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Invited Review Article

Eosinophil-mucus interplay in severe asthma: Implications for treatment with biologicals

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ABSTRACT

Airway mucus is a hydrogel with unique biophysical properties due to its primary water composition and a small proportion of large anionic glycoproteins or mucins. The predominant mucins in human mucus, MUC5AC and MUC5B, are secreted by specialized cells within the airway epithelium both in normal conditions and in response to various stimuli. Their relative proportions are correlated with specific inflammatory responses and disease mechanisms. The dysregulation of mucin expression is implicated in numerous respiratory diseases, including asthma, COPD, and cystic fibrosis, where the pathogenic role of mucus has been extensively described yet often overlooked. In airway diseases, excessive mucus production or impaired mucus clearance leads to mucus plugging, with secondary airway occlusion that contribute to airflow obstruction, asthma severity and poor control. Eosinophils and Charcot Leyden crystals in sputum contribute to the mucus burden and tenacity. Mucin may also contribute to eosinophil survival. Other mechanisms, including eosinophil-independent IL-13 release, mast-cell activation and non-type-2 (T2) cytokines, are also likely to participate in mucus pathobiology. An accurate assessment of mucus and its clinical and functional consequences require a thorough approach that includes evaluation of cellular predominance in sputum, airway cytokines and other inflammatory markers, mucus characteristics and composition and structural and functional impact measured by advanced lung imaging. This review, illustrated with clinical scenarios, provides an overview of current methods to assess mucus and its relevance to the choice of biologics to treat patients with severe asthma.

Introduction

Airway mucus is a heterogeneous combination of proteins, cells and cellular debris originating from proximal and distal airway cells. Its primary role in health is to act as a barrier defense by trapping pathogens and other potentially harmful particles and facilitating their subsequent transport and removal with the aid of the cilia.[1,2] Mucus has the properties of both a solid and a fluid, and thus it is best described as a hydrogel with the ability to maintain shape while softly propelled towards the vocal cords to be expectorated or swallowed. These distinctive viscoelastic characteristics are explained by its unique composition of mainly water with only a minimal proportion of solids, including 1% of proteins.[3] Among these, mucins are the dominant proteins responsible for mucus's biophysical properties, which are crucial for mucociliary transport and clearance.[4] Mucins are large anionic glycoproteins of high molecular weight and remarkably high sugar content synthesized by surface epithelial cells and submucosal glands both in normal conditions and in response to intraluminal danger signals.[5] As illustrated in Figure 1, MUC5B and MUC5AC are the predominant mucins expressed in the airways and their absolute and relative proportions are regulated by a complex mechanism that involves IL-13-regulated transcription, O-glycosylation, and polymerization.[1,6] Dehydrated MUC5B and MUC5AC mucins are stored within intracellular granules and released to the airway lumen upon allergen, infectious or autonomic stimuli, where they undergo rapid hydration that forms the definitive mucus layer.[5,7,8] Although similar, these two glycoproteins play different roles in the mucus clearance system, with predominance of MUC5B secretion by surface club cells of proximal and distal airways and only minimal MUC5AC proximal secretion in the healthy lung.[9] This contrasts with enhanced MUC5AC production and secretion as part of allergic responses in murine and human models[10,11] and thus a relative deficiency of MUC5B that could promote airway hyperresponsiveness (AHR), mucus plugging and eosinophil survival.[12,13]

The objectives of this review are to briefly describe the contribution of mucus to asthma severity, methods to assess mucus in

