exacerbation outcomes across the 10-fold dose range for mepolizumab in the DREAM study. *Collected from 94 patients in the DREAM sputum substudy. †Defined as worsening of asthma requiring use of OCSs for 3 or more days, admission, or a visit to the emergency room *(ER)*. *IV*, Intravenous.

decision to take the lowest dose forward was supported by data that suggested that a dose of 75 mg administered intravenously (and the comparable exposure dose of 100 mg administered subcutaneously) was the minimum dose required to significantly reduce eosinophil counts.[42] In the subsequent clinical trials that have used the 100-mg subcutaneous dose (which has comparable systemic exposure to 75 mg administered intravenously), mepolizumab reduced exacerbation rates similar to the rate reduction achieved with higher doses of mepolizumab.[18,48,49]

In the SIRIUS trial (NCT01691508), mepolizumab at a dose of 100 mg administered subcutaneously was shown to reduce the requirement for daily OCS while significantly improving asthma control, lung function, and quality of life.[18,20] In the MUSCA trial (NCT02281318) patients treated with mepolizumab reported an improvement in Asthma Control Questionnaire scores. This also corresponded to an observed improvement in patients' quality of life, as measured by using the St George Respiratory Questionnaire.[48] Results from the Steroid Reduction with Mepolizumab Study (SIRIUS) trial were comparable with the OCS-sparing effects seen during a trial with mepolizumab at 750 mg administered intravenously,[48] providing further indirect clinical evidence that a

higher dose of mepolizumab is not required to achieve maximum efficacy benefits.

Taken together, pharmacodynamic responses to mepolizumab in the blood and sputum informed the therapeutic dose across a 10-fold dose range. Blood pharmacodynamic responses showed that a dose of 100 mg of mepolizumab administered subcutaneously provides the desired pharmacology to neutralize serum IL-5 levels and reduce blood eosinophil counts to approximately 40 cells/μL, which corresponds to a low normalized blood eosinophil count.[18-20,48] Interestingly, dose-dependent pharmacodynamic responses in blood suggest that complete depletion of eosinophil counts in the blood compartment is not necessary to achieve maximal clinical efficacy. Correspondingly, in clinical trials blood eosinophil counts are consistently reduced to approximately 40 cells/μL rather than fully depleted.[18-20] Furthermore, this reduction occurs with mepolizumab doses ranging from 75 mg administered intravenously/100 mg administered subcutaneously to 750 mg administered intravenously, suggesting that increasing the mepolizumab dose would not further reduce blood eosinophil counts.

Outside of the mepolizumab clinical trial program, recent studies have shown that benralizumab can deplete blood eosinophils to a higher degree than mepolizumab or reslizumab,[50,51] and this differentiated mechanism of action has led some investigators to suggest that benralizumab might prove to be more efficacious than mepolizumab or reslizumab.[52] Although no head-to-head data exist, in the phase 3 SIROCCO (NCT01928771) and CALIMA (NCT01914757) trials, benralizumab reduced the annual rate of exacerbations by 28% to 51% versus placebo in patients with blood eosinophil counts of 300 cells/μL or greater at baseline,[14,15] which does not exceed the exacerbation reductions achieved in similar studies of mepolizumab and reslizumab.[17,18,20] Similarly, in a *post hoc* analysis of SIROCCO and CALIMA trials, benralizumab reduced the annual rate of exacerbations by 36% to 42% in patients with blood eosinophil counts of 150 cells/μL or greater at baseline by using an 8-week dosing regimen.[53] In addition, effects on asthma control and quality of life appeared to be less prominent than in mepolizumab studies when assessed according to blood eosinophil counts at baseline.[14,15,18,20] This further supports the observation from the DREAM study that a maximal degree of eosinophil depletion in the blood is not necessary to achieve maximal clinical efficacy. In fact, recent animal data suggest that the eosinophil population might contain a specific subtype of eosinophils that contribute to homeostatic immune processes, and therefore targeting complete deletion of eosinophils might not be desirable.[54]

