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# Eosinophilic inflammation in COPD: from an inflammatory marker to a treatable trait

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## ABSTRACT

The heterogeneity of chronic obstructive pulmonary disease (COPD) creates many diagnostic, prognostic, treatment and management challenges, as the pathogenesis of COPD is highly complex and the underlying cellular and molecular mechanisms remain poorly understood. A reliable, easy-to-measure, clinically relevant biomarker would be invaluable for improving outcomes for patients. International and national guidance for COPD suggests using blood eosinophil counts as a biomarker to help estimate likely responsiveness to inhaled corticosteroids (ICS) and, potentially, to aid effective management strategies. However, with the mechanism underlying the association between higher eosinophil levels and ICS effect unknown, use of the blood eosinophil count in COPD continues to be widely debated by the respiratory community.

Two international meetings involving respiratory medicine specialists, immunologists and primary and secondary care clinicians were held in November 2018 and March 2019, facilitated and funded by GlaxoSmithKline plc. The aims of these meetings were to explore the role of eosinophils in the disease processes of COPD and as prognostic and diagnostic markers, and to identify areas of deficient knowledge that warrant further research. The consensus views of the attendees on key topics, contextualised with current literature, are summarised in this review article, with the aim of aiding ongoing research into the disease processes of COPD and the development of biomarkers to aid clinical management.

Under certain conditions, eosinophils can be recruited to the lung, and increasing evidence supports a role for eosinophilic inflammation in some patients with COPD. Infiltration of eosinophils across the bronchial vascular epithelium into the airways is promoted by the actions of immunoregulatory cells, cytokines and chemokines, where eosinophil-mediated inflammation is driven by the release of proinflammatory mediators.

Multiple studies and two meta-analyses suggest peripheral blood eosinophils may correlate positively with an increased likelihood of exacerbation reduction benefits of ICS in COPD. The studies, however, vary in design and duration and by which eosinophil levels are viewed as predictive of an ICS response. Generally, the response was seen when eosinophil levels were 100–300 cells/ $\mu$ L (or higher), levels which are traditionally viewed within the normal range. Some success with interleukin-5-targeted therapy suggests that the eosinophilic phenotype may be a treatable trait. The use of biomarkers could help to stratify treatment for COPD—the goal of which is to improve patient outcomes. Some evidence supports eosinophils as a potential biomarker of a treatable trait in COPD, though it is still lacking and research is ongoing. A unified

consensus and a practical, accessible and affordable method of utilising any biomarker for COPD was thought to be of most importance. Challenges around its utilisation may include presenting a clear and pragmatic rationale for biomarker-driven therapy, guidance on ICS withdrawal between primary and secondary care and a lack of financial incentives supporting broad application in clinical practice. Future treatments should, perhaps, be more targeted rather than assuming the primary disease label (COPD or asthma) will define treatment response.

## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an umbrella term for a variety of lung conditions that the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2020 recommendations define as: ‘a common, preventable and treatable disease that is characterised by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases’.<sup>1</sup> For reasons that are unclear, inflammation is no longer part of the GOLD definition of COPD; however, GOLD has introduced the blood eosinophil count as a biomarker for estimating the efficacy of inhaled corticosteroids (ICS) for the prevention of exacerbations.<sup>1</sup> GOLD advises that a threshold of  $\geq 100$  cells/ $\mu$ L should be considered for ICS treatment in patients with COPD experiencing one exacerbation despite long-acting muscarinic antagonist/long-acting beta<sub>2</sub>-agonist (LAMA/LABA) treatment.<sup>1</sup> Although the benefits of ICS have been found to outweigh the risks, it is important to be mindful of these risks, particularly with respect to pneumonia.<sup>2,3</sup>

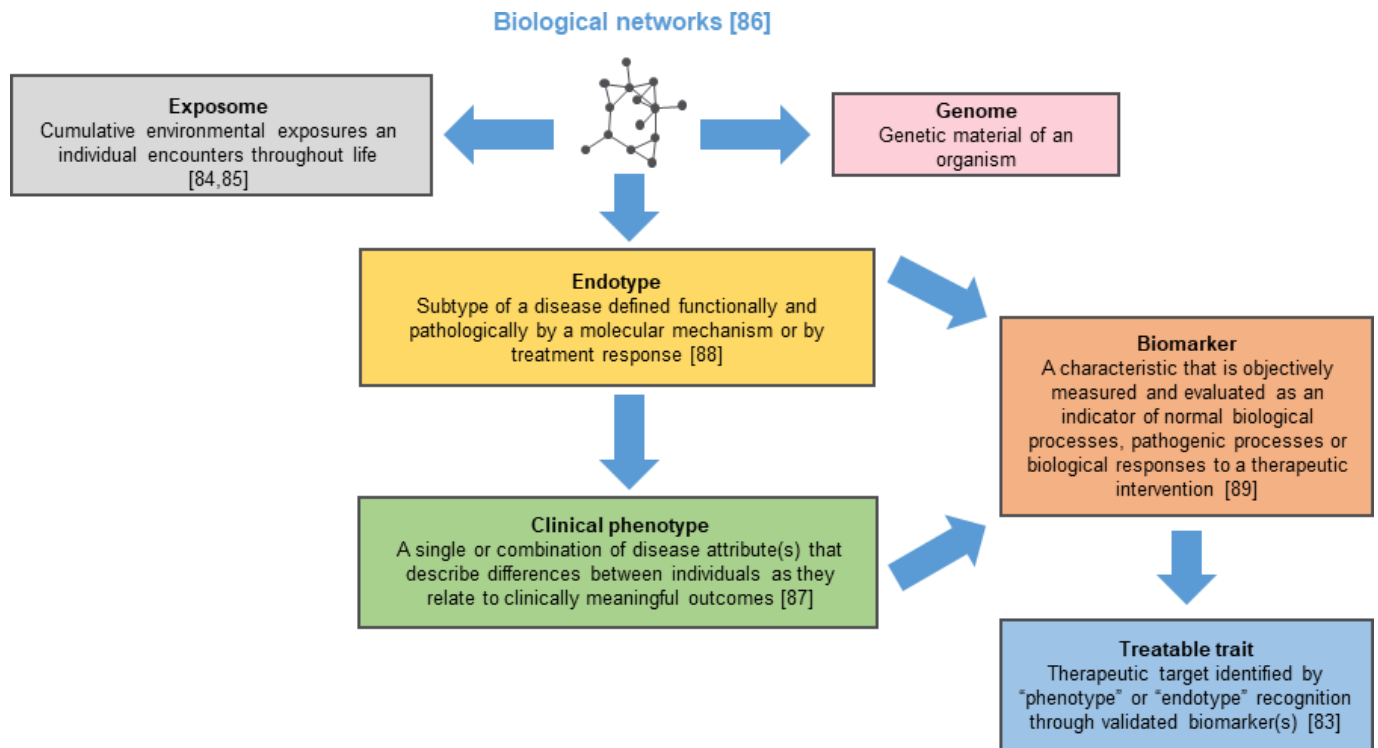
The pathogenesis of COPD is highly complex and the underlying cellular and molecular mechanisms remain poorly understood.<sup>4</sup> GOLD<sup>1</sup> identifies three main mechanisms underlying COPD pathology: small airway disorders/abnormalities, emphysema and systemic effects. Additionally, Turner *et al*<sup>5</sup> describe a larger series of clinically relevant characteristics in COPD; these include conditions (frequent exacerbator; chronic bronchitis; alpha-1 antitrypsin deficiency (A1ATD); upper zone dominant emphysema and bullous emphysema; type 1 respiratory failure; type 2 respiratory failure; eosinophilic COPD; and biomass COPD) with clear implications for treatment strategy, and conditions (pulmonary hypertension; bronchiectasis; systemic inflammation; and bacterial colonisation) that have implications for prognosis but where the therapeutic approach is less clear. Some phenotypes demonstrate discrete biochemical and clinical profiles.



