THERAPEUTIC ADVANCES in
Respiratory Disease

Review

Eosinophils in chronic obstructive pulmonary disease

Marco Vanetti, Dina Visca, Francesco Ardesi, Martina Zappa, Patrizia Pignatti and Antonio Spanevello

Ther Adv Respir Dis

2025, Vol. 19: 1–18

DOI: 10.1177/17534666251335800

***Abstract*:** Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous lung condition characterised by chronic respiratory symptoms, fixed airway obstruction and persistent inflammation that leads to a progressive airflow limitation. Although COPD has traditionally been linked to neutrophilic inflammation, recent studies have identified a subset of patients – approximately 20%–40% – with elevated eosinophil levels in blood and sputum. Emerging evidence suggests that eosinophilic inflammation has a pivotal role in a subset of COPD patients and may influence disease progression, exacerbation frequency and therapeutic responses. This narrative review provides a comprehensive analysis of the role of eosinophils in COPD with particular attention to their role as biomarkers in blood and sputum. We evaluate the prevalence of eosinophilic inflammation in COPD exanimating different thresholds used in blood and in sputum to define it. In addition, we focus on eosinophilic COPD phenotype as a treatable trait, emphasising recent evidence that supports the effectiveness of biological target therapy.

Keywords: biologics, biomarkers, COPD, eosinophil, type-2 inflammation

Received: 31 October 2024; revised manuscript accepted: 1 April 2025.

Correspondence to:
Marco Vanetti
Department of Pulmonary Rehabilitation, Istituti Clinici Scientifici Maugeri IRCCS, Via Crotto Roncaccio 16, Tradate 12049, Italy

Department of Medicine and Surgery, University of Insubria, via Ravasi 2, Varese 21100, Italy
marco.vanetti@icsmaugeri.it

Dina Visca
Antonio Spanevello
Department of Pulmonary Rehabilitation, Istituti Clinici Scientifici Maugeri IRCCS, Tradate, Italy

Department of Medicine and Surgery, University of Insubria, Varese, Italy

Francesco Ardesi
Department of Medicine and Surgery, University of Insubria, Varese, Italy

Martina Zappa
Department of Pulmonary Rehabilitation, Istituti Clinici Scientifici Maugeri IRCCS, Tradate, Italy

Patrizia Pignatti
Allergy and Immunology Unit, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous lung disease characterised by chronic respiratory symptoms and persistent lung inflammation, which leads to fixed and progressive airflow obstruction.[1] COPD occurs in approximately 10% of the population over 40 and causes over 3 million deaths a year globally.[2] In developed countries, cigarette smoking is the main cause of COPD. However, the pathogenesis of the disease involves complex host-environment interactions where multiple factors such as genetic predisposition, respiratory infections, air pollution and preterm births can interact from the earliest years of life and contribute to disease development.[3] From the inflammatory point of view, patients with COPD were historically considered characterised by neutrophilic airway inflammation driven by Th1/Th17 pathways, completely distinct from the T2 eosinophilic airway inflammation, which characterises most asthmatic patients.[4] However, in recent years, increasing evidence shows that almost 20%–40% of COPD patients have increased sputum and/or blood eosinophils, which highlights a possible role of Type-2 inflammation in a subgroup of COPD patients.[5,6] To date there are significant data regarding the physiopathological characteristics of these patients. Indeed, some studies report that eosinophilic phenotype in COPD patients is associated with distinctive clinical characteristics, including a higher exacerbations frequency,[7] greater responsiveness to inhaled short β2-agonist[8] and a better response to inhaled corticosteroids (ICS).[9] It is well known that eosinophilic inflammation in the blood and in sputum has significant clinical implications, particularly in the context of corticosteroid treatment, precision medicine and targeted therapeutic interventions. To optimise therapeutic strategies, Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommends using a blood eosinophil

threshold of ⩾300 cells/μL to identify patients with a history of exacerbations (⩾1 severe or ⩾2 moderate) who are most likely to benefit from ICS therapy.[1] COPD patients with eosinophilic inflammation respond better to ICS therapy in terms of exacerbation reduction and in terms of quality of life and lung function improvement.[1,9] The development of Type-2-targeted monoclonal antibodies provides the opportunity for targeted treatments in this subgroup of COPD patients with eosinophilic inflammation. For this reason, identifying and validating new biomarkers to monitor and phenotype eosinophilic COPD patients would be an important area of study.[10]

Although eosinophilic COPD is considered a treatable trait of the disease, there is currently no uniform consensus on its definition in terms of blood and sputum eosinophil cut-off values.[11,12] Moreover, evidence regarding the role of blood and sputum eosinophils in disease outcomes is conflicting, highlighting the need for further research to clarify their role.[13]

This narrative review aims to provide a comprehensive analysis of the role of eosinophils in COPD. We have considered epidemiological data on the prevalence of eosinophilic inflammation in COPD, exploring the molecular mechanisms underlying eosinophil-mediated lung damage and discussing their clinical implications for diagnosis, prognosis and treatment.

To collect all significative data on the subject, a non-systematic search of the scientific literature in English was carried out without time restrictions. PubMed, EMBASE, Web of Science and Cochrane Library databases were consulted. A combination of MeSH and free-text terms was used for the search (COPD, eosinophils, eosinophilia, sputum eosinophils and type-2 inflammation). Only articles published in English were considered for inclusion. No limitations on the research or type of publication were applied throughout the literature search. A second analysis of the grey literature was also conducted to cover every spectrum of the disease entity and every possible reference to it.

Pathophysiological role of eosinophils

Eosinophils are granulocytic leukocytes constituting 1%–4% of circulating white blood cells. They are characterised by a large bilobed nucleus and acidophilic cytoplasmic granules containing specific basic proteins that include major basic protein, eosinophil cationic protein, eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin.[14] Eosinophils originate from the pluripotent cells of the bone marrow CD 34+, their differentiation is initially driven by granulocyte-monocyte colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) in the early stages and by interleukin-5 (IL-5) in the later stage.[15] IL-5 is one of the most important cytokines involved in eosinophil activation and inflammation process, indeed it promotes the survival and migration of eosinophils from the bone marrow to the bloodstream.[16] Eosinophils spend only a short time in the peripheral blood (half-life between 3 and 24 h) before migrating to the target organs (e.g. gastrointestinal tract, lungs, thymus and adipose tissue) where they reside as homeostatic cells.[15,17] Under physiological conditions, the bone marrow releases only a small number of eosinophils. However, during T2 cell-mediated responses associated with helminth infections or allergic diseases such as asthma, eosinophilopoiesis is markedly increased and eosinophils can be activated and recruited to tissues in response to eotaxin and chemokines.[18] Eosinophils typically remain in tissues for 2–5 days before undergoing apoptosis. However, their lifespan can be extended by pro-eosinophilic cytokines, such as IL-5, which promote eosinophil survival. Most eosinophils undergo apoptosis within the tissue and do not re-enter the circulation.[19]

Migration of eosinophils to the lungs involves a complex regulated process of adhesion and transmigration across the vascular endothelium. This process is mediated by interactions between integrins on eosinophils and adhesion molecules on endothelial cells, including P-selectin glycoprotein ligand-1 and vascular cell adhesion molecule-1 (VCAM-1).[20] Chemokines CCL5, 7, 11, 13, 15, 24 and 26 interact with the CCR3 receptor guiding eosinophil recruitment toward airway sites of inflammation. In addition, CRTH2, expressed on T-helper 2 (Th2) cells and its ligand, prostaglandin D2, also contribute to regulate this process.[21,22] IL-4 and IL-13 can upregulate the expression of VCAM-1 and CCR3, facilitating eosinophil adhesion and promoting eosinophilic airway inflammation.[23] The activation, proliferation and survival of eosinophils in the airway tissue are regulated by type-2 (T2) inflammation mediators, such as IL-4, IL-5 and