PREDICTIVE BIOMARKERS FOR MEPOLIZUMAB TREATMENT RESPONSE

Identifying a predictive biomarker for response to treatment was one of the main aims of the mepolizumab clinical development program. As such, initial modeling analyses of the DREAM study were carried out to identify characteristics that could predict treatment response. In the exploration for biomarkers predictive of treatment response, the frequency and severity of exacerbations were considered the principal clinically relevant end points. Although other highly relevant measures, such as lung function, are known to be improved during mepolizumab treatment,[18,20,48] these end points were secondary to the frequency of exacerbations. Analyses investigated various clinical characteristics as individual covariates in the DREAM study (ie, sex, age, weight, geographic region, baseline forced expiratory volume, airway reversibility at screening, number of exacerbations in the previous year, baseline blood eosinophil count, baseline use of maintenance OCSs, and IgE count) to distinguish which variables were predictive of more frequent exacerbations and differential efficacy of mepolizumab (Fig 1).[19] The model identified blood eosinophil counts as the strongest predictor of reduction in exacerbation rates with mepolizumab compared with placebo.[19] Consequently, in a *post hoc* analysis of the DREAM study, it was found that a blood eosinophil count threshold of 150 cells/μL or greater combined with other clinical characteristics, such as exacerbation rate, could predict response to treatment with mepolizumab. Blood eosinophil counts at baseline were also a better predictor of response than sputum eosinophil counts. Furthermore, treatment-related effects on blood eosinophil counts related more closely to clinical benefits than effects on sputum eosinophil counts, leading us to believe that suppression of blood eosinophil counts is crucial to efficacy.[19]

The initial modeling analysis from the DREAM study was repeated for the phase 3 Mepolizumab Adjunctive Therapy in Subjects with Severe Uncontrolled Refractory Asthma (MENSA) study (NCT01691521)[18] and also showed that there is a direct relationship between baseline blood eosinophil counts and reduction in exacerbation rates (Fig 3).[55] This was confirmed in a clinical evaluation of the alternative anti–IL-5 therapies reslizumab[56] and benralizumab[14] in which patients with blood eosinophil counts of greater than 400 or 300 cells/μL, respectively, derived greater clinical benefit from the therapy compared with those with eosinophil counts of less than the threshold.[14,56] At 150 cells/μL, the predicted reduction in exacerbation rate was estimated to be 30% and 39% in the DREAM and MENSA studies, respectively, both representing clinically meaningful reductions in exacerbation rates.[55] Thus although both studies showed a reduction in exacerbation rates of approximately 50% in the overall population,[18,19] the model predicted a clinically important 30% or greater reduction in exacerbation rates in patients with baseline blood eosinophil counts of 150 cells/μL. This was similar to a subanalysis of 2 benralizumab studies focusing on patients with eosinophil counts of greater than 150 cells/μL, in which the therapy reduced exacerbation rates by 32% and 42%.[53] Although 150 cells/μL is the minimal cutoff to observe meaningful clinical benefit, the majority of the patients in the mepolizumab program have blood eosinophil counts of 300 cells/μL or greater. However, using a blood eosinophil count threshold of 300 cells/μL to identify patients for treatment might exclude 25% of patients with severe asthma with an eosinophil count in the range of 150 to 300 cells/μL who might benefit from treatment. A meta-analysis of patients from the DREAM and MENSA studies showed that patients with blood eosinophil counts of 150 or greater to less than 300 cells/μL at baseline achieved a 33% reduction in annual clinically significant exacerbations (rate ratio [RR], 0.67; 95% CI, 0.45-1.01).[55]

Because the DREAM and MENSA studies had different inclusion criteria to identify patients with severe eosinophilic asthma (Table I), additional analyses have been carried out to