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**Figure 1** Relationships between the exposome and the genome, the emergence of endotypes and phenotypes and the possibility of identifying them through validated biomarkers of treatable traits. Redrawn with permission from 'Precision medicine in airway diseases: moving to clinical practice,' by Agusti, Bafadhel M, Beasley R, *et al. Eur Respir J* 2017;50(4):1701655, 2017 European Respiratory Society<sup>82–89</sup>

The limitations of using diagnostic labels such as 'COPD' or 'asthma', however, are becoming increasingly apparent; Agusti *et al*<sup>6</sup> proposed a precision medicine strategy based on the presence (or absence) of 'treatable traits'. These traits may be based on 'phenotypic' recognition or on knowledge of the underlying pathways (eg, 'endotypes').<sup>6</sup> Rather than treating a patient diagnostically labelled with COPD or asthma, precision medicine treats a patient with airway disease based on the treatable traits present (figure 1). Agusti *et al*<sup>6</sup> highlighted three sets of treatable traits in airway disease: pulmonary treatable traits (eg, eosinophilic airway inflammation), extrapulmonary traits (eg, cardiovascular disease) and treatable behaviour/lifestyle risk factors of airway diseases (eg, exposure to sensitising agents/pollution).<sup>6</sup>

A predominant characteristic of COPD is neutrophilic inflammation,<sup>4</sup> with a subset of patients (20%–40%) demonstrating an eosinophilic phenotype.<sup>7–10</sup> The latter is associated with a pattern of expression of type-2 mediators in the airways, as can also be seen in patients with asthma.<sup>11</sup> There may even be combined neutrophil/eosinophil phenotypes with varying degrees of each type of inflammation. Bafadhel *et al*<sup>12</sup> (online supplemental table 1) demonstrated that the profile of airway inflammatory mediators in COPD and asthma are broadly similar, and that differences observed between eosinophilic and non-eosinophilic phenotypes are independent of disease. These data further highlight the limitations of using diagnostic labels.

The heterogeneity of COPD poses many diagnostic, prognostic and management challenges (box 1). Reliable, easily measurable, clinically relevant biomarkers to identify a COPD phenotype would be invaluable for improving patient outcomes. Recent guidance on the management of COPD<sup>1</sup> suggest using the blood eosinophil count as part of follow-up management in patients with a blood eosinophil count of  $\geq 100$  cells/ $\mu$ L who are not responding satisfactorily to long-acting inhaled

bronchodilator(s). In addition, data from epidemiological studies and a number of post-hoc analyses of clinical trials have demonstrated that blood eosinophil levels are associated not only with response to ICS but also systemic corticosteroids, and with the risk of exacerbations, mortality and length of hospitalisation.<sup>13–17</sup> Different pathological mechanisms of COPD, however, may coexist in the same patient.<sup>6</sup> While guidance including use of the blood eosinophil count is a welcome addition to COPD management, its utility nevertheless continues to garner much debate within the COPD and wider respiratory community.

Two international meetings, facilitated by GlaxoSmithKline plc (online supplemental file A), involving respiratory medicine specialists, immunologists and primary/secondary care physicians, took place in November 2018 and March 2019. The aims were to reappraise available data to encourage scientific debate and discussion around the role of immune cells in the disease

#### Box 1 COPD poses many diagnostic, prognostic and management challenges

- ▶ A heterogeneous disease—with respect to symptoms, physiology, inflammation, extrapulmonary effects, response to treatment and disease trajectory, thus patients have variable responses to treatments.
- ▶ Patient phenotypes can change during the course of COPD.
- ▶ By the time COPD is diagnosed, the disease is overt and irreversible.
- ▶ Many target proteins along the underlying biochemical and molecular pathways have yet to be identified.
- ▶ The need to standardise blood and sputum eosinophil cut-off levels.

processes of COPD and as prognostic and diagnostic biomarkers, and to identify areas of scientific knowledge that are currently deficient and warrant further research. A significant proportion of the meetings focused on the utility of eosinophil-related biomarkers and the identification of clinical biomarkers relevant for COPD. The potential of such a biomarker was discussed in terms of contribution to the assessment of patients, risk prediction, treatment guidance and assessment of response.<sup>18</sup> The attendees' views on key topics, contextualised with current literature, are summarised in this review article, with the aim of aiding ongoing research into the disease processes of COPD and the development of biomarkers to aid clinical management.