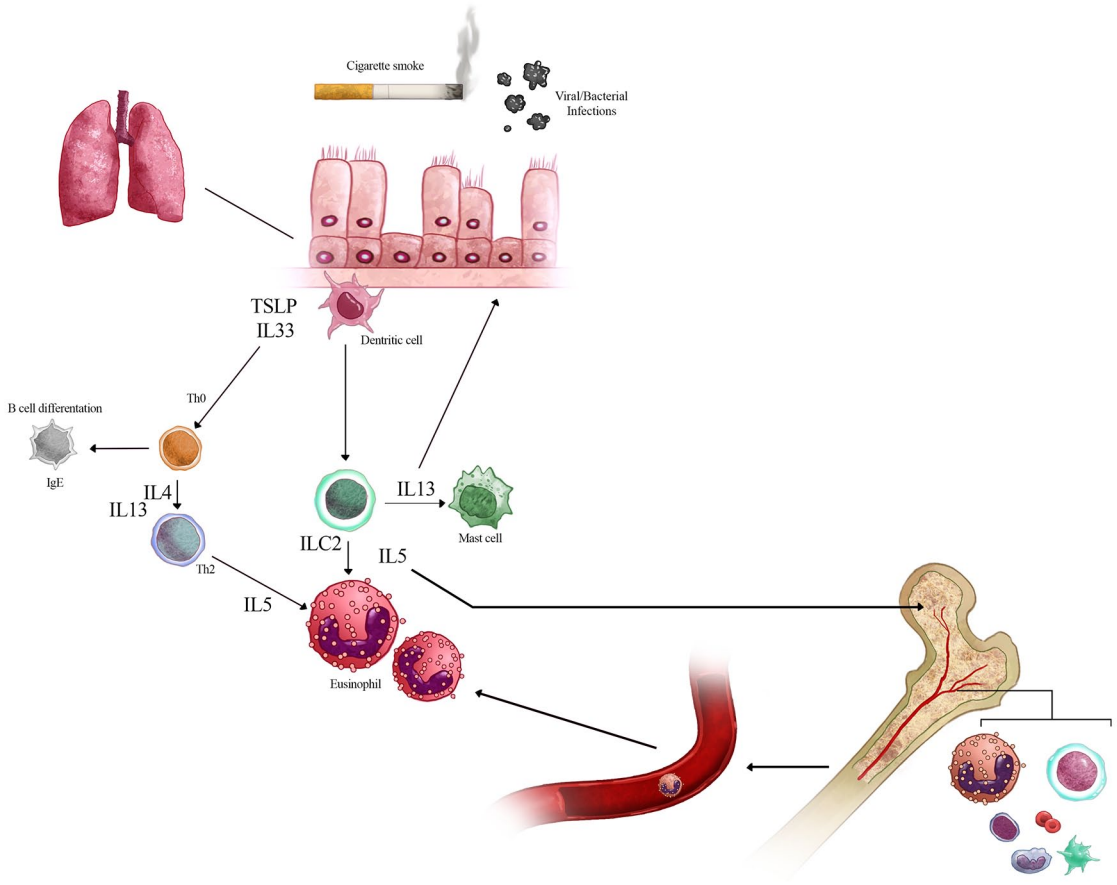

Figure 1. Eosinophilic airway inflammation in COPD.
IL, interleukin; ILC2, type 2 innate lymphoid cells; TH0, naive T cell; TH2, T-helper 2 cell; TLSP, thymic stromal lymphopoietin.

IL-13. During degranulation, eosinophils release their major basic proteins which contribute to viral and bacterial clearance but also lead to tissue damage, remodelling and mucus plugging.[24,25] Eosinophils also secrete other pro-inflammatory mediators, including IL-2, IL-4, IL-5, IL-10, IL-13, CCL5, CCL11 and CCL13 which amplify inflammatory responses by recruiting and activating other immune cell mediators.[14,17] Growth factors such as tumour necrosis factor and transforming growth factor (TGF) α/β released by eosinophils are involved in airway remodelling and fibrosis. Thymic stromal lymphopoietin, an IL-7-like cytokine associated with chronic airway inflammation, can further modulate eosinophil activity by upregulating inflammatory cytokine expression.[26,27] In Figure 1, we have summarised the complex role of eosinophils in COPD, highlighting their involvement in type-2 inflammation.

While both asthma and a subset of COPD patients exhibit eosinophilic inflammation, different studies highlight key differences in their pathophysiological mechanisms.[28,29] Histopathological studies have shown a higher number of large airway eosinophils in asthma than in COPD.[30] Nevertheless, there are no studies directly comparing the small airway eosinophils in these two diseases. Gene expression studies have identified distinct inflammatory signatures in asthma and COPD. Although both diseases share an overlap in T2-related mechanisms,[28,31] data from EvA (emphysema vs airway disease) and Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED) studies identified 12 genes

associated with blood eosinophil counts (BEC) in patients with COPD and 1197 genes that were associated with BEC in asthma patients, and only one of these genes (CST1) overlapped with COPD gene expression changes.[32] Furthermore, in asthma mast cells play a key role in allergic inflammation response,[33] nevertheless the role of mast cells in COPD is still unclear.[34,35] Some recent studies showed that in COPD patients with type 2 inflammation mast cell activation appears to occur through an independent IgE mechanism[35] and may contribute to tissue damage by increased tryptase activity, which compromises epithelial barrier integrity.[36] These findings suggest a potential pathological role for mast cells in the progression of eosinophilic COPD.

Recent data from animal and human models suggest the existence of eosinophil subsets with distinct functions. Tissue-resident eosinophils (rEos) have homeostatic roles, maintaining normal tissue function. In contrast, inflammatory eosinophils (iEos) are recruited into airways during the inflammatory process and are implicated in disease pathophysiology.[37] These subsets of eosinophils differ in surface receptor expression and tissue distribution.[38] In animal and human models, CD62L (L-selectin) has been used as a marker to distinguish iEos (bronchial eosinophils) and rEos (parenchymal eosinophils) and indeed is only present in parenchymal eosinophils.[39] Recently, Cabrera López et al. have used flow cytometry to investigate the presence of distinct eosinophil subtypes (iEos or rEos) in patients with asthma, COPD and healthy controls. The authors have reported that the proportions of inflammatory eosinophils (Siglec-8^{+}CD62L^{low}IL-3R^{high}) and resident eosinophils (Siglec-8^{+}CD62L^{high}IL-3R ^{low}) in total BECs were comparable in patients with COPD and control groups (smokers and non-smokers). However, patients with asthma have exhibited a significantly higher percentage of iEos, but no notable difference in rEos. These findings highlight that there are different circulating eosinophils subtypes between patients with asthma and those with COPD.[40] These differences may have clinical implications in eosinophilia interpretation and could play a role in the development of new tailored therapies.

Biomarkers in eosinophilic COPD

Evidence indicates that elevated blood eosinophil counts in COPD patients are linked to increased Type-2 (T2) inflammation.[41] Higher BECs are associated with greater expression of T2 inflammatory cytokines, such as interleukin-5 (IL-5), IL-4 and IL-13 in sputum.[42,43] In addition, gene expression analysis using bronchial epithelial brush samples identified a signature of 100 T2-related genes that correlate with elevated BECs.[28] Identifying reliable and clinically applicable biomarkers to detect T2 inflammation in COPD patients is necessary for optimising disease management and targeting therapeutic strategies. In Figure 2 the principal techniques to detect the biomarkers of eosinophilic inflammation are summarised.

Airway and blood eosinophils

Eosinophilic airway inflammation can be detected using non-invasive and invasive methods such as induced sputum and bronchoalveolar lavage (BAL) respectively. Bronchoscopy is an invasive procedure that allows the study of inflammation through bronchial wall biopsy in the proximal airways and enables the assessment of the distal airways by BAL. The eosinophil count is expressed as a percentage of total leucocytes in BAL and in a number of cells of a specific area in bioptic samples.[44,45] Bronchial biopsies and bronchoalveolar lavage allow accurate identification of Th1/Th2 inflammatory cell effectors in the proximal and distal airways of COPD patients.[28,46] While these invasive techniques provide an efficient analysis of inflammatory cellularity, they are expensive, time-consuming and can present risks of complications for the patients. For this reason, they are not routinely used in clinical practice.[47] The evaluation of inflammatory cells in sputum has proven to be a promising method for studying endobronchial inflammation in patients with chronic inflammatory airway diseases.[48] Induced sputum is the only non-invasive procedure that allows direct evaluation of airway inflammation by differential cell counts.[49] Sputum induction with hypertonic solution is a safe technique, well tolerated by the patient and with rare complications.[50] In healthy non-smokers, eosinophils in sputum range from 0.3% to 1.4%,[51] whereas COPD patients have higher sputum eosinophil counts (1.0% and 10.4%).[52] A threshold of 3% is typically used to define sputum eosinophilic inflammation in COPD;[53–56] however, some studies have used threshold values of 1% and 2%.[5,57] Using sputum eosinophil cut-off of 3% it is possible to identify the eosinophilic phenotype

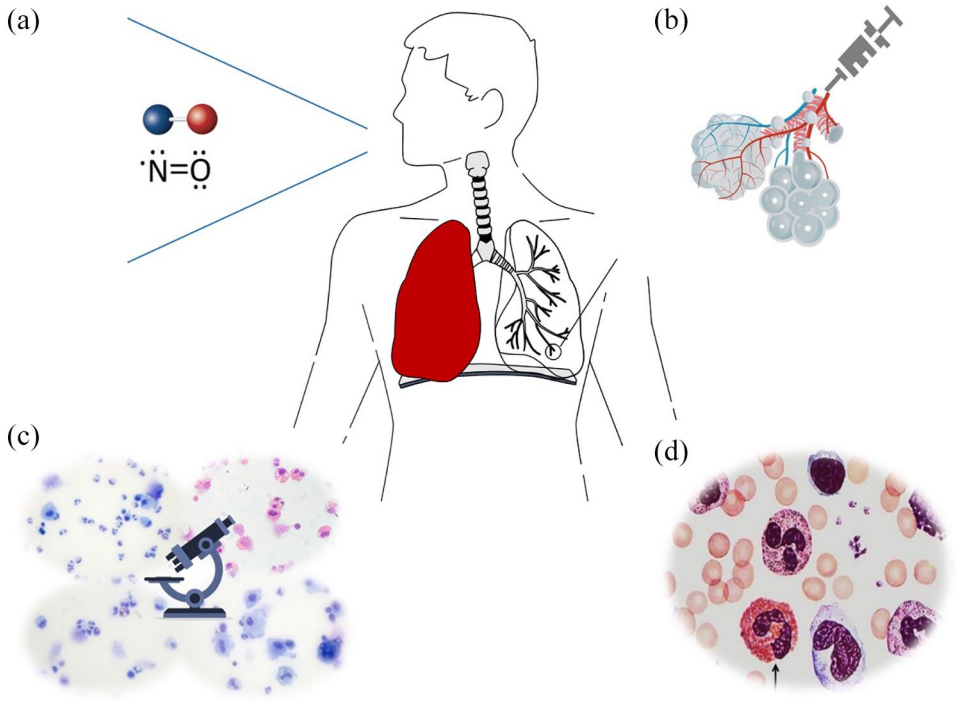

Figure 2. Technique to detect biomarkers of eosinophilic inflammation: (a) fractional exhaled nitric oxide (FeNO), (b) tissue biopsy or bronchoalveolar lavage by bronchoscopy examinations, (c) induced sputum and (d) blood cells count.