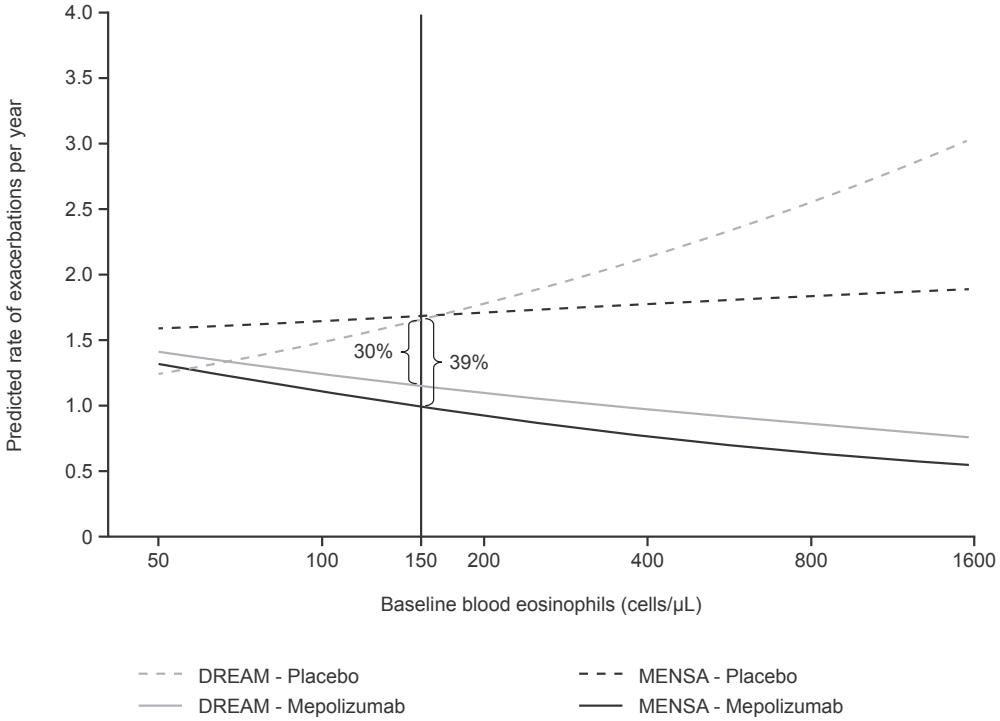

FIG 3. Modeling analysis: predicted rate of exacerbations per year against baseline blood eosinophil count (DREAM and MENSA studies). Data are from the intention-to-treat populations from the DREAM and MENSA studies. The graph shows percentage differences in exacerbation rates (mean per person per year) between placebo and mepolizumab at a baseline blood eosinophil count of 150 cells/μL. Reprinted from Ortega et al[55] with permission from Elsevier.

TABLE I. Exacerbation reduction in the DREAM study analyzed according to MENSA study enrollment criteria

	Exacerbation rate (events/y)		Reduction in mepolizumab vs placebo (%)	RR, mepolizumab vs placebo (95% CI)
	Placebo rate/y	Mepolizumab rate/y		
≥150 cells/μL at treatment start or 300 cells/μL in previous 12 mo				
Yes	n = 137 2.42	n = 185 1.18	51	0.49 (0.38-0.63)
No	n = 27 2.07	n = 76 1.82	10	0.90 (0.49-1.64)

corroborate the clinical benefit of mepolizumab based on blood eosinophil counts. The DREAM study used 4 criteria to identify patients with eosinophilic inflammation, as shown in Fig 1. In contrast, the MENSA study defined eosinophilic inflammation as a blood eosinophil count of 150 cells/μL or greater at screening or 300 cells/μL or greater at some time during the previous year. A *post hoc* analysis of the primary end point for the DREAM study was conducted by using the blood eosinophil criteria used in the MENSA study to assess the effect of these different criteria. This analysis showed that patients treated with mepolizumab who met the blood eosinophil criteria had a 50% reduction in exacerbation rates over those receiving placebo compared with a 10% reduction in patients who did not meet either blood eosinophil criteria (Table I).[55]

Further analysis of the combined DREAM and MENSA study population showed that clinically relevant reductions in exacerbations were also achieved in patients who met either of the 2 blood eosinophil criteria (Table II). Taken together, these data show that either criterion (historical or baseline) is predictive of response.[55] These results support the utility of the evaluated blood eosinophil counts as a biomarker to identify a patient likely to benefit from treatment with mepolizumab.

Although sputum eosinophil measurements have been correlated with airway eosinophilia, further analysis of the DREAM study showed that sputum eosinophil counts did not predict response mepolizumab treatment.[38] In the 94 patients from the sputum substudy of the overall DREAM study, exacerbation rates were reduced by approximately 70% in both patients with a sputum eosinophil count of 3% or greater (RR, 0.31; 95% CI, 0.17-0.59) and those with a count of less than 3% (RR, 0.34; 95% CI, 0.13-0.93).[36] In the whole study, when analyzed based on blood eosinophil counts, exacerbation rate reductions were 72% for counts of 150 cells/μL or greater (RR, 0.28; 95% CI, 0.14-0.54) compared with 30% for counts of less than 150 cells/μL (RR, 0.70; 95% CI, 0.21-2.34).[38] This lack of differential response with sputum eosinophil counts has been debated because it seems more intuitive that sputum eosinophil counts would be more predictive of treatment response than blood eosinophil counts. Although the DREAM sputum substudy was based on 94 adult patients, a recent study in patients with severe asthma suggested that exacerbation frequency is associated with increasing sputum eosinophil counts.[57]

An extension of the knowledge that blood eosinophil counts of greater than 150 cells/μL are predictive of response to mepolizumab is that a blood eosinophil count of less than 150 cells/μL might be considered predictive of nonresponse

TABLE II. Exacerbation reduction stratified by categories of blood eosinophil criteria in the DREAM and MENSA studies (intent-to-treat populations)*

	Exacerbation rate (events/y)		Reduction in mepolizumab vs placebo (%)	RR, mepolizumab vs placebo (95% CI)
	Placebo	Mepolizumab		
All patients	n = 346 1.91	n = 846 1.01	47	0.53 (0.44-0.62)
All patients ≥150 cells/μL at treatment start	n = 278 1.94	n = 642 0.92	52	0.48 (0.39-0.58)
All patients ≥300 cells/μL in previous 12 mo	n = 253 1.96	n = 608 1.01	49	0.51 (0.42-0.63)

*All mepolizumab doses were pooled for analysis.