### Development of COPD

Little is known about the early onset of COPD, nor the potential role of eosinophils in this process. Clearly, it takes decades for the disease to develop.<sup>19</sup> Exposure to noxious particles/gases (eg, via smoking, household wood burners, fires or environmental pollutants) is the main risk factor for COPD, although host factors (eg, genetic background, abnormal lung development and accelerated ageing) are also known to predispose individuals to COPD.<sup>1</sup> As only a small fraction of smokers (10%–20%) develop COPD,<sup>20</sup> other factors must also be involved; indeed, some life-long non-smokers go on to develop COPD-like pathophysiology.<sup>21</sup> Poverty is consistently associated with airflow obstruction<sup>22</sup> and individuals of lower socioeconomic status are more likely to develop COPD.<sup>23</sup> Asthma and airway hyper-responsiveness (without asthma) are also risk factors for COPD.<sup>24</sup> The underlying mechanisms of COPD, including the relative contributions of neutrophil-mediated and/or eosinophil-mediated inflammation, likely differ dependent on a patient's specific disease subtype.

### Genetic background contributing to COPD

Individuals with hereditary A1ATD or with defects in other single genes, such as that coding for matrix metalloproteinase-12 (MMP-12), provoke a decline in lung function and/or increased risk for COPD.<sup>25 26</sup> Smokers and non-smokers with severe A1ATD develop aggressive emphysema, though the process occurs faster in smokers.<sup>26</sup> A1AT is synthesised in the liver and is important as a circulating antiprotease balancing the actions of proteases. In A1ATD, the activity of neutrophil elastases is no longer tightly controlled due to the deficiency of antiproteases, resulting in destruction of elastin in the lung tissue, a process associated with the development of emphysema; acute infection also tends to provoke abnormal processes in A1AT glycosylation.<sup>26</sup>

### Emphysema in COPD

Emphysema is characterised by enlargement of airspace beyond the terminal bronchioles as a result of airway wall destruction.<sup>27</sup> Centrilobular emphysema is mostly associated with smoking while panlobular emphysema is associated with A1ATD.<sup>27 28</sup> Emphysema pathogenesis may be provoked by infection and it responds poorly to anti-inflammatory agents.<sup>4</sup>

### Bronchitis in COPD

Overproduction and hypersecretion of mucus by goblet cells, and its reduced elimination, are the primary mechanisms responsible for excessive mucus in chronic bronchitis; however, its precise contribution to the airflow limitation in COPD is still uncertain.<sup>27</sup> While alveolar pathology is dominant in emphysema, in bronchitis, damage to the airways is largely caused by chronic

inflammation<sup>29</sup> and is often treated using anti-inflammatory agents (eg, corticosteroids).

### Small-airway disease

Increased airway resistance is principally localised in the small airways of <2 mm in internal diameter. In healthy individuals, the small airways have a much larger collective cross-sectional area compared with the central airways so that, physiologically, they contribute approximately 20% of total airflow resistance. This is the reason why >80% of the small airways need to be occluded before there is any demonstrable airflow impairment and why many cigarette smokers develop progressive small airway disease long before airflow obstruction is detected.<sup>30 31</sup> Small-airway disease results from injury (by factors such as cigarette smoke and viral infection), leading to inflammation, airway remodelling and mucous plugging.<sup>28</sup>

### COPD—therapeutic intervention

Current therapies for COPD are largely ineffective. This partly reflects a failure to appreciate the different pathologies underlying COPD. It has proved difficult to find an effective treatment for emphysema, which tends to be corticosteroid resistant.<sup>4 32</sup> Applying a universal/broad-spectrum treatment to different patient phenotypes or endotypes can cause harm, so identification of treatable traits is desirable, and a precision medicine strategy preferable. Embracing this, the clinical community in COPD is moving away from the 'one size fits all' approach to treatment suggested by prior guidelines. There is an increasing urgency for biomarker targets along the underlying inflammatory pathway, towards the diagnosis of airway disease with a specific inflammatory phenotype or endotype. Perhaps one of the most apt descriptions of a treatable traits approach is that it deconstructs airway disease into its component parts for targeting, including airway eosinophilia, cough reflex hypersensitivity and airway structural damage.<sup>33</sup> Heaney and McGarvey<sup>33</sup> comment that some traits are more treatable than others, citing the example that it is easier to target eosinophil dysfunction than it is to treat cough reflex hypersensitivity; nevertheless, novel therapies are emerging all the time.

While noxious stimuli drive the disease in otherwise healthy individuals,<sup>27</sup> similar exposures can provoke different pulmonary processes in different individuals, implying that certain individuals may be more susceptible to the inciting stimuli than others. Two attractive—but not mutually exclusive—hypotheses have emerged to try and explain the course of disease in COPD: tissue imbalances of proteases/antiproteases and tissue imbalances of oxidants/antioxidants.<sup>27</sup> The disease processes can occur over many years without the individual suffering any signs or symptoms of COPD ('allostasis').

Allotaxis associated with subclinical disease is the key stage at which to identify early and potentially reversible airway damage, and attempt to remove noxious (or other inciting) stimuli. Theoretically, it may also be the point at which treatment could stop the destructive processes and prevent the development of overt COPD, a state which can be coined as 'pathostasis'.<sup>27</sup> Opinion is clear that the identification of biomarkers in COPD is key to being able to discriminate between 'healthy' smokers and smokers in allotaxis. For this to happen, a thorough understanding of the early biochemical and molecular processes of COPD is required. After the point of allotaxis, the disease is irreversible, slowly progressive and, crucially, difficult to treat. If no intervention is made at allotaxis, the mechanisms underlying the inflammatory responses pass a 'point of no return' and

even when inciting agents subside (eg, smoking cessation), the inflammation remains. As yet, it is unclear when, where or how the transformation to 'persistent inflammation' occurs, or even whether there is a change in phenotype at this stage; more data are needed to explain these aspects. Specific longitudinal tests to determine the extent to which the immune system is activated may help to predict which patients will go on to develop COPD. Novel therapeutics should be targeted at the different phases of disease.