in approximately 22% to 44% of COPD patients.[54,55,58,59] Induced sputum is a reproducible technique that requires specialised personnel and is limited to specialised centres.[60] For this reason, the use of blood eosinophils as surrogate markers of sputum eosinophils is now extensively used.[61] Blood eosinophils are quantified as a percentage of all blood leukocytes or as absolute count/µl of blood. These data give us an idea of how intense the recruitment of eosinophils from the bone marrow is, often reflecting the engagement in tissues.[61] In healthy individuals, median BECs have been reported to be approximately 100 cells/µL for women and 120 cells/µL for men, while the COPD population has a median BEC of 200 cells/µL.[62] This elevation in eosinophil count among COPD patients has been confirmed by another study, which has demonstrated higher BECs in patients with COPD compared to age-matched participants without disease.[63] Currently, there is no standard blood eosinophil threshold to define eosinophilic COPD. BEC $\geqslant$ 300 cells/µL is frequently used as a cut-off, nevertheless, some studies have used different threshold values, such as blood eosinophils $\geqslant$150 cells/µL or $>$ 2%.[64] Since 2019, the GOLD report recommends $<$100 and $\geqslant$ 300 eosinophil/µL thresholds to identify patients with a history of exacerbations who are the least and the most likely, respectively, to benefit from ICS treatment.[1] The stability of blood eosinophil levels over time is a key consideration for the reliable use of this biomarker in patients with COPD.[11] In a post-hoc analysis of the COPDMAP observational cohort study Long et al. demonstrated that blood eosinophils had good stability after 1 year of observation (rho: 0.71, $p < 0.001$, ICC 0.84). Notably, 69.3% of patients remained in the same eosinophil GOLD category and 85.3% of patients with baseline eosinophils $<$100 cells/µL maintained stable levels at the end of the follow-up. While the greatest variability was observed in patients with higher eosinophil counts, transitions between the low and high eosinophil groups (BEC $<$100 and BEC $\geqslant$300 respectively) were rare and were reported in less than 1% of the cohort.[65] A good stability of blood eosinophils was observed by Beech et al. (rho = 0.76, $p < 0.001$, ICC 0.89) and by Ellingsen et al. (ICC 0.69; 95% CI 0.64–0.73) after 6 and 24 months respectively.[52,66]

Different studies have reported that the correlation between BECs and sputum eosinophils varied from weak to moderate (rho = 0.04–0.54).[5,43,61,67–70] Schleich et al. found that in stable COPD patients the best cut-off, which reflects sputum eosinophils ⩾3%, was 215 eosinophils/μL (AUC 0.76, sensitivity 60% and specificity 93%) or 2.3% blood eosinophils (AUC of 0.7, sensitivity 62% and specificity 94%).[53] On the other hand, data on the correlation between blood and sputum eosinophils are discordant: in COPD patients with concomitant cardiovascular disease (ischaemic heart disease, atrial fibrillation hypertension) no correlation between blood and sputum eosinophils has been found.[61] In a biotical study, Turato et al. reported a weak or even absent correlation between airway and blood eosinophils – indeed tissue biopsies did not reflect the amount of blood eosinophils.[71] The variable correlation strength between blood and sputum eosinophil counts has raised reservations about the reliability of using blood eosinophils as surrogate biomarkers for airway eosinophilic inflammation. However, the role of blood eosinophils in predicting the response to ICS is supported by high-quality evidence.[72] A recent study has shown that the low correlation between blood and sputum eosinophils in COPD could be due to the accumulation of these cells in the small airways other than in the central airways, which are sampled by the induced sputum technique.[73] In addition, BEC exhibits circadian variations with higher levels observed in the morning.[74] The low correlation between blood and sputum eosinophils in COPD could also be due to the heterogeneity of these cells and their different functions. Different eosinophil subtypes (rEos and iEos) have been recently described. In an animal model, Mesnil et al. demonstrated that both rEos and iEos are detectable in the blood, indicating that their differentiation occurs before the extravasation into tissues. Therefore, blood eosinophils constitute a heterogeneous population with different roles, and probably only a part of them is recruited into the airways.[61,75]

As previously reported, in COPD patients with cardiovascular comorbidities the correlation between blood and sputum eosinophils is weaker or absent compared to patients without these diseases. Blood eosinophils could increase because of systemic inflammation due to chronic ischaemic heart disease, heart failure and hypertension.[61] Increased BEC is a risk factor for coronary heart disease and it is positively correlated with coronary artery calcification.[76,77] In addition, eosinophil proteins could activate platelets and promote thrombus formation.[78] COPD is not considered an organ-specific disease limited to the lung; it is a multisystemic and multimorbid condition where the pulmonary disease is associated with numerous extrapulmonary comorbidities. Among these factors, cardiovascular disease has the greatest prognostic impact, as a matter of fact, more patients with a diagnosis of COPD will die from cardiovascular causes or from lung cancer rather than from respiratory failure.[79] Few preliminary data of a prospective cohort of 63 patients suggest that patients with a history of ischaemic heart disease show increased blood eosinophils.[80] If we consider the role that eosinophils can play in cardiovascular disease,[81] it could be an intriguing prospect for future research to investigate the role these cells have in COPD patients with cardiovascular disease.

Fraction-exhalated nitric oxide

Nitric oxide (NO) is a gas produced by inducible nitric oxide synthase (iNOS) in airway epithelial cells. During type-2 inflammation processes, the production of NO is upregulated by stimulation of interleukins IL-4 and IL-13. The increased level of NO in airways could be detected as fractional exhaled nitric oxide (FeNO).[82] American Thoracic Society /European Respiratory Society guidelines have summarised data on FeNO measurement showing that FeNO levels >50 ppb are representative for T2 inflammation in adults, while FeNO < 25 ppb for lack or suppression of it. Intermediate levels should be cautiously evaluated considering possible factors lowering (smoking habit) or increasing (atopy; viral infections) FeNO levels.[83] FeNO is a well-established and widely used biomarker for detecting T2 airway inflammation in asthma and it can be used to assess the patient's response and adherence to ICS therapy.[84] Different studies have evaluated the correlation between FeNO and sputum eosinophils to use this biomarker as a surrogate for sputum eosinophils in asthmatic patients. The correlation between these two biomarkers is significant but weak or moderate since sputum eosinophils and FeNO only partially share T2 mechanisms of inflammation.[85,86] FeNO levels appear to correlate more strongly with interleukins IL-4 and IL-13 than with eosinophil counts

and IL-5. Notably, IL-13 is either one of the main drivers of iNOS activation[87] or one of the interleukins involved in the process of eosinophil extravasation.[88] The role of FeNO in COPD is less well established, but it appears to be related to T2 eosinophilic inflammation of the airway. A systematic review and meta-analysis have reported significant variability in FeNO levels on the basis of several studies ($I^2 = 96\%$). Despite this variation, the analysis has demonstrated that patients with stable COPD had mildly elevated FeNO levels compared with healthy controls (SMD 1.28, 95% CI 0.60–1.96).[89] Although an association between FeNO levels and the airway eosinophils in COPD patients has been observed, the strength of this correlation is often weak.[90,91] Balazs et al. have found that FeNO measurement had a sensitivity of 63% and a specificity of 91% in identifying airway eosinophilia (defined as sputum eosinophils >3%) in stable COPD patients with a high negative predictive value (>90%).[91] Active smoking is one of the main factors that lowers FeNO levels. Recently, Higham et al. have highlighted that in current smokers with COPD, there is no correlation between FeNO and sputum eosinophils (rho = 0.3, $p = 0.2$). [92]

Several studies have investigated the relationship between COPD exacerbations and FeNO levels and have shown that FeNO levels increase significantly during exacerbations.[93,94] However, it is important to emphasise that COPD exacerbations are often due to underlying viral infections,[95] which are themselves one of the primary factors leading to increased FeNO levels.[82] For this reason, the detection of FeNO during a COPD exacerbation may directly reflect the effects of the viral infection. Alcázar et al. have investigated the predictive role of FeNO in COPD exacerbations in a prospective study involving 226 patients. The study has shown that persistently elevated FeNO levels (⩾20 ppb) in stable COPD patients significantly increased the risk of exacerbation.[96] Recently the BOREAS study has highlighted that treatment with dupilumab 300 mg reduced the exacerbation rates by 30% in COPD patients with BECs ⩾300 cells/μL. In addition, the study has highlighted that the subgroups of patients with FeNO levels >20 ppb had greater lung function improvement, suggesting that FeNO could be a potential biomarker to predict the response to the biological therapy in these patients.[97]