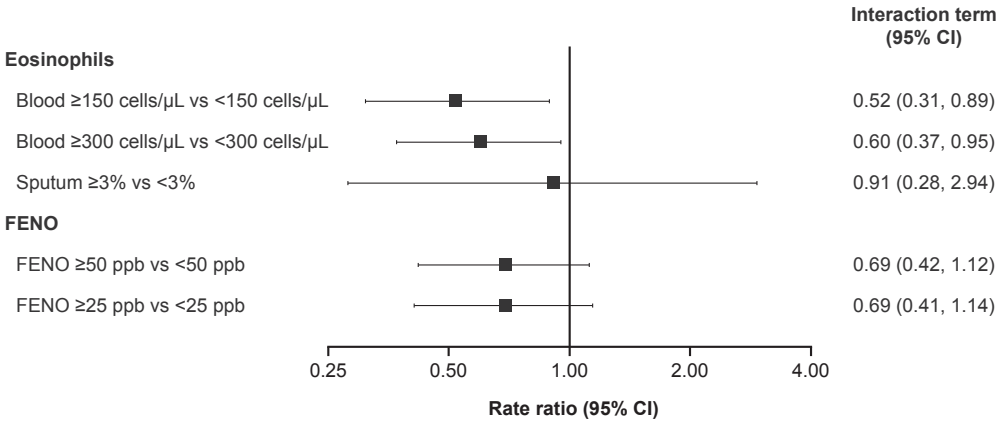

FIG 4. *Post hoc* analysis of exacerbation reduction according to baseline biomarkers (DREAM study).[19]

(Gunsoy et al, personal communication). FENO has also been evaluated as a predictive biomarker for treatment with mepolizumab. However, additional analysis of the DREAM study has also shown that a FENO value of 50 ppb or greater does not act as a predictive biomarker for reductions in exacerbation rates with mepolizumab (Fig 4).[19] In the analysis of the DREAM data, no other early response markers were identified that provided additional predictive value compared with blood eosinophil counts (Gunsoy et al, personal communication).

DIAGNOSTIC BIOMARKERS FOR AIRWAY EOSINOPHILIA

In patients managed according to GINA Step 4 therapeutic interventions[1] who continue to have poorly controlled severe asthma, a detailed investigation is required. This might include evaluation of IgE levels and eosinophil counts to identify whether a specific asthma phenotype is present and to determine the optimal GINA Step 5 course of therapy.[1] Although sputum eosinophil counts of 2% or greater to 3% have traditionally been correlated with airway eosinophilia,[19,58,59] better biomarkers might exist. The utility of blood eosinophil counts as a diagnostic biomarker for airway eosinophilia has been evaluated by assessing the relationship between blood and sputum eosinophil counts.[18,19,21,23]

In the DREAM study, using 76 paired samples at screening of blood and sputum eosinophil counts from the sputum substudy, a screening blood eosinophil count of 150 cells/μL or greater was associated with sputum eosinophilia (≥2%) in 9 of 10 patients.[39] A blood eosinophil count of 150 cells/μL or greater also correctly predicted sputum eosinophilia with a sensitivity of 85% (95% CI, 73% to 93%) and specificity of 75% (95% CI, 48% to 93%; Fig 5).[39] At this threshold, only 4 (5%) patients did not have sputum counts of 2% or greater. If the blood threshold was increased to 300 cells/μL, this excluded only 1 patient who did not have a sputum count of 2% or greater but conversely excluded 25 patients who did have a sputum count of 2% or greater.[39] This indicates that not only does the blood eosinophil count act as a pharmacodynamic and predictive biomarker for mepolizumab treatment but that it also acts as a diagnostic biomarker for severe eosinophilic asthma in patients with a history of 2 or more exacerbations in the past 12 months despite the use of high-dose ICSs plus additional controllers.

Independent studies corroborate that a blood eosinophil count threshold of approximately 150 cells/μL is associated with sputum eosinophilia,[60,61] whereas other studies report that a threshold of 150 cells/μL is not associated with sputum eosinophilia and generally place the threshold around 300 cells/μL.[62,63] It is important to recognize that the threshold is very much dependent on the populations studied. Recent analyses have demonstrated that in patients with severe asthma, blood and sputum eosinophil counts can be increased by reducing doses of ICSs and OCSs and *vice versa*.[64,65] This has been corroborated by a *post hoc* analysis of the SIRIUS trial, which showed that the OCS dose is inversely correlated with blood eosinophil counts.[66] For the studies that showed sputum eosinophilia to be associated with a blood eosinophil count of approximately 300 cells/μL, patients were not necessarily receiving high-dose ICSs, and the use of OCSs was minimal.[62,63] Conversely, for the studies that showed a robust association between airway eosinophilia and a blood eosinophil count