### COPD and biomarkers

Current consensus is that a reliable, easy-to-measure and clinically relevant biomarker would be invaluable in improving patient outcomes in COPD. The WHO defines a biomarker as: 'any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease'.<sup>34</sup> Clinical and immunological biomarkers are now beginning to emerge in COPD and asthma, which will help to inform disease prognosis and response to therapy, as well as identify new therapeutic targets. These biomarkers could help to improve targeting of treatments and could identify patients who are likely to respond to novel treatments.<sup>33</sup>

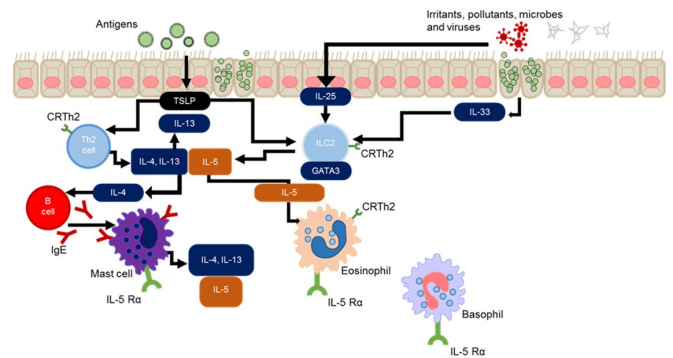
### Neutrophilic inflammation in COPD

Most studies have focused on the underlying neutrophilic inflammation in COPD, and neutrophil activity has been well characterised.<sup>35–38</sup> Patients with COPD with recurrent infective exacerbations have high rates of bacterial colonisation and neutrophilic inflammation.<sup>39</sup> Although this does not imply causality, it suggests that neutrophil host defence mechanisms are impaired. This concept is supported by studies showing alterations in neutrophil migration, degranulation and production of reactive oxygen species in cells isolated from patients with COPD.<sup>37</sup> The role of neutrophils in COPD and airway disease has been much studied and has been reviewed extensively elsewhere.<sup>36 38 40</sup>

### Eosinophilic inflammation in COPD

Recently, it has been recognised that eosinophils may be involved in the inflammatory response in COPD. Under certain circumstances, inflammatory cues promote eosinophil recruitment to the lungs, where secretion of a variety of chemokines (eg, CCL5, CCL11, CCL13), cytokines (eg, interleukin (IL)-2, IL-3, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-25) and cytotoxic granular products (major basic protein, eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin) contributes to inflammation.<sup>41–44</sup> As yet, the eosinophilic inflammatory response is not completely understood, though it appears to enhance host defences in allergic disease and may make certain individuals more susceptible to exacerbations.<sup>16</sup> In the remainder of this article, we highlight relevant clinical studies that support the eosinophil as a potential biomarker, and eosinophilia as a treatable trait.

Eosinophils are inflammatory leucocytes consisting of bi-lobed nuclei and large acidophilic cytoplasmic granules. They are produced in healthy bone marrow from CD34+ myeloid progenitors<sup>45</sup> and the number of eosinophils generated is typically low, with circulating levels ranging from 1% to 4% of the total white blood cell count.<sup>46</sup> Differentiation from a haematopoietic stem cell into a mature eosinophil is promoted by IL-5, while a role in vivo for granulocyte/macrophage-colony-stimulating factor has been suggested<sup>47 48</sup> (figure 2). Once mature, eosinophils enter the systemic circulation and mainly migrate to the gastrointestinal



**Figure 2** The T2 inflammatory pathway (eosinophilic COPD and asthma) and the target sites for anti-IL-5 agents. Adapted with permission of 2019 Dove Medical Press Limited, from 'Targeting IL-5 in COPD,' by Narendra DK and Hanania Na, *Int J COPD* 2019;14:1045–51.<sup>11</sup> COPD, chronic obstructive pulmonary disease; IL, interleukin.

tract and thymus.<sup>46</sup> In the context of the inflammatory response, the quality and activation state of eosinophils is likely more important than absolute eosinophil numbers.

Circulating eosinophils are recruited into the airways by immunoregulatory cells and chemokines.<sup>49</sup> In homeostasis, eosinophils flow along in the blood stream and roll across the bronchial vascular endothelium. Infiltration of eosinophils into the airways only occurs when inflammatory signals induce expression and/or activation of appropriate adhesion molecules on both the bronchial vascular endothelium and epithelium. This recruitment to the airway is under the control of the chemokines CCL5, 7, 11, 13, 15, 24 and 26 and their cognate receptors, such as CCR1, CCR2 and CCR3.<sup>50</sup> This chemokine/receptor interaction plays a critical role, together with chemoattractant receptor homologous molecule expressed on T helper type 2 cells and its ligand, prostaglandin D<sub>2</sub>.<sup>50</sup> Using an in vitro model, Doyle *et al*<sup>51</sup> showed that eosinophil-derived IL-13 promoted alveolar macrophage MMP-12 and that airspace enlargement in a transgenic mouse model was dependent on MMP-12; similarly, in patients with chronic airways disease, pulmonary eosinophilia was associated with elevated MMP-12 levels, predictive of emphysema.

Recent guidance for COPD<sup>1 16</sup> refers to the use of eosinophils as a marker of a patient's phenotype and/or predicted responsiveness to ICS. Eosinophil numbers in the blood of patients with COPD and asthma are similar and are predictive of risk of exacerbations and response to ICS during stable disease and to oral corticosteroids during disease exacerbations.<sup>52</sup> Patients with fixed airflow obstruction often have no response to  $\beta$ -agonists or corticosteroids. It appears that serum eosinophil counts per se do not provide the sensitivity, specificity or accuracy in the identification of the multiple COPD phenotypes or assist in early diagnosis.

### Could enhanced eosinophil numbers be a biomarker of a treatable trait?

Sputum studies of patients with COPD indicate that eosinophil numbers of >3% are found in a subset of patients.<sup>53</sup> Airway biopsies and sputum samples taken during acute exacerbations of COPD also show an increased number of eosinophils.<sup>11 54</sup>

Database studies investigating the role of eosinophils in COPD (including the COPD population in general) can sometimes lead to the surprising conclusion that there is only a weak link between eosinophils and COPD. As the whole COPD population includes not only mild COPD but also patients already

on an ICS (which will reduce the exacerbation frequency/risk in eosinophilic patients on appropriate treatment), the results of these studies may miss or underestimate the link(s) between COPD and this potential biomarker.