Clinical characteristics, outcomes and ICS responsiveness in eosinophilic COPD

Clinical outcomes and characteristics are key aspects in the management of COPD patients and eosinophilia seems to have an impact on both features. Indeed, eosinophilic patients showed different clinical characteristics compared with non-eosinophilic in previous studies.[5,98–100] Data from the ECLIPSE study highlighted that COPD patients with BECs ⩾2% were more frequently male, older, former smokers with fewer symptoms (lower St George's Respiratory Questionnaire and mMRC) and with a lower BODE (body mass index, airflow obstruction, dyspnoea and exercise capacity) index.[5] Moreover, eosinophilic COPD patients showed increased bronco-responsiveness.[8] Growing evidence in mucus plugs is providing new insights into the clinical outcomes of respiratory diseases. In COPD, mucus plugs have previously been associated with sputum neutrophils.[101] However, recent preliminary data, including findings from both the COPDGene and ECLIPSE cohorts, suggest that BEC is associated with a higher risk of mucus plug formation, highlighting a potential role of eosinophilic inflammation in airway obstruction.[102]

Acute exacerbations of COPD (AECOPD) are the most important events in the natural history of the disease.[1] COPD exacerbations are associated with rapid lung function decline,[103] deteriorating quality of life and increased mortality.[104] History of exacerbations is the strongest predictor of subsequent exacerbation risk.[105] There is growing evidence that COPD exacerbations significantly increase the risk of cardiovascular events. The cardiovascular risk in patients with COPD remains elevated not only during the exacerbation but can remain elevated up to a year later.[106,107] Several studies have evaluated the link between blood eosinophils and the risk of exacerbations in COPD patients. Nevertheless, published data have reported contradictory findings. Observational studies have not shown a clear relationship between BECs in stable patients and exacerbation risk.[108,109] Data from the CHAIN and BODE cohorts have indicated that persistently elevated blood eosinophils do not significantly correlate with an increased risk of exacerbations.[109] On the other hand, analysis from ECLIPSE and COPDGene studies have highlighted a significant correlation between blood

eosinophils and exacerbation risks, particularly in patients with a BEC >300 cell/μL.[64] In the SPIROMICS cohort only the sputum eosinophils, but not those in blood, have been associated with an increased rate of exacerbations requiring corticosteroids. Furthermore, only sputum eosinophils could identify a specific subset of COPD patients with more severe airflow obstruction.[70] A metanalysis of 11 RCT, involving 11,215 patients, has found no significant relationship between the BEC in stable patients and the risk of exacerbation in COPD patients.[11] Eosinophils in blood and in sputum could be elevated only during the exacerbations. An interesting prospective study deeply investigated the COPD exacerbations phenotype recognising four clusters by unbiased clustering and factor analyses: bacterial-predominant, virus-predominant, eosinophil-predominant and pauci-inflammatory. The T2-driven exacerbations occurred approximately in 30% of COPD exacerbations and may respond favourably to a target treatment.[55] Data from a CORTICO-COP trial suggests that eosinophils might be used as a biomarker during exacerbations to identify patients who are less likely to benefit from a systemic corticosteroid treatment (eosinophils < 300). Nevertheless, eosinophil-guided therapy is non-inferior to standard care.[110] However, eosinophilic inflammation has been evaluated only through blood samples and sputum cytology has not been performed. History of exacerbations remains the main predictor of future exacerbation risk and the BECs need to be integrated with this information to increase the prediction of ICS responsiveness.[64] Recent data showed that eosinophilic exacerbations in both asthma and COPD could benefit from IL5 R therapy. Indeed, in the ABRA study benralizumab was used in single dose (100 mg) to treat acute eosinophilic exacerbations achieving better outcomes than the current standard of care with prednisolone alone.[111] Despite this evidence, there is an increasing need for biomarkers to better phenotype acute COPD exacerbations and improve target treatment.[112]

Lung function decline in COPD is a key marker of disease progression and severity. Identifying reliable biomarkers that can predict lung function decline is essential for optimising patient management and tailoring treatment strategies.[1] One potential biomarker is the BEC, whose role in lung function decline merits attention. In the Dunedin Multidisciplinary Health and Development Study involving healthy young adults ($n = 971$), elevated BECs have been significantly associated with a more rapid decline in FEV1, even after adjusting for smoking status. Similar findings have been recently reported in a retrospective cohort analysis from the Kangbuk Samsung Health Study (KSCS), evaluating 629,784 healthy subjects. In this study, both never-smokers and ever-smokers with higher BECs have had faster FEV1 decline than those with lower eosinophil counts.[113] Higher blood eosinophils may also represent an independent risk factor for developing obstructive lung disease in healthy subjects without a history of asthma and COPD as reported in a large cohort study ($n = 359.456$).[114] Data from the Canadian Cohort Obstructive Lung Disease (CANCOLD; $n = 1.120$) study highlight that BEC ⩾ 300 cells/μL is an independent risk factor for accelerated lung function decline in COPD patients. Particularly those patients with BEC ⩾ 300 cells/μL have had a greater FEV1 decline (−67.30 mL/year) than those with eosinophil counts of <150 cells/μL (−38.78 mL/year).[115] In addition, the use of ICS has reduced lung function decline in COPD patients with frequent exacerbations and elevated BECs.[116] These data suggest that higher BEC could correlate with FEV1 decline in different populations, highlighting a possible key role of T2 inflammation in airway remodelling in this subgroup of patients. Nevertheless, in COPD patients, both peripheral eosinophilia and exacerbation frequency significantly contribute to lung function decline. These two factors should be considered together to determine the most appropriate therapeutic strategy.

ICS is anti-inflammatory therapy used in combination with the long-acting beta-agonist (LABA) and long-acting muscarinic antagonist (LAMA) to reduce exacerbation frequency in COPD patients with increased exacerbation risk.[1] The mechanism of action of ICS therapy is not fully understood, it appears that ICS diffuses into eosinophils, where they bind to cytoplasmic glucocorticoid receptors. This complex is subsequently transported into the nucleus, where it inhibits the transcription of pro-inflammatory genes and promotes eosinophil apoptosis.[117] It has been shown that patients with sputum eosinophils ⩾3% have a better response to treatment with systemic or inhaled corticosteroids in terms

of symptoms and lung function improvement than the patients without sputum eosinophilia.[7,54,56] Post-hoc analyses of RCTs comparing the safety and effectiveness of ICS/LABA versus LAMA in COPD patients with increased exacerbation risk alone have investigated the potential role of BEC as a predictor of ICS response. These studies have found that patients with higher baseline BECs and a history of exacerbations in the previous year (⩾1 severe or ⩾2 moderate) have a significant exacerbation reduction with ICS treatment.[9,118] Post-hoc analyses of the WISDOM trial highlight that COPD patients with baseline BEC ⩾300 cells/μL had a significantly higher rate of exacerbations following ICS withdrawal compared with those who continued ICS therapy, particularly in patients with a history of ⩾2 exacerbations/year.[119] Similarly, data from the SUNSET trial showed that patients on long-term triple therapy with ⩽1 exacerbation in the previous year and BEC ⩾300 cells/μL had an increased risk of disease deterioration, including a higher exacerbation frequency, after ICS discontinuation.[120] RCTs of inhaled triple therapy (ICS/LABA/LAMA) have consistently demonstrated greater reductions in exacerbation rates in patients with elevated eosinophil counts and a history of exacerbations in the previous year. The IMPACT[121] trial has reported more significant exacerbation reductions in patients with BEC ⩾150 cells/μL. On the other hand, TRIBUTE[122] has shown more significant exacerbation reductions in those with BEC ⩾2% while ETHOS[123] has reported more significant exacerbation reductions in patients with BEC ⩾100 cells/μL. Most of the data focus on the benefit of ICS/LABA/LAMA in reducing exacerbations in patients with higher BECs, but in addition to this benefit, there is also supporting evidence that triple therapy improves lung function and reduces mortality.[124,125] Based on this evidence GOLD document recommends BEC thresholds <100 and >300 cells/μL to guide ICS use in clinical practice, suggesting that there is an increased probability of benefit in BEC thresholds of 100–300 cells/μL.[1] No benefit from ICS treatment is reported in COPD patients with BECs <100 cells/μL. Indeed these patients present a higher risk of developing pneumonia and chronic bacterial infection.[126] Once ICS therapy is initiated, changes in BEC have demonstrated predictive value as a biomarker of treatment response in COPD. Post-hoc analyses of both the ISOLDE and/or the FLAME studies revealed that a decrease in BEC following ICS treatment was associated with a reduced exacerbation rate[127,128] and slower lung function decline.[128] On the other hand, an increase in BEC was linked to worsened outcomes. However, changes in BEC did not predict improvements in health status assessed by SGRQ.[127,128] These emerging findings suggest that BEC could play a role as a therapeutic response biomarker, offering new perspectives for the management of COPD.[129] Prospective studies are warranted to validate these observations further.