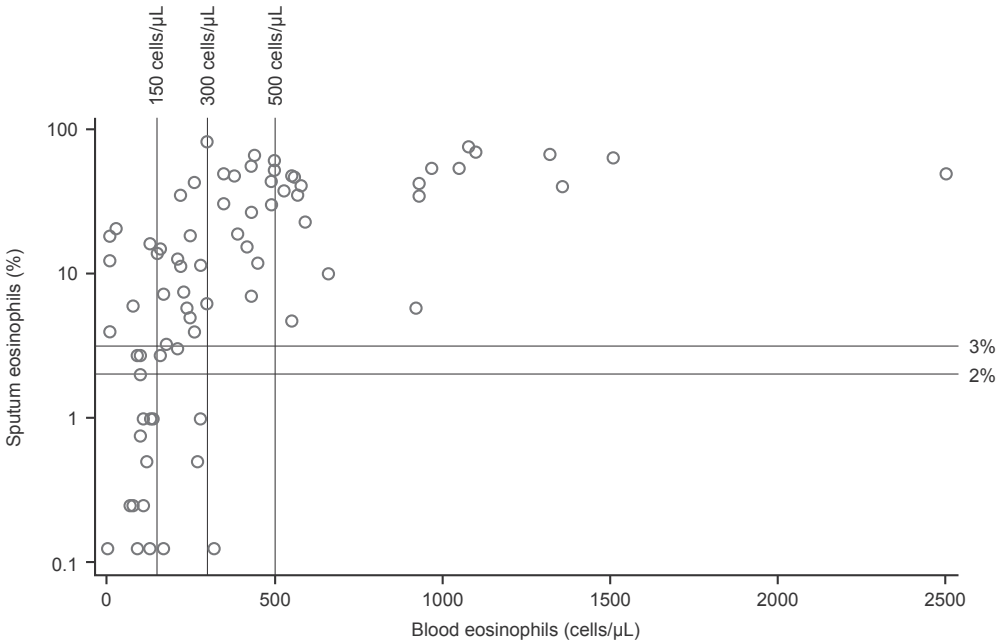

FIG 5. Display of sputum eosinophil versus blood eosinophil counts at the screening visit in the DREAM study. Reprinted from Ortega et al[39] with permission from Elsevier.

threshold of approximately 150 cells/μL, nearly all patients were receiving high-dose ICSs, and about a quarter of patients were also receiving OCS doses typical for patients with severe asthma.[60,67] Overall, the evidence shows that a blood eosinophil threshold of 150 cells/μL identifies patients with severe eosinophilic asthma receiving guideline-directed treatment.

Because blood eosinophil counts can vary on a daily basis, it is important to understand the effect of this variability. For this reason, the reproducibility of blood eosinophil counts as a diagnostic biomarker was assessed in *post hoc* analyses of the DREAM and MENSA studies for patients receiving placebo.[38,68] In the DREAM study, of the 115 patients with a blood eosinophil count of 150 cells/μL or greater at screening, 98 (85%) remained at greater than this threshold in their postscreening average, with a mean postscreening average of 168 cells/μL (95% CI, 149-189 cells/μL) across the year.[38] Similar results were obtained from the 167 patients from the MENSA study with a blood eosinophil count of 150 cells/μL or greater at screening, with 86% having a count of 150 cells/μL or greater over the following 8 months.[68] In both analyses multiple measurements only marginally increased sensitivity, meaning that a single measurement was adequate to identify patients with severe eosinophilic asthma. Overall, this suggests that a single blood eosinophil count of 150 cells/μL or greater in concert with other clinical markers of severe disease can be used effectively as a diagnostic biomarker for airway eosinophilia in a population with severe asthma that continues to exacerbate despite the use of high-dose ICSs plus additional controllers.

DISCUSSION AND CONCLUSIONS

Validation and qualification of biomarkers is crucial to their successful use and should include confirmation of sensitivity, specificity, and reproducibility.[69] Through the mepolizumab clinical development program, the blood eosinophil count has been assessed for use as a pharmacodynamic and predictive biomarker. First, it was established that blood eosinophil counts, rather than sputum eosinophil counts, are the more useful pharmacodynamic biomarker for mepolizumab.[19,42] Second, and crucially for clinical decision making, blood eosinophil counts can be used to identify a patient likely to benefit from mepolizumab treatment in terms of reduction in exacerbation rates, as demonstrated by the direct relationship between baseline blood eosinophil counts and the predicted exacerbation rate shown in analyses of the DREAM and MENSA studies.[18,19,55]