Consensus is that eosinophilic inflammation is a treatable trait in COPD. Asthma and COPD, which were first linked over five decades ago,<sup>55</sup> are complex, heterogeneous diseases that are increasingly recognised as overlapping syndromes sharing similar pathophysiological mechanisms and treatable traits.<sup>56</sup> Eosinophilic inflammation in the airways could prove the most treatable trait of COPD. A number of monoclonal antibodies and small molecule therapies have recently been designed to target this inflammatory pathway. For example, there are monoclonal antibodies against IL-5 (eg, mepolizumab), IL-5 receptor-alpha (eg, benralizumab), IL-13 (eg, tralokinumab) and IL-4 receptor-alpha (eg, dupilumab).<sup>56-59</sup> Responses to these agents in COPD have been mixed. Pavord *et al*<sup>60</sup> (online supplemental table 1) investigated mepolizumab in patients with COPD with a history of moderate to severe exacerbations in two randomised controlled trials, one in which patients were stratified by blood eosinophil count (METREX) and one in which all patients had an eosinophilic phenotype (METREO). In patients with an eosinophilic phenotype, the mean annual rate of moderate or severe exacerbations (primary endpoint) was lower with mepolizumab versus placebo in both trials (METREX: rate ratio (RR)=0.82; METREO: RR=0.80 (100 mg), RR=0.86 (300 mg); this was statistically significant only in METREX (adjusted p=0.04). A greater effect of mepolizumab compared with placebo was observed in patients with higher blood eosinophil counts at screening (RR: 0.77; 95% CI, 0.63 to 0.94).<sup>60</sup> These findings suggest that eosinophilic inflammation contributes to exacerbations.<sup>60</sup> What was challenging in these two trials, however, was that not all patients responded (showed reduced exacerbations) despite similarities in clinical, functional and inflammatory features. Possibly, this could be related to their different endotypes with different underlying processes, which have not yet been identified. Similar results were observed in two randomised controlled trials of benralizumab in patients with COPD who had elevated blood eosinophil counts ( $\geq 220$  cells/ $\mu$ L) and a history of moderate or severe exacerbations while taking inhaled dual or triple maintenance therapy (GALATHEA (30 mg and 100 mg) and TERRANOVA (10 mg, 30 mg and 100 mg), online supplemental table 1). In both trials, annualised COPD exacerbation RRs (primary endpoint) were lower with benralizumab versus placebo, but statistical significance was not achieved (GALATHEA: RR=0.96 (p=0.65) and 0.83 (p=0.05); TERRANOVA: RR=0.85 (p=0.06), 1.04 (p=0.66) and 0.93 (p=0.40)).<sup>61</sup> Although elevated blood eosinophil counts at baseline was a key factor for predicting a greater treatment effect of benralizumab, this characteristic alone was not sufficient to determine treatment effect with antieosinophil therapy.<sup>62</sup>

Please refer to the online supplemental file B for a discussion on the 'Standardisation of measurement of eosinophils'.

### Response to corticosteroids

Blood eosinophil counts are useful for predicting response to ICS and may represent a treatable trait for exacerbation frequency with ICS/LABA in patients with COPD and a history of moderate/severe exacerbations.<sup>63-65</sup> In other respiratory pathologies, the link between eosinophil levels and corticosteroid response has also been shown. Shim *et al*<sup>66</sup> were among the first investigators to show that airway eosinophilia indicated responsiveness to corticosteroids. In a small trial in patients with

chronic bronchitis, treated either with prednisolone or placebo, patients with increased levels of eosinophils were more likely to respond to corticosteroids (p<0.001).<sup>66</sup> Since then, this finding has been repeated many times and, generally, applies to patients with airway eosinophils of >3% with stable COPD.<sup>8</sup> Pavord *et al*<sup>63</sup> conducted a post-hoc analysis of three randomised controlled studies each of at least 1 year in duration and found that there was a greater response to ICS/LABA compared with placebo or LAMA, in patients with a pretreatment blood eosinophil level of  $\geq 2\%$  (RR=0.75 and 0.63; p=0.006 and <0.001) compared with those with a level of <2% (RR=0.75 and 0.63; p=0.006 and <0.001). While these post-hoc analyses used a binary cut-off for eosinophil levels, a recent post-hoc analysis by Bafadhel *et al*<sup>65</sup> (online supplemental table 1) comprising a large dataset from three randomised controlled studies comparing ICS/LABA with LABA in patients with COPD, showed that the eosinophil count is a continuous variable. Moreover, these authors reported a significant treatment effect for ICS (p=0.015) that increased with blood eosinophil counts from >100 cells/ $\mu$ L.<sup>65</sup> The use of the eosinophil count threshold to aid clinical decision-making regarding ICS treatment is seen in the recent GOLD 2020<sup>1</sup> recommendations, whereby patients with a blood eosinophil count of >300 cells/ $\mu$ L have an improved chance of responding to treatment with ICS.<sup>1</sup> However, eosinophilic airway inflammation is not always responsive to ICS.<sup>67</sup> Despite ICS therapy, many patients with severe eosinophilic asthma have persistent airway type 2 inflammation.<sup>68</sup>

The ISOLDE study<sup>69</sup> (online supplemental table 1) investigated the rate of decline in forced expiratory volume in one second (FEV<sub>1</sub>) with the use of ICS. As this study took place before widespread usage of LABAs, it is unique in that it analysed the effects of ICS alone, rather than in combination with other inhaled medications. Reanalysis of the core ISOLDE data<sup>70</sup> showed that use of ICS in patients with higher blood eosinophil counts ( $\geq 2\%$ ) was associated with a slower rate of decline in FEV<sub>1</sub> (unusually, however, there appeared to be no impact on exacerbation rate).<sup>70</sup> This effect is not seen in all studies, however.<sup>71</sup> Smokers, whether they suffer from asthma or COPD, generally do not respond as well to ICS as non-smokers. However, findings from post-hoc analyses of randomised controlled trials of ICS/LABA<sup>65</sup> and ICS/LABA/LAMA<sup>72</sup> have shown that the magnitude of response to ICS in smokers, in the context of exacerbation reduction, is greatest in those with higher eosinophil levels. Even in patients with COPD who do not respond to ICS, primary care physicians may be reluctant to stop this medication, possibly reflecting the influence of prior national guidance.