Road to biologics

The accurate phenotyping of COPD has made it possible to identify a subgroup of patients characterised by eosinophilic inflammation who may benefit from targeted biological therapies. Several studies have evaluated the safety and efficacy of these therapies – various already used in severe asthma – in terms of reducing exacerbations and improving clinical and functional outcomes in COPD patients (Table 1).[41]

Mepolizumab is a humanised monoclonal antibody that reduces eosinophil counts in blood and tissues by blocking IL-5. The safety and the efficacy of mepolizumab versus placebo in COPD patients with a history of ⩾2 exacerbations have been evaluated in METREX and in METREO. Mepolizumab significantly reduces annual exacerbation rates in patients with BEC ⩾150 cells/μL in METREX, but not in METREO.[130] A post-hoc analysis of the studies indicates that the efficacy of mepolizumab is higher when BECs are ⩾ 300 cells/μL.[131] A large phase III clinical trial MATINEE (NCT04133909) is currently underway to evaluate the efficacy of the drug in patients with BEC ⩾ 300 cells/μL. In GALATHEA and TERRANOVA trials, no significant reduction of exacerbation has been observed in COPD patients with BEC ⩾220 cells/μL treated with benralizumab (Anti-IL5R alpha receptor antibody) over 56 weeks.[132] However, a subsequent analysis suggests that patients with a history of more than three exacerbations in the previous year may benefit from treatment with benralizumab 100 mg.[133] The RESOLUTE (NCT04053634) trial is currently underway to further investigate this outcome. The positive results from the phase III BOREAS study indicate that a 52-week treatment with dupilumab (anti-IL-4R alpha receptor antibody) significantly reduces exacerbations compared with placebo in COPD patients with BEC

Table 1. Effect of biologics on the reduction of annualised exacerbation rate in patients with COPD: evidence from RCTs.

RCT	No of patients	Asthma diagnosis	Eosinophils threshold (cells/μL)	Treatment regimen	Biological therapy	Annualised moderate or severe exacerbation rate (treatment vs placebo)
METREX (NCT02105948) Phase III	Total: 837 Mepolizumab 100 mg: 417 Placebo: 420	Exclusion Criteria	⩾150 at screening or ⩾ 300 in the previous year	Every 4 weeks for 52 weeks	Mepolizumab 100 mg	1.40 versus 1.71 RR = 0.82 $p = 0.04$
METREO (NCT02105961) Phase III	Total: 675 Mepolizumab 100 mg: 223 Mepolizumab 300 mg: 226 Placebo: 226	Exclusion Criteria	⩾150 at screening or ⩾ 300 in the previous year	Every 4 weeks for 52 weeks	Mepolizumab 100 mg Mepolizumab 300 mg	1.19 versus 1.49 RR = 0.80 $p = 0.07$ 1.27 versus 1.49 RR = 0.86 $p = 0.14$
GALATHEA (NCT02138916) Phase III	Total: 1120 Bernalizumab 30 mg: 382 Benralizumab 100 mg: 379 Placebo: 359	Current: 5.4% Past: 8.3%	⩾220 at baseline or < 220 at baseline	Every 4 weeks for the first three doses, then every 8 weeks for 56 weeks	Benralizumab 30 mg Benralizuamb 100 mg	1.19 versus 1.24 RR = 0.96 $p = 0.65$ 1.03 versus 1.24 RR = 0.83 $p = 0.05$
TERRANOVA (NCT02155660) Phase III	Total: 1545 Benralizumab 10 mg: 377 Benralizumab 39 mg: 394 Benralizumab 100 mg: 386 Placebo: 388	Current: 3.3% Past: 6.1%	⩾220 at baseline or < 220 at baseline	Every 4 weeks for the first three doses, then every 8 weeks for 56 weeks	Benralizumab 10 mg Benralizumab 30 mg Benralizuamb 100 mg	0.99 versus 1.17 RR = 0.85 $p = 0.06$ 1.21 versus 1.17 RR 1.04 $p = 0.66$ 1.09 versus 1.17 RR = 0.93 $p = 0.40$
BOREAS (NCT03930732) Phase III	Total: 939 Dupilumab mg: 468 Placebo: 471	Exclusion Criteria	⩾ 300 at screening	Every 2 weeks for 52 weeks	Dupilumab 300 mg	0.78 versus 1.10 RR = 0.70 $p < 0.001$
NOTUS (NCT04456673) Phase III	Total: 935 Dupilumab mg: 470 Placebo: 465	Exclusion Criteria	⩾300 at screening	Every 2 weeks for 52 weeks	Dupilumab 300 mg	0.86 versus 1.30 RR 0.66 $p < 0.001$
COURSE (NCT04039113) Phase IIA	Total: 333 Tezepelumab mg: 165 Placebo 168	Exclusion Criteria	⩾300 at baseline ⩾150 and <300 at baseline <150 at baseline	Every 4 weeks for 52 weeks	Tezepelumab 420 mg	1.75 versus 2.11 RR 0.83 $p = 0.10$
(NCT03546907) Phase IIA	343 Itepekimab mg: 172 Placebo 171	Exclusion Criteria	⩾250 at screening < 250 at screening	Every 2 weeks for 24-52 weeks	Itepekimab 300 mg	1.30 versus 1.61 RR 0.81 $p = 0.13$
COPD-ST2OP NCT03615040. Phase IIA	Total: 81 Astegolimab mg: 42 Placebo: 39	Not reported	No Thresholds	Every 4 weeks for 44 weeks	Astegolimab 490 mg	2.18 versus 2.81 RR 0.78 $p = 0.19$

COPD, chronic obstructive pulmonary disease.

⩾300 cells/μL.[97] Furthermore, patients treated with dupilumab have shown significant lung function improvement (FEV1 + 83 mL). As previously reported, the subgroups of patients with FeNO levels >20 ppb had a greater lung function improvement.[97] These findings have been further confirmed by the NOTUS study, which demonstrates a 34% reduction in exacerbation rate in COPD patients treated with dupilumab.[134,135] The effectiveness of dupilumab may be attributed to its dual inhibition of IL-4 and IL-13 pathways, both central to type 2 inflammation. By targeting these cytokines, dupilumab addresses multiple inflammatory processes and demonstrates its efficacy on mucus hypersecretion and airway remodelling,[136] which play a key role in the pathogenesis of eosinophilic COPD. Biological agents targeting TLSP and IL-33 have been studied as novel therapeutic approaches in COPD. Indeed, targeting TLSP and IL-33 with biologic therapies aims to modulate key inflammatory pathways, potentially reducing disease progression and exacerbations and improving clinical outcomes in COPD patients.[137,138] TLSP plays a pivotal role in the regulation of immune responses and inflammatory pathways, and its inhibition has been shown to attenuate airway inflammation in COPD models.[139,140] Recent evidence from the phase IIA COURSE study did not show a significant reduction in moderate-to-severe exacerbation in COPD patients, but additional analysis suggested a potential role of TSLP in patients with BEC over 150 cells.[137] IL-33, a pro-inflammatory cytokine, is involved in the activation of innate and adaptive immune cells and contributes to the pathogenesis of COPD.[141] Data from a phase IIA study showed a reduction in exacerbation rate in ex-smoker patients treated with anti-IL-33 itepekimab.[138] Another study investigated targeting ST2, the IL-33 receptor, to reduce COPD exacerbations but did not find a significant difference compared to placebo.[142] Given the promising evidence provided by these biologic therapies,[143] ongoing clinical trials are further investigating their efficacy and safety to optimise treatment strategies and implement precision medicine for COPD patients.

Conclusion

Eosinophils play a significant role in a substantial subset of COPD patients, challenging the traditional view of COPD as a neutrophilic disease. BECs are an emerging biomarker to identify patients who are more likely to benefit from ICS therapy and potentially from biological treatments. However, the utility of blood eosinophils as a surrogate marker for airway inflammation remains complex due to a variable correlation with sputum eosinophils, particularly in patients with comorbid cardiovascular conditions. Despite various therapeutic strategies, including both pharmacological treatments and non-pharmacological interventions such as pulmonary rehabilitation, COPD management remains challenging. This complexity could be due to the complex inflammatory mechanisms underlying the disease and its associated syndemic comorbidities. Eosinophilic inflammation may play a pivotal role in optimising clinical management in these patients. Increasing data regarding eosinophils and T2 inflammation encourage a treatable trait approach and could have a pivotal role in the context of precision medicine. A combined assessment of both systemic and airway inflammation may be essential to identify stable biomarkers that can reliably identify patients at high risk for disease progression and predict therapeutic response. Further research on the relationship between systemic and airway inflammation is needed to explore the relationship between COPD pathophysiology and clinical outcomes.

Author's note

An Associate Editor of Therapeutic Advances in Respiratory Disease is an author of this paper. Therefore, the peer review process was managed by alternative members of the Editorial Board and the submitting Editor had no involvement in the decision-making process.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Marco Vanetti: Conceptualisation; Investigation; Methodology; Project administration; Writing – original draft; Writing – review & editing.

Dina Visca: Conceptualisation; Writing – original draft; Writing – review & editing.

Francesco Ardesi: Methodology; Writing – original draft; Writing – review & editing.

Martina Zappa: Methodology; Writing – original draft; Writing – review & editing.