Although several diverse approaches were taken during the development of mepolizumab to examine whether novel protein or genetic markers were an indicator of eosinophilia, no marker emerged that was more predictive of treatment effect than the blood eosinophil count obtained from a simple complete blood count. Importantly for its routine clinical use, measurement of blood eosinophil counts is practical and minimally invasive in comparison to collection of sputum samples. These qualities mean that information to support clinical decision making can be easily accessible to health care providers during routine care. A blood examination is also relatively inexpensive, and the results can be available in a short period of time after collection. Importantly, the data suggest that a single measurement is sufficient to identify a blood eosinophil count of 150 cells/μL or greater in approximately 85% of patients.[38,68] This compares favorably with the use of hemoglobin A_{1C} in patients with diabetes, where a single measurement can be used to assess average blood glucose control over 3 months.[70,71]

In addition to biomarker evaluation, the DREAM and MENSA studies refined the target population for mepolizumab treatment to patients with severe asthma with frequent exacerbations despite high-dose ICSs plus additional controllers or dependency on OCSs and quantified the thresholds required to predict treatment response. Taking into account that patients treated with high doses

of ICSs (and OCSs) might have reduced blood eosinophil counts, from the model developed, the threshold of 150 cells/μL provides a reliable means to identify the patient population with severe asthma that is likely to benefit from mepolizumab treatment.[55] Additionally, historical blood eosinophil counts (≥300 cells/μL) allow flexibility to start a patient with severe asthma immediately on treatment if deemed appropriate by the health care provider.[55] Of course, as with all biomarker use, deriving a credible health effect with the use of blood eosinophil counts requires the use of sound clinical judgement to incorporate biomarker measurement information into a clinically relevant patient management strategy.[72] This is particularly important in clinical practice, where a range of blood eosinophil counts can be predictive of exacerbation risk given the heterogeneous nature of asthma populations.[60,73-75] As such, individual symptoms, disease patterns, and treatment goals must be considered as part of the treatment decision-making process, and exacerbation history is key in this regard.

With the introduction of further predictive biomarkers for use in patients with severe asthma, as well as the growing number of targeted biological therapies available, decisions regarding biomarker use in a particular patient are complex. The decision to treat a patient with severe eosinophilic asthma who is likely to benefit from an anti–IL-5 therapy might not be straightforward because the underlying mechanisms responsible for poor asthma control and airway eosinophilia might be due to heterogeneous T2 inflammatory pathways.[41] It is also documented that some patients with severe asthma have both activated T2 and T1 inflammatory pathways.[76] It is possible that future research can identify a composite of different biomarkers rather than a single biomarker to help target biological therapies to individual pathology. Currently, there are no head-to-head studies comparing the efficacy of anti-IgE and anti–IL-5 biologic therapies, but such studies might be difficult because of sample size estimates. Therefore use of clinical end points and available biomarkers should help select the appropriate patients for these treatment options.

Several aspects of treatment with mepolizumab require further investigation. Mepolizumab is indicated currently in adults and adolescents, but further research is needed to understand its efficacy, pharmacokinetics, and pharmacodynamics in children. As more biological therapies become available, the effect of switching from one therapy to another should be evaluated. Finally, a deeper understanding of the pharmacoeconomics of the use of biologics in the management of severe eosinophilic asthma is warranted.

In conclusion, stratification of patients with severe asthma to guide treatment decisions is complex, and reliable and sensitive biomarkers are a valuable tool for physicians in this regard. Data from the mepolizumab clinical trial program support the use of baseline blood eosinophil counts as a sensitive and practical predictive biomarker for mepolizumab treatment effect in patients with severe eosinophilic asthma with frequent exacerbations, despite optimal standard of care or are dependent on maintenance OCS therapy. In combination with sound clinical judgement, use at a baseline blood eosinophil count threshold of 150 cells/μL or greater and/or a historical blood eosinophil count threshold of 300 cells/μL or greater will allow selection of patients who are most likely to achieve clinically significant reductions in the rate of exacerbations with mepolizumab treatment.

Editorial support (in the form of writing assistance during development of the subsequent drafts, assembling tables and figures, collating authors comments, grammatical editing, and referencing) was provided by Elizabeth Hutchinson, PhD, CMPP, at Fishawack Indicia Ltd and funded by GlaxoSmithKline.