### Relation to COPD exacerbations

Patients with higher blood eosinophil levels during stable disease tend to suffer from more frequent and severe exacerbations. A prospective, single-centre study used blood eosinophil levels to direct systemic steroids during an exacerbation.<sup>14</sup> Eosinophilic exacerbations were associated with rapid symptomatic recovery and fewer treatment failures than non-eosinophilic exacerbations.<sup>14 73</sup> By contrast, a low eosinophil count during an exacerbation predicted the risk of worse outcomes.<sup>16 74</sup> In cohort studies of patients hospitalised for exacerbations, blood eosinophil counts of <50 cells/ $\mu$ L were more strongly associated with infection (91% vs 52%, p=0.001), distinguished patients with longer median hospital stays (7 vs 4 days, p<0.001) and were associated with lower 12-month survival (82.4% vs 90.7%, p=0.028) than patients with an eosinophil count of >150 cells/ $\mu$ L.<sup>17</sup>

Many current clinical trial inclusion criteria stipulate that patients must have experienced a prespecified number of exacerbations over a given period of time; by taking into account the level of blood eosinophils, clinicians will have to adopt a completely different approach. Furthermore, some investigators<sup>75</sup> have found that blood eosinophil levels show significant variability throughout the course of COPD, during both stable periods and acute exacerbations, such that a single measurement may not be a reliable predictor of ICS response.

Please refer to the online supplemental file C for a discussion on the 'Relationship with infectious disease'

### COPD—is eosinophil inflammation ready to be used as a treatable trait?

The 2020 GOLD report<sup>1</sup> states: 'A number of recent studies have shown that blood eosinophil counts predict the magnitude of the effect of ICS (added on top of regular maintenance bronchodilator treatment) in preventing future exacerbations.' There is a continuous relationship between blood eosinophil counts and ICS effects; no and/or small effects are observed at lower eosinophil counts, with incrementally increasing effects observed at higher eosinophil counts.<sup>16 76</sup> Blood eosinophil levels have been found to correlate with sputum eosinophils, though to a lesser extent than is seen in patients with asthma.<sup>77</sup> The SPIROMICS investigation<sup>78 79</sup> (online supplemental table 1) used eosinophil cut-offs >1.25% for sputum and 200 cells/ $\mu$ L for blood as the threshold to categorise high and low eosinophil counts. This allowed prediction of clinical differences between patients, such as response to ICS, positive impact on quality of life, improved lung function and the level of emphysema (greater in patients with sputum eosinophilia), but not total yearly exacerbation rate. This investigation suggested that high concentrations of sputum eosinophils were a better biomarker than high concentrations of blood eosinophils to identify a patient subgroup with more severe disease, more frequent exacerbations, and increased emphysema by quantitative CT. Blood eosinophils alone were not a reliable biomarker for COPD severity or exacerbations, or for sputum eosinophils.<sup>80</sup> Once the cut-off level for high eosinophil count in sputum was raised to 2%, however, a link with the total number of exacerbations was established. A blood eosinophil count of >100 cells/ $\mu$ L to >300 cells/ $\mu$ L identifies the continuous relationship between eosinophils and ICS; indicating first, patients likely to benefit from ICS treatment, and second, patients with the greatest likelihood of treatment benefit with ICS.<sup>66</sup> These thresholds, of >100 cells/ $\mu$ L and >300 cells/ $\mu$ L, are estimates that can predict treatment benefit with ICS, rather than being considered precise cut-off values.<sup>1</sup> Also, given the chance of steroid-induced morbidity with continued corticosteroid treatment, the measuring of blood eosinophils would offer simple and useful insights.<sup>2 3</sup>

The consensus from the meeting attendees was that blood eosinophilia may assist clinicians in making clinical decisions for patients with COPD, including estimating the likelihood of a beneficial response with regard to exacerbation risk and, possibly, the risk of infection, to the addition of ICS to regular bronchodilator treatment. Thus, eosinophils can be used as a biomarker in conjunction with clinical assessment. Notably, based on available data, the COPD Biomarker Qualification Consortium has submitted a letter of intent to the Food and Drug Administration proposing blood eosinophils for qualification as a drug development tool.<sup>81</sup>

### CONCLUSIONS

The potential areas for future research in COPD are large and wide ranging (box 2). There is a drive towards personalised

### Box 2 Potential goals of future research in COPD

- ▶ A better understanding of the existence of different phenotypes of COPD and that patients may display more than one phenotype, or that their phenotype may change over time.
- ▶ Eosinophil kinetics and activation in the setting of COPD to study intravascular kinetics and the physiological fate, and the stability of eosinophil levels longitudinally.
- ▶ Develop/identify methods for achieving white cell count normalisation and restoration of white cell homeostasis rather than inhibition.
- ▶ Identification of differences in circulating and/or resident eosinophil levels between patients with COPD, individuals in allostasis and healthy subjects.
- ▶ A better understanding of the behaviour, existence and location of different white cell phenotypes and the role they, and their interactions, play in acute exacerbations and stable state COPD.
- ▶ Investigation of the interactions between neutrophils and eosinophils, cell surface proteins and their actions in different compartments/tissues.
- ▶ Targeting mucus hypersecretion and the effects of IL-5 inhibition (eg, with mepolizumab, benralizumab).

medicine in the treatment of COPD, as with many other diseases. Ultimately, many biomarkers are likely to become available to aid the diagnosis, prognosis and management of patients with COPD. Some evidence exists to support eosinophils as a biomarker of a treatable trait in COPD, though it is still lacking and research is ongoing. Nevertheless, widespread introduction of blood eosinophil count in COPD management would be worthwhile. A unified consensus and a practical, accessible and affordable method of using any biomarker for COPD was thought to be of the utmost importance. Challenges around its utilisation include presenting a clear and pragmatic rationale for biomarker-driven therapy, guidance on ICS withdrawal between primary and secondary care and a lack of financial incentives for its widespread clinical application. It seems likely that, in the near future and based on a clear understanding of the underlying pathogenetic pathways in COPD, clinical biomarkers of treatable traits will be able to guide clinicians in their decisions regarding the most effective treatments for patients with airway diseases.