Patrizia Pignatti: Methodology; Supervision; Writing – original draft; Writing – review & editing.

Antonio Spanevello: Conceptualisation; Investigation; Supervision; Validation; Writing – review & editing.

Acknowledgements
The authors sincerely thank Susan Koller for her contribution to the linguistic revision of the manuscript and Filippo Vacca for his support in the graphic development of Figure 1.

Funding
The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: It has been partially funded by the Ricerca Corrente scheme of the Ministry of Health, Italy.

Competing interests
The authors declare that there is no conflict of interest.

Availability of data and materials
All data are available on request.

ORCID iDs
Marco Vanetti https://orcid.org/0009-0007-3782-5021

Dina Visca https://orcid.org/0000-0003-2298-1623

Francesco Ardesi https://orcid.org/0000-0003-3420-4012

Martina Zappa https://orcid.org/0000-0001-8443-5316

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for diagnosis, management and prevention of chronic obstructive pulmonary disease: 2024 report. https://goldcopd.org/2024-gold-report/ (accessed September 2024).
2. Adeloye D, Song P, Zhu Y, et al. Global, regional, and national prevalence of, and risk factors for, chronic obstructive pulmonary disease (COPD) in 2019: a systematic review and modelling analysis. *Lancet Respir Med* 2022; 10: 447–458.
3. Agustí A, Melén E, DeMeo DL, et al. Pathogenesis of chronic obstructive pulmonary disease: understanding the contributions of gene-environment interactions across the lifespan. *Lancet Respir Med* 2022; 10: 512–524.
4. Stănescu D, Sanna A, Veriter C, et al. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996; 51: 267–271.
5. Singh D, Kolsum U, Brightling CE, et al. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. *Eur Respir J* 2014; 44: 1697–700.
6. Zanini A, Cherubino F, Zampogna, et al. Bronchial hyperresponsiveness, airway inflammation, and reversibility in patients with chronic obstructive pulmonary disease. *Int J Chronic Obstruct Pulmon Dis* 2015; 10: 1155–1161.
7. Siva R, Green RH, Brightling CE, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. *Eur Respir J* 2007; 29: 906–913.
8. Papi A, Romagnoli M, Baraldo S, et al. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 162: 1773–1777.
9. Pascoe S, Locantore N, Dransfield MT, et al. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. *Lancet Respir Med* 2015; 3: 435–442.
10. Polverino F and Sin DD. Type 2 airway inflammation in COPD. *Eur Respir J* 2024; 63: 2400150.
11. David B and Bafadhel M. Eosinophilic inflammation in COPD: from an inflammatory marker to a treatable trait. *Thorax* 2021; 76: 188–195.
12. Kolsum U, Southworth T, Jackson N, et al. Blood eosinophil counts in COPD patients

compared to controls. *Eur Respir J* 2019; 54: 1900633.

13. Singh D, Agusti A, Martinez FJ, et al. Blood eosinophils and chronic obstructive pulmonary disease: a global initiative for chronic obstructive Lung disease science committee 2022 review. *Am J Respir Crit Care Med* 2022; 206: 17–24.
14. George L and Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. *Ther Adv Chronic Dis* 2016; 7: 34–51.
15. Rothenberg ME and Hogan SP. The eosinophil. *Ann Rev Immunol* 2006; 24: 147–174.
16. Molfino NA, Gossage D, Kolbeck R, et al. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clin Exper Allergy* 2012; 42: 712–737.
17. Hogan SP, Rosenberg HF, Moqbel R, et al. Eosinophils: biological properties and role in health and disease. *Clin Exper Allergy* 2008; 38: 709–750.
18. Rosenberg HF, Dyer KD and Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol* 2013; 13: 9–22.
19. Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991; 324: 1110–1118.
20. Johansson MW, Lye MH, Barthel SR, et al. Eosinophils adhere to vascular cell adhesion molecule-1 via podosomes. *Am J Respir Cell Mol Biol* 2004; 31: 413–422.
21. Tashkin DP and Wechsler ME. Role of eosinophils in airway inflammation of chronic obstructive pulmonary disease. *Int J Chronic Obstruct Pulm Dis* 2018; 13: 335–349.
22. Smit JJ and Lukacs NW. A closer look at chemokines and their role in asthmatic responses. *Eur J Pharmacol* 2006; 533: 277–288.
23. Nakagome K and Nagata M. The possible roles of IL-4/IL-13 in the development of eosinophil-predominant severe asthma. *Biomolecules* 2024; 14: 546.
24. Acharya KR and Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem* 2014; 289: 17406–17415.
25. Dunican EM, Elicker BM, Gierada DS, et al. Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. *J Clin Investig* 2018; 128: 997–1009.
26. Davoine F and Lacy P. Eosinophil cytokines, chemokines, and growth factors: emerging roles in immunity. *Front Immunol* 2014; 5: 570.
27. Moqbel R, Levi-Schaffer F and Kay AB. Cytokine generation by eosinophils. *J Allergy Clin Immunol* 1994; 94: 1183–1188.
28. Christenson SA, Steiling K, van den Berge M, et al. Asthma-COPD overlap. Clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015; 191: 758–766.
29. Beech A and Higham A. Type 2 inflammation in COPD: is it just asthma? *Breathe (Sheff)* 2024; 20(3): 230229.
30. Fabbri LM, Romagnoli M, Corbetta L, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 167: 418–424.
31. Higham A, Beech A, Wolosianka S, et al. Type 2 inflammation in eosinophilic chronic obstructive pulmonary disease. *Allergy* 2021; 76: 1861–1864.
32. George L, Taylor AR, Esteve-Codina A, et al. Blood eosinophil count and airway epithelial transcriptome relationships in COPD versus asthma. *Allergy* 2020; 75: 370–380.
33. Bradding P, Walls AF and Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; 117: 1277–1284.
34. Faiz A, Pavlidis S, Kuo CH, et al. Th2 high and mast cell gene signatures are associated with corticosteroid sensitivity in COPD. *Thorax* 2023; 78: 335–343.
35. Higham A, Dungwa J, Pham TH, et al. Increased mast cell activation in eosinophilic chronic obstructive pulmonary disease. *Clin Transl Immunol* 2022; 11: e1417.
36. Ramu S, Akbarshahi H, Mogren S, et al. Direct effects of mast cell proteases, tryptase and chymase, on bronchial epithelial integrity proteins and anti-viral responses. *BMC Immunol* 2021; 22: 35.
37. Wechsler ME, Munitz A, Ackerman SJ, et al. Eosinophils in health and disease: a state-of-the-art review. *Mayo Clin Proc* 2021; 96: 2694–707.
38. Geslewitz WE, Percopo CM and Rosenberg HF. FACS isolation of live mouse eosinophils at high purity via a protocol that does not target Siglec F. *J Immunol Methods* 2018; 454: 27–31.
39. Vultaggio A, Accinno M, Vivarelli E, et al. Blood CD62L(low) inflammatory eosinophils are related to the severity of asthma and reduced by mepolizumab. *Allergy* 2023; 78: 3154–3165.

40. Cabrera López C, Sánchez Santos A, Lemes Castellano A, et al. Eosinophil subtypes in adults with asthma and adults with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2023; 208: 155–162.

41. Rabe KF, Rennard S, Martinez FJ, et al. Targeting type 2 inflammation and epithelial alarmins in chronic obstructive pulmonary disease: a biologics outlook. *Am J Respir Crit Care Med* 2023; 208: 395–405.

42. Fricker M, McDonald VM, Winter NA, et al. Molecular markers of type 2 airway inflammation are similar between eosinophilic severe asthma and eosinophilic chronic obstructive pulmonary disease. *Allergy* 2021; 76: 2079–2089.

43. Kolsum U, Damera G, Pham TH, et al. Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. *J Allergy Clin Immunol* 2017; 140: 1181–4.e7.

44. Maestrelli P, Saetta M, Di Stefano A, et al. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. *Am J Respir Crit Care Med* 1995; 152: 1926–1931.

45. Barnes PJ, Chowdhury B, Kharitonov SA, et al. Pulmonary biomarkers in chronic obstructive pulmonary disease. *Am J Resp Crit Care Med* 2006; 174: 6–14.

46. Saetta M, Di Stefano A, Turato G, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; 157: 822–826.

47. Bellinger CR, Khan I, Chatterjee AB, et al. Bronchoscopy safety in patients with chronic obstructive lung disease. *J Bronchol Interventional Pulmonol* 2017; 24: 98–103.

48. Dragonieri S, Tongoussouva O, Zanini A, et al. Markers of airway inflammation in pulmonary diseases assessed by induced sputum. *Monaldi Arch Chest Dis* 2009; 71: 119–126.

49. Spanevello A, Confalonieri M, Sulotto F, et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. *Am J Respir Critical Care Med* 2000; 162: 1172–1174.

50. Jones PD, Hankin R, Simpson J, et al. The tolerability, safety, and success of sputum induction and combined hypertonic saline challenge in children. *Am J Respir Crit Care Med* 2001; 164: 1146–1149.

51. Balbi B, Pignatti P, Corradi M, et al. Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: Values in healthy adults. *Eur Respir J* 2007; 30: 769–781.