REFERENCES

1. Global Initiative For Asthma. Global strategy for asthma management and prevention, 2017. Available at: www.ginasthma.org. Accessed July 1, 2017.
2. Genentech. Omalizumab prescribing information. South San Francisco (CA): Genentech; 2017.
3. Lin CH, Cheng SL. A review of omalizumab for the management of severe asthma. Drug Des Devel Ther 2016;10:2369-78.
4. Novartis. Omalizumab summary of product characteristics. Basel (Switzerland): Novartis; 2015.
5. Wahn U, Martin C, Freeman P, Blogg M, Jimenez P. Relationship between pretreatment specific IgE and the response to omalizumab therapy. Allergy 2009;64:1780-7.
6. Bousquet J, Rabe K, Humbert M, Chung KF, Berger W, Fox H, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. Respir Med 2007;101:1483-92.
7. Buttner 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.
8. Castro M, Mathur S, Hargreave F, Boulet LP, Xie F, Young J, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. Am J Respir Crit Care Med 2011;184:1125-32.
9. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. Am J Respir Crit Care Med 2003;167:199-204.
10. Menzies-Gow A, Flood-Page P, Sehmi R, Burman J, Hamid Q, Robinson DS, et al. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. J Allergy Clin Immunol 2003;111:714-9.
11. GlaxoSmithKline. Mepolizumab prescribing information. Research Triangle Park (NC): GlaxoSmithKline; 2017.
12. GlaxoSmithKline. Mepolizumab summary of product characteristics. Research Triangle Park (NC): GlaxoSmithKline; 2016.
13. Teva. Reslizumab summary of product characteristics. Petach Tikva (Israel): Teva; 2016.
14. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta2-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. Lancet 2016;388:2115-27.
15. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet 2016;388:2128-41.
16. Ghazi A, Trikha A, Calhoun WJ. Benralizumab—a humanized mAb to IL-5Ralpha with enhanced antibody-dependent cell-mediated cytotoxicity—a novel approach for the treatment of asthma. Expert Opin Biol Ther 2012;12:113-8.
17. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet Respir Med 2015;3:355-66.
18. 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.
19. 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.
20. 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.
21. Nair P, Wenzel S, Rabe KF, Bourdin A, Lugogo NL, Kuna P, et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. N Engl J Med 2017;376:2448-58.
22. Albers FC, Mullerova H, Gunsoy NB, Shin JY, Nelsen LM, Bradford ES, et al. Biologic treatment eligibility for real-world patients with severe asthma: the IDEAL study. J Asthma 2017 [Epub ahead of print].

23. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69: 89-95.
24. Moore KT, Byra W, Vaidyanathan S, Natarajan J, Ariyawansa J, Salih H, et al. Switching from rivaroxaban to warfarin: an open label pharmacodynamic study in healthy subjects. Br J Clin Pharmacol 2015;79:907-17.
25. de Groote P, Pinet F, Bauters C. New technologies, new therapies: toward personalized medicine in heart failure patients? Eur Heart J 2013;34:636-7.
26. Duffy MJ, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E, et al. Clinical use of biomarkers in breast cancer: updated guidelines from the European Group on Tumor Markers (EGTM). Eur J Cancer 2017;75:284-98.
27. Raterman HG, Vosslamber S, de Ridder S, Nurmohamed MT, Lems WF, Boers M, et al. The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. Arthritis Res Ther 2012;14:R95.
28. Gaggin HK, Januzzi JL Jr. Biomarkers and diagnostics in heart failure. Biochim Biophys Acta 2013;1832:2442-50.
29. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-9.
30. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9-16.
31. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. J Allergy Clin Immunol 2001;108:753-8.
32. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001-8.
33. Gheorghiade M, Thyssen A, Zolynas R, Nadar VK, Greenberg BH, Mehra M, et al. Pharmacokinetics and pharmacodynamics of rivaroxaban and its effect on biomarkers of hypercoagulability in patients with chronic heart failure. J Heart Lung Transplant 2011;30:218-26.
34. Lemiere C, Ernst P, Olivenstein R, Yamauchi Y, Govindaraju K, Ludwig MS, et al. Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. J Allergy Clin Immunol 2006;118:1033-9.
35. Chu HW, Balzar S, Westcott JY, Trudeau JB, Sun Y, Conrad DJ, et al. Expression and activation of 15-lipoxygenase pathway in severe asthma: relationship to eosinophilic phenotype and collagen deposition. Clin Exp Allergy 2002;32: 1558-65.
36. Grootendorst DC, Sont JK, Willems LN, Kluin-Nelemans JC, Van Krieken JH, Veselic-Charvat M, et al. Comparison of inflammatory cell counts in asthma: induced sputum vs bronchoalveolar lavage and bronchial biopsies. Clin Exp Allergy 1997;27:769-79.
37. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med 1997;155:449-53.
38. Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. Ann Am Thorac Soc 2014;11:531-6.
39. Ortega H, Katz L, Gunsoy N, Keene O, Yancey S. Blood eosinophil counts predict treatment response in patients with severe eosinophilic asthma. J Allergy Clin Immunol 2015;136:825-6.
40. Hart TK, Cook RM, Zia-Amirhosseini P, Minthorn E, Sellers TS, Maleeff BE, et al. Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys. J Allergy Clin Immunol 2001;108:250-7.
41. Chung KF. Targeting the interleukin pathway in the treatment of asthma. Lancet 2015;386:1086-96.
42. Pouliquen IJ, Kornmann O, Barton SV, Price JA, Ortega HG. Characterization of the relationship between dose and blood eosinophil response following subcutaneous administration of mepolizumab. Int J Clin Pharmacol Ther 2015; 53:1015-27.
43. 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.
44. Stein ML, Collins MH, Villanueva JM, Kushner JP, Putnam PE, Buckmeier BK, et al. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. J Allergy Clin Immunol 2006;118:1312-9.
45. Straumann A, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. Gut 2010; 59:21-30.
46. Kim S, Marigowda G, Oren E, Israel E, Wechsler ME. Mepolizumab as a steroid-sparing treatment option in patients with Churg-Strauss syndrome. J Allergy Clin Immunol 2010;125:1336-43.
47. Rothenberg ME, Klion AD, Roufosse FE, Kahn JE, Weller PF, Simon HU, et al. Treatment of patients with the hypereosinophilic syndrome with mepolizumab. N Engl J Med 2008;358:1215-28.
48. 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.
49. Yancey SW, Ortega HG, Keene ON, Mayer B, Gunsoy NB, Brightling CE, et al. Meta-analysis of asthma-related hospitalization in mepolizumab studies of severe eosinophilic asthma. J Allergy Clin Immunol 2017;139:1167-75.e2.
50. Brightling CE, Bleecker ER, Panettieri RA Jr, Bafadhel M, She D, Ward CK, et al. Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study. Lancet Respir Med 2014;2:891-901.
51. Castro M, Wenzel SE, Bleecker ER, Pizzichini E, Kuna P, Busse WW, et al. Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. Lancet Respir Med 2014;2:879-90.
52. Menzella F, Lusuardi M, Galeone C, Facciolongo N, Zucchi L. The clinical profile of benralizumab in the management of severe eosinophilic asthma. Ther Adv Respir Dis 2016;10:534-48.
53. Goldman M, Hirsch I, Zangrilli JG, Newbold P, Xu X. The association between blood eosinophil count and benralizumab efficacy for patients with severe, uncontrolled asthma: subanalyses of the Phase III SIROCCO and CALIMA studies. Curr Med Res Opin 2017;33:1605-13.
54. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirottin D, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. J Clin Invest 2016;126:3279-95.
55. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. Lancet Respir Med 2016;4:549-56.
56. Corren J, Weinstein S, Janka L, Zangrilli J, Garin M. Phase 3 study of reslizumab in patients with poorly controlled asthma: effects across a broad range of eosinophil counts. Chest 2016;150:799-810.
57. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Cardet JC, et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. Am J Respir Crit Care Med 2017;195:302-13.
58. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 2007;176:1062-71.
59. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. J Allergy Clin Immunol 2004;113:101-8.
60. Schleich F, Brusselle G, Louis R, Vandenplas O, Michils A, Pilette C, et al. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). Respir Med 2014;108:1723-32.
61. McKeon S, Saglani S, Bush A, Fleming L. P98 The relationship between invasive and non-invasive measures of inflammation in children with severe therapy-resistant asthma. Thorax 2015;70(suppl):A124-5.
62. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. J Allergy Clin Immunol 2015;135: 822-4.e2.
63. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.
64. Austin D, Pouliquen IJ, Keene O, Yancey S. Blood eosinophil dose response to oral corticosteroids in a population of patients with severe asthma. Eur Respir J 2016;48(suppl 60):PA1110.
65. Austin D, Pouliquen IJ, Yancey S, Bleecker ER. A blood eosinophil count of greater than 150 cells/uL predicts sputum eosinophilia ≥3% in patients with severe asthma with other markers of inflammatory lung disease. Am J Respir Crit Care Med 2016;193:A4338.
66. Prazma C, Bel E, Price R, Bradford E, Albers F, Yancey S. Oral corticosteroid dose modulation in severe asthma: impact on peripheral blood eosinophil count. Am J Respir Crit Care Med 2017;195:A1363.
67. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. Lancet Respir Med 2015;3:290-300.

68. Ortega H, Gleich G, Mayer B, Yancey S. Reproducibility of a single blood eosinophil measurement as a biomarker in severe eosinophilic asthma. Ann Am Thorac Soc 2015;12:1896-7.
69. Wagner JA. Overview of biomarkers and surrogate endpoints in drug development. Dis Markers 2002;18:41-6.
70. American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care 2017;40(suppl 1):S11-24.
71. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Position statement executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34:1419-23.
72. Pletcher MJ, Pignone M. Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. Circulation 2011;123:1116-24.
73. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe adult-onset asthma: a distinct phenotype. J Allergy Clin Immunol 2013; 132:336-41.
74. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respir Med 2015;3:849-58.
75. Zeiger RS, Schatz M, Li Q, Chen W, Khatry DB, Gossage D, et al. High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. J Allergy Clin Immunol Pract 2014;2: 741-50.
76. Hansbro PM, Kaiko GE, Foster PS. Cytokine/anti-cytokine therapy—novel treatments for asthma? Br J Pharmacol 2011;163:81-95.