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## ONLINE SUPPLEMENT

Supplementary Table 1

Study	Study design	Sample size	Main outcomes
<b>Bafadhel et al[1]</b>	Prospective study examining sputum mediators of subjects with asthma and COPD	Subjects with asthma and COPD (n = 54 and n = 49)	Recovery of sputum mediators sensitive to DTT can be improved using the described sputum processing technique. Within airway inflammatory sub-phenotypes there is a differential pattern of mediator expression that is independent of disease.
<b>Pavord et al[2]</b>	Two phase 3, randomized, placebo-controlled, double-blind, parallel-group trials comparing mepolizumab (100 mg in METREX, 100 or 300 mg in METREO) with placebo	Patients were 40 years of age or older and had a documented diagnosis of COPD for at least 1 year (based on the ATS-ERS 2004 definition)  METREX = 462 patients  METREO = 674 patients	Mepolizumab at a dose of 100 mg was associated with a lower annual rate of moderate or severe exacerbations than placebo among patients with COPD and an eosinophilic phenotype.
<b>Criner et al [3]</b>	GALATHEA and TERRANOVA were phase 3, randomized, double-blind, placebo-controlled, parallel-group trials	Patients who were 40 to 85 years of age and had moderate to very severe COPD  Primary analysis populations (1120 patients in GALATHEA and 1545 patients in TERRANOVA had baseline blood eosinophil counts of $\geq 220$ per cubic millimeter) and in the overall study populations (1656 in GALATHEA and 2254 in TERRANOVA)	Add-on benralizumab was not associated with a lower annualized rate of COPD exacerbations than placebo among patients with moderate to very severe COPD, a history of frequent moderate or severe exacerbations, and blood eosinophil counts of 220 per cubic millimeter or greater
<b>Bafadhel et al[4]</b>	Post-hoc analysis of three randomised,	Patients with COPD, who had blood eosinophils	In patients with COPD treated with formoterol,

	double-blind, double-dummy, parallel-group, multicentre trials of budesonide–formoterol fixed-dose combination	collected at the screening visit  Of the 4612 patients (excluding patients allocated to budesonide 160 µg alone) randomised in the three studies, 4528 had available baseline eosinophil counts and were included in the pooled analysis	blood eosinophil count predicts exacerbation risk and the clinical response to ICS
<b>Burge et al (The ISOLDE study) [5]</b>	Randomised, Double Blind, Placebo Controlled Study	A total of 751 men and women aged between 40 and 75 years with mean forced expiratory volume in one second (FEV <sub>1</sub> ) 50% of predicted normal	Fluticasone propionate 500 microgram twice daily did not affect the rate of decline in FEV <sub>1</sub> but did produce a small increase in FEV <sub>1</sub> . Patients on fluticasone propionate had fewer exacerbations and a slower decline in health status
<b>Hastie et al and Couper et al (The SPIROMICS investigation) [6,7]</b>	Prospective cohort study	3,200 participants were split into four strata (Non-smokers, Smokers without airflow obstruction, Mild/Moderate COPD, and Severe COPD)	High concentrations of sputum eosinophils were a better biomarker than high concentrations of blood eosinophils to identify a patient subgroup with more severe disease, more frequent exacerbations, and increased emphysema by quantitative computed tomography

**A. Summary of GlaxoSmithKline plc.'s scientific engagement protocols and reinforcement of non-promotional nature of collaboration/sponsorship**

Scientific collaboration between GlaxoSmithKline plc. and diverse groups of experts (academia, industry, regulators, public health authorities, etc) is necessary to advance key medical/scientific discussions and to contribute and share GlaxoSmithKline plc.'s broad scientific knowledge and experience in various settings.

GlaxoSmithKline plc.'s scientific engagement may take the form of a scientific workshop as a standalone meeting with the purpose of discussing or debating disease-related scientific topics. These topics are driven by healthcare professionals/other healthcare staff needs and interests. Participants are healthcare professionals/other healthcare staff or other experts who have relevant expertise and recent or current interest in the proposed topics. GlaxoSmithKline plc. staff with a defined role to play may participate and GlaxoSmithKline plc. may arrange and pay for reasonable travel and accommodation for participants of a meeting.

#### **B. Standardisation of measurement of eosinophils**

The eosinophil has a role in host defence against parasitic infections, which affect millions of individuals worldwide and the natural distribution of which goes far beyond endemic areas.[8] While the prevalence of parasitic infections may differ between populations and geographic regions, the range of peripheral blood eosinophils reported across diverse countries is generally very similar in 'healthy' individuals. Commonly, blood eosinophil levels are considered within the normal range when the percentage of eosinophils in the blood is 1–4%[9] or the absolute blood eosinophil count is 30–350 cells/ $\mu$ L.[10] These ranges may vary slightly between laboratories. Studies investigating the relationship between eosinophils and COPD have variably used sputum or blood and absolute or relative counts to measure eosinophil levels. While each approach has its merits, the discordance in methods makes it difficult to compare studies or conduct meta-analyses. Furthermore, neither absolute nor relative cell counts predict phenotype or determine eosinophil activation state, while the importance of the relationship between blood eosinophil count and eosinophil levels in the tissues is currently under debate. The development and use of standardised parameters, which are likely to differ dependent on the outcome of interest, could be an aid to the effective adoption of eosinophils as a biomarker in COPD, to be used in conjunction with other factors such as clinical assessment. The suitability of the eosinophil count to be incorporated into a composite clinical scoring system that includes additional clinical parameters, analogous to the QRISK approach in cardiovascular disease,[11] remains to be determined.

### C. Relationship with infectious disease

Bacterial and viral respiratory infections are thought to have an important role in most COPD exacerbations.[12-15] Exacerbation phenotypes associated with bacteria, virus and sputum eosinophilia have been described, with co-existence of bacteria- and sputum eosinophil-associated exacerbations rarely observed.[16] Patients with eosinophilic exacerbations have been shown to have an altered and distinct lung microbiome profile compared with other exacerbation phenotypes that discriminated these events from bacterial exacerbations.[17] One UK study showed patients with COPD with blood eosinophils  $\geq 2\%$  at exacerbation and eosinophil predominance during stable disease had a lower risk of bacterial presence at exacerbation;[18] there was seasonality in the occurrence of bacterial infection at exacerbation (winter vs summer, odds ratio 4.74,  $p=0.011$ ), which was most apparent in the predominantly eosinophilic patients.[18] Eosinophil counts of  $< 2\%$  are potential indicators of bacterial infection in acute exacerbations, implying that eosinophil count may be helpful in deciding whether to prescribe antibiotics.[19]