52. Beech A, Jackson N and Singh D. Identification of COPD inflammatory endotypes using repeated sputum eosinophil counts. *Biomedicines* 2022; 10: 2611.

53. Schleich F, Corhay JL and Louis R. Blood eosinophil count to predict bronchial eosinophilic inflammation in COPD. *Eur Respir J* 2016; 47: 1562–1564.

54. Leigh R, Pizzichini MM, Morris MM, et al. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *Eur Respir J* 2006; 27: 964–971.

55. Bafadhel M, McKenna S, Terry S, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med* 2011; 184: 662–671.

56. Brightling CE, McKenna S, Hargadon B, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. *Thorax* 2005; 60: 193–198.

57. Basanta M, Ibrahim B, Dockry R, et al. Exhaled volatile organic compounds for phenotyping chronic obstructive pulmonary disease: a cross-sectional study. *Respir Res* 2012; 13: 72.

58. Kim VL, Coombs NA and Staples KJ. Impact and associations of eosinophilic inflammation in COPD: analysis of the AERIS cohort. *Eur Respir J* 2017; 50: 1700853.

59. Saha S and Brightling CE. Eosinophilic airway inflammation in COPD. *Int J Chron Obstruct Pulmon Dis* 2006; 1: 39–47.

60. Spanevello A, Migliori GB, Sharara A, et al. Induced sputum to assess airway inflammation: a study of reproducibility. *Clin Exper Allergy* 1997; 27: 1138–1144.

61. Pignatti P, Visca D, Cherubino F, et al. Do blood eosinophils strictly reflect airway inflammation in COPD? Comparison with asthmatic patients. *Respir Res* 2019; 20: 145.

62. Hartl S, Breyer MK, Burghuber OC, et al. Blood eosinophil count in the general population: typical values and potential confounders. *Eur Respir J* 2020; 55.

63. Miravitlles M, Soler-Cataluña JJ, Soriano JB, et al. Determinants of blood eosinophil levels in the general population and patients with COPD: a population-based, epidemiological study. *Respir Res* 2022; 23: 49.

64. Yun JH, Lamb A, Chase R, et al. Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2018; 141: 2037–47.e10.

65. Long GH, Southworth T, Kolsum U, et al. The stability of blood Eosinophils in chronic obstructive pulmonary disease. *Respir Res* 2020; 21: 15.

66. Ellingsen J and Janson C. Neutrophil-to-lymphocyte ratio, blood eosinophils and COPD exacerbations: a cohort study. *ERJ Open Res* 2021; 7: 00471–2021.

67. Negewo NA, McDonald VM, Baines KJ, et al. Peripheral blood eosinophils: a surrogate marker for airway eosinophilia in stable COPD. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 1495–504.

68. Proboszcz M, Mycroft K, Paplinska-Goryca M, et al. Relationship between blood and induced sputum eosinophils, bronchial hyperresponsiveness and reversibility of airway obstruction in mild-to-moderate chronic obstructive pulmonary disease. *Copd* 2019; 16: 354–361.

69. Singh D, Watz H, Beeh KM, et al. COPD sputum eosinophils: relationship to blood eosinophils and the effect of inhaled PDE4 inhibition. *Eur Respir J* 2020; 56: 2000237.

70. Hastie AT, Martinez FJ, Curtis JL, et al. Association of sputum and blood eosinophil concentrations with clinical measures of COPD severity: an analysis of the SPIROMICS cohort. *Lancet Respir Med* 2017; 5: 956–967.

71. Turato G, Semenzato U and Bazzan E. Blood eosinophilia neither reflects tissue eosinophils nor worsens clinical outcomes in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2018; 197: 1216–1219.

72. Harries TH, Rowland V, Corrigan CJ, et al. Blood eosinophil count, a marker of inhaled corticosteroid effectiveness in preventing COPD exacerbations in post-hoc RCT and observational studies: systematic review and meta-analysis. *Respir Res* 2020; 21: 3.

73. Abdo M, Pedersen F and Trinkmann F. Association of airway eosinophilia with small airway dysfunction in patients with mild and at risk for COPD. *Int J Chron Obstruct Pulmon Dis* 2022; 17: 1403–1408.

74. Van Rossem I, Hanon S, Verbanck S, et al. Blood eosinophil counts in chronic obstructive pulmonary disease: adding within-day variability to the equation. *Am J Respir Crit Care Med* 2022; 205: 727–729.

75. Mesnil C, Raulier S, Paulissen G, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Investig* 2016; 126: 3279–3295.

76. Sweetnam PM, Thomas HF, Yarnell JW, et al. Total and differential leukocyte counts as predictors of ischemic heart disease: the Caerphilly and Speedwell studies. *Am J Epidemiol* 1997; 145: 416–421.

77. Tanaka M, Fukui M, Tomiyasu K, et al. Eosinophil count is positively correlated with coronary artery calcification. *Hypertens Res* 2012; 35: 325–328.

78. Mukai HY, Ninomiya H, Ohtani K, et al. Major basic protein binding to thrombomodulin potentially contributes to the thrombosis in patients with eosinophilia. *Brit J Haematol* 1995; 90: 892–899.

79. Fabbri LM, Celli BR, Agustí A, et al. COPD and multimorbidity: recognising and addressing a syndemic occurrence. *Lancet Respir Med* 2023; 11: 1020–1034.

80. Pignatti P, Visca D, Zappa M, et al. Monitoring COPD patients: systemic and bronchial eosinophilic inflammation in a 2-year follow-up. *BMC Pulmon Med* 2024; 24: 247.

81. Xu J, Guo J, Liu T, et al. Differential roles of eosinophils in cardiovascular disease. *Nat Rev Cardiol* 2025; 22(3): 165–182.

82. Pignatti P, Visca D, Loukides S, et al. A snapshot of exhaled nitric oxide and asthma characteristics: Experience from high to low income countries. *Pulmonology* 2022; 28: 44–58.

83. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; 171: 912–930.

84. Global Initiative for Asthma (GINA). 2024 GINA report – global strategy for asthma management and prevention, https://ginasthma.org/2024-report/ (accessed September 2024).

85. Pavlidis S, Takahashi K, Ng Kee Kwong F, et al. "T2-high" in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. *Eur Respir J* 2019; 53: 1800938.

86. Schleich FN, Chevremont A, Paulus V, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014; 44: 97–108.

87. Chibana K, Trudeau JB, Mustovich AT, et al. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. *Clin Exper Allergy* 2008; 38: 936–946.

88. Brightling CE, Saha S and Hollins F. Interleukin-13: prospects for new treatments. *Clin Exper Allergy* 2010; 40: 42–49.

89. Lu Z, Huang W, Wang L, et al. Exhaled nitric oxide in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Int J Chron Obstruct Pulm Dis* 2018; 13: 2695–2705.

90. Chou KT, Su KC, Huang SF, et al. Exhaled nitric oxide predicts eosinophilic airway inflammation in COPD. *Lung* 2014; 192: 499–504.

91. Antus B, Paska C and Barta I. Predictive value of exhaled nitric oxide and blood eosinophil count in the assessment of airway eosinophilia in COPD. *Int J Chronic Obstruct Pulmon Dis* 2020; 15: 2025–2035.

92. Higham A, Beech A, Dean J, et al. Exhaled nitric oxide, eosinophils and current smoking in COPD patients. *ERJ Open Res* 2023; 9: 00686–2023.

93. Bhowmik A, Seemungal TA, Donaldson GC, et al. Effects of exacerbations and seasonality on exhaled nitric oxide in COPD. *Eur Respir J* 2005; 26: 1009–1015.

94. Agustí AG, Villaverde JM, Togores B, et al. Serial measurements of exhaled nitric oxide during exacerbations of chronic obstructive pulmonary disease. *Eur Respir J* 1999; 14: 523–528.

95. Papi A, Bellettato CM, Braccioni F, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med* 2006; 173: 1114–1121.

96. Alcázar-Navarrete B, Ruiz Rodríguez O, Conde Baena P, et al. Persistently elevated exhaled nitric oxide fraction is associated with increased risk of exacerbation in COPD. *Eur Respir J* 2018; 51: 1701457.

97. Bhatt SP, Rabe KF, Hanania NA, et al. Dupilumab for COPD with type 2 inflammation indicated by eosinophil counts. *N Engl J Med* 2023; 389: 205–214.

98. Wu HX, Zhuo KQ and Cheng DY. Prevalence and baseline clinical characteristics of eosinophilic chronic obstructive pulmonary disease: a meta-analysis and systematic review. *Front Med* 2019; 6: 282.

99. Albanna A, Almuyidi FM, Beitar NF, et al. Clinical characteristics and outcome related to blood eosinophilic chronic obstructive pulmonary disease (COPD) patients. *Cureus* 2022; 14: e27998.

100. Wu X, Deng Z, Wu F, et al. Clinical characteristics and 2-year outcomes of chronic obstructive pulmonary disease patients with high blood eosinophil counts: a population-based prospective cohort study in China. *Archivos de Bronconeumología*. 2024; 60: 402–409.

101. Dunican EM, Elicker BM, Henry T, et al. Mucus plugs and emphysema in the pathophysiology of airflow obstruction and hypoxemia in smokers. *Am J Respir Crit Care Med* 2021; 203: 957–968.