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## ONLINE SUPPLEMENT

Supplementary Table 1

Study	Study design	Sample size	Main outcomes
<b>Bafadhel et al[1]</b>	Prospective study examining sputum mediators of subjects with asthma and COPD	Subjects with asthma and COPD (n = 54 and n = 49)	Recovery of sputum mediators sensitive to DTT can be improved using the described sputum processing technique. Within airway inflammatory sub-phenotypes there is a differential pattern of mediator expression that is independent of disease.
<b>Pavord et al[2]</b>	Two phase 3, randomized, placebo-controlled, double-blind, parallel-group trials comparing mepolizumab (100 mg in METREX, 100 or 300 mg in METREO) with placebo	Patients were 40 years of age or older and had a documented diagnosis of COPD for at least 1 year (based on the ATS-ERS 2004 definition)  METREX = 462 patients  METREO = 674 patients	Mepolizumab at a dose of 100 mg was associated with a lower annual rate of moderate or severe exacerbations than placebo among patients with COPD and an eosinophilic phenotype.
<b>Criner et al [3]</b>	GALATHEA and TERRANOVA were phase 3, randomized, double-blind, placebo-controlled, parallel-group trials	Patients who were 40 to 85 years of age and had moderate to very severe COPD  Primary analysis populations (1120 patients in GALATHEA and 1545 patients in TERRANOVA had baseline blood eosinophil counts of $\geq 220$ per cubic millimeter) and in the overall study populations (1656 in GALATHEA and 2254 in TERRANOVA)	Add-on benralizumab was not associated with a lower annualized rate of COPD exacerbations than placebo among patients with moderate to very severe COPD, a history of frequent moderate or severe exacerbations, and blood eosinophil counts of 220 per cubic millimeter or greater
<b>Bafadhel et al[4]</b>	Post-hoc analysis of three randomised,	Patients with COPD, who had blood eosinophils	In patients with COPD treated with formoterol,

	double-blind, double-dummy, parallel-group, multicentre trials of budesonide–formoterol fixed-dose combination	collected at the screening visit  Of the 4612 patients (excluding patients allocated to budesonide 160 µg alone) randomised in the three studies, 4528 had available baseline eosinophil counts and were included in the pooled analysis	blood eosinophil count predicts exacerbation risk and the clinical response to ICS
<b>Burge et al (The ISOLDE study) [5]</b>	Randomised, Double Blind, Placebo Controlled Study	A total of 751 men and women aged between 40 and 75 years with mean forced expiratory volume in one second (FEV <sub>1</sub> ) 50% of predicted normal	Fluticasone propionate 500 microgram twice daily did not affect the rate of decline in FEV <sub>1</sub> but did produce a small increase in FEV <sub>1</sub> . Patients on fluticasone propionate had fewer exacerbations and a slower decline in health status
<b>Hastie et al and Couper et al (The SPIROMICS investigation) [6,7]</b>	Prospective cohort study	3,200 participants were split into four strata (Non-smokers, Smokers without airflow obstruction, Mild/Moderate COPD, and Severe COPD)	High concentrations of sputum eosinophils were a better biomarker than high concentrations of blood eosinophils to identify a patient subgroup with more severe disease, more frequent exacerbations, and increased emphysema by quantitative computed tomography

**A. Summary of GlaxoSmithKline plc.'s scientific engagement protocols and reinforcement of non-promotional nature of collaboration/sponsorship**

Scientific collaboration between GlaxoSmithKline plc. and diverse groups of experts (academia, industry, regulators, public health authorities, etc) is necessary to advance key medical/scientific discussions and to contribute and share GlaxoSmithKline plc.'s broad scientific knowledge and experience in various settings.



GlaxoSmithKline plc.'s scientific engagement may take the form of a scientific workshop as a standalone meeting with the purpose of discussing or debating disease-related scientific topics. These topics are driven by healthcare professionals/other healthcare staff needs and interests. Participants are healthcare professionals/other healthcare staff or other experts who have relevant expertise and recent or current interest in the proposed topics. GlaxoSmithKline plc. staff with a defined role to play may participate and GlaxoSmithKline plc. may arrange and pay for reasonable travel and accommodation for participants of a meeting.

#### **B. Standardisation of measurement of eosinophils**

The eosinophil has a role in host defence against parasitic infections, which affect millions of individuals worldwide and the natural distribution of which goes far beyond endemic areas.[8] While the prevalence of parasitic infections may differ between populations and geographic regions, the range of peripheral blood eosinophils reported across diverse countries is generally very similar in 'healthy' individuals. Commonly, blood eosinophil levels are considered within the normal range when the percentage of eosinophils in the blood is 1–4%[9] or the absolute blood eosinophil count is 30–350 cells/ $\mu$ L.[10] These ranges may vary slightly between laboratories. Studies investigating the relationship between eosinophils and COPD have variably used sputum or blood and absolute or relative counts to measure eosinophil levels. While each approach has its merits, the discordance in methods makes it difficult to compare studies or conduct meta-analyses. Furthermore, neither absolute nor relative cell counts predict phenotype or determine eosinophil activation state, while the importance of the relationship between blood eosinophil count and eosinophil levels in the tissues is currently under debate. The development and use of standardised parameters, which are likely to differ dependent on the outcome of interest, could be an aid to the effective adoption of eosinophils as a biomarker in COPD, to be used in conjunction with other factors such as clinical assessment. The suitability of the eosinophil count to be incorporated into a composite clinical scoring system that includes additional clinical parameters, analogous to the QRISK approach in cardiovascular disease,[11] remains to be determined.

### C. Relationship with infectious disease

Bacterial and viral respiratory infections are thought to have an important role in most COPD exacerbations.[12-15] Exacerbation phenotypes associated with bacteria, virus and sputum eosinophilia have been described, with co-existence of bacteria- and sputum eosinophil-associated exacerbations rarely observed.[16] Patients with eosinophilic exacerbations have been shown to have an altered and distinct lung microbiome profile compared with other exacerbation phenotypes that discriminated these events from bacterial exacerbations.[17] One UK study showed patients with COPD with blood eosinophils  $\geq 2\%$  at exacerbation and eosinophil predominance during stable disease had a lower risk of bacterial presence at exacerbation;[18] there was seasonality in the occurrence of bacterial infection at exacerbation (winter vs summer, odds ratio 4.74,  $p=0.011$ ), which was most apparent in the predominantly eosinophilic patients.[18] Eosinophil counts of  $< 2\%$  are potential indicators of bacterial infection in acute exacerbations, implying that eosinophil count may be helpful in deciding whether to prescribe antibiotics.[19]

**SUPPLEMENTARY REFERENCES**

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