102. Diaz AA, Grumley S, Yen A, et al. Mucus plugs, eosinophils, and exacerbations in copd: findings from two cohorts. *CHEST* 2024; 166: A4747–A8.

103. Donaldson GC, Seemungal TA, Bhowmik A, et al. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax* 2002; 57: 847–852.

104. Rothnie KJ, Müllerová H, Smeeth L, et al. Natural history of chronic obstructive pulmonary disease exacerbations in a general practice-based population with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2018; 198: 464–471.

105. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med* 2010; 363: 1128–1138.

106. Graul EL and Nordon C. Temporal risk of nonfatal cardiovascular events after chronic obstructive pulmonary disease exacerbation: a population-based study. 2024; 209: 960-72.

107. Beghe B, Spanevello A and Fabbri LM. Risk and Prevention of cardiovacular events after exacerbations of respiratory symptoms in patients with COPD. *Am J Respir Crit Care Med* 2024; 209: 901–972.

108. Vedel-Krogh S, Nielsen SF, Lange P, et al. Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study. *Am J Respir Crit Care Med* 2016; 193: 965–974.

109. Casanova C, Celli BR, de-Torres JP, et al. Prevalence of persistent blood eosinophilia: relation to outcomes in patients with COPD. 2017; 50.

110. Sivapalan P, Lapperre TS, Janner J, et al. Eosinophil-guided corticosteroid therapy in patients admitted to hospital with COPD exacerbation (CORTICO-COP): a multicentre, randomised, controlled, open-label, non-inferiority trial. *Lancet Respir Med* 2019; 7: 699–709.

111. Ramakrishnan S, Russell REK, Mahmood HR, et al. Treating eosinophilic exacerbations of asthma and COPD with benralizumab (ABRA): a double-blind, double-dummy, active placebo-controlled randomised trial. *Lancet Respir Med* 2025; 13: 59–68.

112. Leung JM, Chen V, Hollander Z, et al. COPD exacerbation biomarkers validated using multiple reaction monitoring mass spectrometry. *PloS One* 2016; 11: e0161129.

113. Hong YS, Park HY and Ryu S. The association of blood eosinophil counts and FEV(1) decline: a cohort study. *Eur Respir J* 2024; 63(5): 2301037.

114. Park HY, Chang Y, Kang D, et al. Blood eosinophil counts and the development of obstructive lung disease: the Kangbuk Samsung Health Study. *Eur Respir J* 2021; 58(4): 2003823.

115. Tan WC and Bourbeau J. High eosinophil counts predict decline in FEV(1): results from the CanCOLD study. *Eur Respir J* 2021; 57(5): 2000838.

116. Barnes NC, Sharma R, Lettis S, et al. Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. *Eur Respir J* 2016; 47: 1374–1382.

117. Lea S and Higham A. How inhaled corticosteroids target inflammation in COPD. *Eur Respir Rev* 2023; 32(170): 230084.

118. Bafadhel M, Peterson S, De Blas MA, et al. Predictors of exacerbation risk and response to budesonide in patients with chronic obstructive pulmonary disease: a post-hoc analysis of three randomised trials. *Lancet Respir Med* 2018; 6: 117–126.

119. Watz H, Tetzlaff K, Wouters EF, et al. Blood eosinophil count and exacerbations in severe chronic obstructive pulmonary disease after withdrawal of inhaled corticosteroids: a post-hoc analysis of the WISDOM trial. *Lancet Respir Med* 2016; 4: 390–398.

120. Chapman KR, Hurst JR, Frent SM, et al. Long-term triple therapy de-escalation to indacaterol/glycopyrronium in patients with chronic obstructive pulmonary disease (SUNSET): a randomized, double-blind, triple-dummy clinical trial. *Am j Respir Crit Care Med*. 2018; 198: 329–39.

121. Lipson DA, Barnhart F, Brealey N, et al. Once-daily single-inhaler triple versus dual therapy in patients with COPD. *N Engl J Med* 2018; 378: 1671–1680.

122. Papi A, Vestbo J, Fabbri L, et al. Extrafine inhaled triple therapy versus dual bronchodilator therapy in chronic obstructive pulmonary disease (TRIBUTE): a double-blind, parallel group, randomised controlled trial. *Lancet (London, England)*. 2018; 391: 1076–1084.

123. Rabe KF, Martinez FJ, Ferguson GT, et al. Triple inhaled therapy at two glucocorticoid doses in moderate-to-very-severe COPD. *N Engl J Med* 2020; 383: 35–48.

124. Martinez FJ, Rabe KF, Ferguson GT, et al. Reduced all-cause mortality in the ETHOS trial of budesonide/glycopyrrolate/formoterol for chronic obstructive pulmonary disease. A randomized, double-blind, multicenter, parallel-group study. *Am J Respir Critl Care Med* 2021; 203: 553–564.

125. Pascoe S, Barnes N, Brusselle G, et al. Blood eosinophils and treatment response with triple and dual combination therapy in chronic obstructive pulmonary disease: analysis of the IMPACT trial. *Lancet Respir Med* 2019; 7: 745–56.

126. Martinez-Garcia MA, Faner R, Oscullo G, et al. Inhaled steroids, circulating eosinophils, chronic airway infection, and pneumonia risk in chronic obstructive pulmonary disease. a network analysis. *Am J Respir Crit Care Med* 2020; 201: 1078–1085.

127. Mathioudakis AG, Bate S, Sivapalan P, et al. Rethinking blood eosinophils for assessing inhaled corticosteroids response in COPD: a post hoc analysis from the FLAME trial. *Chest* 2024; 166: 987–997.

128. Mathioudakis AG, Bikov A, Foden P, et al. Change in blood eosinophils following treatment with inhaled corticosteroids may predict long-term clinical response in COPD. *Eur Respir J* 2020; 55(5): 1902119.

129. Chen S and Miravitlles M. Patients with chronic obstructive pulmonary disease and evidence of eosinophilic inflammation experience exacerbations despite receiving maximal inhaled maintenance therapy. *Int J Chron Obstruct Pulmon Dis* 2022; 17: 2187–2200.

130. Pavord ID, Chanez P, Criner GJ, et al. Mepolizumab for eosinophilic chronic

obstructive pulmonary disease. *N Engl J Med* 2017; 377: 1613–1629.

131. Pavord ID and Chapman KR. Mepolizumab for eosinophil-associated COPD: analysis of METREX and METREO. *Int J Chron Obstruct Pulmon Dis* 2021; 16: 1755–1770.

132. Criner GJ, Celli BR, Brightling CE, et al. Benralizumab for the prevention of COPD exacerbations. *N Engl J Med* 2019; 381: 1023–1034.

133. Singh D, Criner GJ, Agustí A, et al. Benralizumab prevents recurrent exacerbations in patients with chronic obstructive pulmonary disease: a post hoc analysis. *Int J Chron Obstruct Pulmon Dis* 2023; 18: 1595–1599.

134. Bhatt SP, Rabe KF, Hanania NA, et al. Dupilumab for COPD with Blood Eosinophil Evidence of Type 2 Inflammation. *N Engl J Med* 2024; 390(24): 2274–2283.

135. Bhatt SP, Rabe KF, Hanania NA, et al. Dupilumab for chronic obstructive pulmonary disease with type 2 inflammation: a pooled analysis of two phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med* 2025; 13: 234–243.

136. Castro M, Papi A, Porsbjerg C, et al. Effect of dupilumab on exhaled nitric oxide, mucus plugs, and functional respiratory imaging in patients with type 2 asthma (VESTIGE): a randomised, double-blind, placebo-controlled, phase 4 trial. *Lancet Respir Med* 2025; 13: 208–220.

137. Singh D, Brightling CE, Rabe KF, et al. Efficacy and safety of tezepelumab versus placebo in adults with moderate to very severe chronic obstructive pulmonary disease (COURSE): a randomised, placebo-controlled, phase 2a trial. *Lancet Respir Med* 2025; 13: 47–58.

138. Rabe KF, Celli BR, Wechsler ME, et al. Safety and efficacy of itepekimab in patients with moderate-to-severe COPD: a genetic association study and randomised, double-blind, phase 2a trial. *Lancet Respir Med* 2021; 9: 1288–1298.

139. Smelter DF, Sathish V, Thompson MA, et al. Thymic stromal lymphopoietin in cigarette smoke-exposed human airway smooth muscle. *J Immunol (Baltimore, Md : 1950)*. 2010; 185: 3035–3040.

140. Yamada H, Hida N, Masuko H, et al. Effects of lung function-related genes and TSLP on COPD phenotypes. *COPD* 2020; 17: 59–64.

141. Byers DE, Alexander-Brett J, Patel AC, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. *J Clin Investig* 2013; 123: 3967–3982.

142. Yousuf AJ, Mohammed S, Carr L, et al. Astegolimab, an anti-ST2, in chronic obstructive pulmonary disease (COPD-ST2OP): a phase 2a, placebo-controlled trial. *Lancet Respir Med* 2022; 10: 469–477.

143. Pitre T, Lupas D, Mah J, et al. Biologic therapies for chronic obstructive pulmonary disease: a systematic review and network meta-Analysis of randomized controlled trials. *Copd*. 2025; 22: 2449889.