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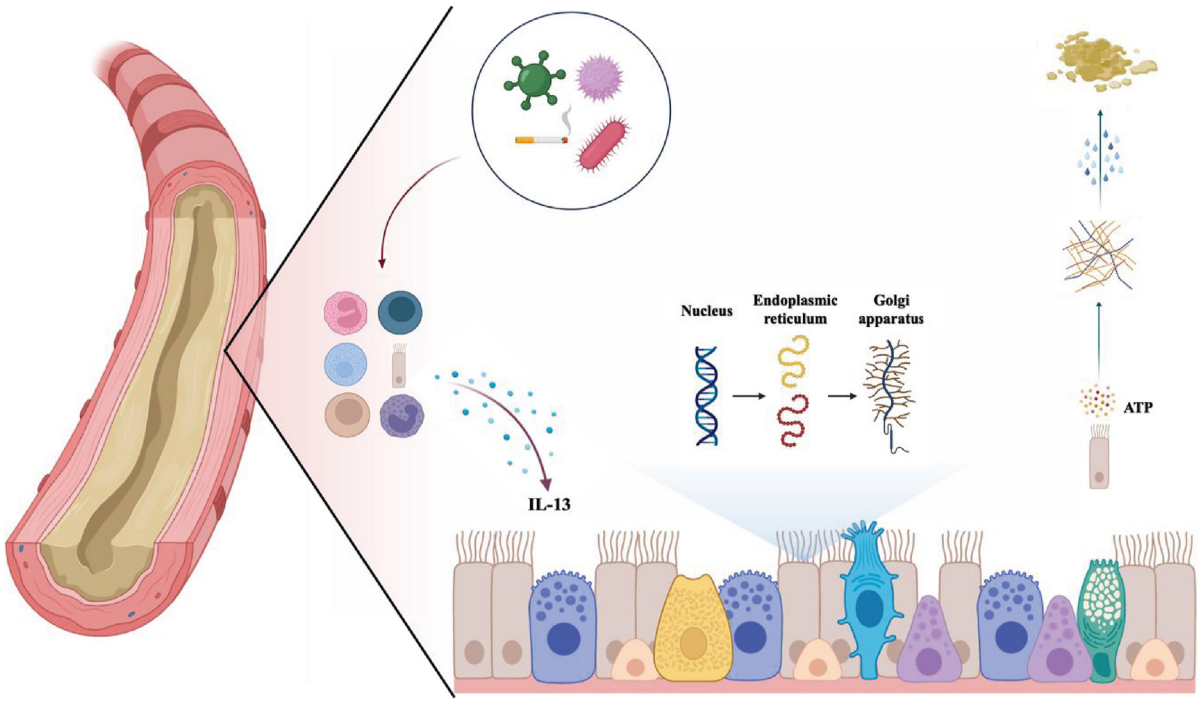

Fig. 1. Production and secretion of mucins by airway epithelial cells. MUC5B and MUC5AC are the main mucins produced by the airway epithelium. The relative proportions of MUC5B and MUC5AC change depending on the state of health, with a predominance of MUC5B and almost no MUC5AC secreted in normal conditions. IL-13 released by CD4 cells, eosinophils, ILC2, mast cells, basophils and epithelial cells stimulate the transcription of mucin proteins by secretory cells (such as goblet and cube cells) from both the superficial epithelium and the submucosal glands. Mucin monomers are dimerized in the endoplasmic reticulum and subsequently polymerized and glycosylated in the Golgi apparatus. Mucin polymers are later stored in granules in a dehydrated form and secreted to the airway lumen in response to ATP and other secretagogues, where they rapidly hydrate and increase their volume dramatically to form the mucus gel layer.

clinical practice, the relationships between mucus and other markers of T2 inflammation such as eosinophils and exhaled nitric oxide in breath (FeNO) and illustrate with three clinical case scenarios our approach in clinical practice to choose biologics or other therapies based on detailed imaging and immunophenotyping.

Mucus biology and clinical relevance

Inflammation plays a pivotal role in the pathophysiology of asthma, and the accumulation and plugging of the airway lumen due to mucus has been recognized as a feature of fatal asthma since the early 20th century.[14] The mucus plugging (MP) can be due to several complex pathophysiologic mechanisms, including triggering its hypersecretion (increased goblet cells), altered mucus composition and hydration affecting the clearance, or impaired clearance due to abnormalities of the mucociliary apparatus or ineffective cough. In asthma, mucus hyperproduction is remarkably frequent even in milder asthmatics, irrespective of treatment,[15] and it is correlated with worse airflow obstruction, uncontrolled airway inflammation, and frequent exacerbations.[15,16] Goblet cell metaplasia and subsequent mucin hypersecretion are typically mediated by T2 cytokines, such as IL-4/IL-13 (through a JAK-1/STAT-6-driven path, and further enhanced by IL-6 and IL-17[17–19]). Severe asthma patients with persistent mucus plugs have elevated T2 inflammation (i.e., IL-4, IL-5, and IL-13), including sputum eosinophils and FeNO.[20] In fact, the IL-4/IL-13-axis is thought to be one of the prime drivers of mucus plugs. Studies in mice have shown that IL-4/IL-13 signalling can induce mucus production even in the absence of IL-5,[21] highlighting the importance of IL-4/IL-13 in patients unresponsive to anti-IL-5 biologics.[22]

MP can be tenacious and persistent within the same airways, causing localized occlusion and atelectasis that could potentially lead to fatal asthma events.[20,23,24] Mucus is particularly relevant in severe forms of asthma, including the ones associated with fungal sensitization, such as allergic bronchopulmonary aspergillosis (ABPA), where it is almost invariably observed, often associated with Charcot Leyden crystals (CLCs).[25] The direct pathophysiologic role of mucus plugging has been confirmed by hyperpolarized gas magnetic resonance imaging (MRI) studies that demonstrated impaired ventilation in the whole lung spatially colocalized with computed tomography (CT)-observed mucus plugs.[26,27]

Pathologic mucus plays a very similar role in COPD, where it is likewise frequent and anatomically persistent both in the short term and at 5 years.[28,29] However, it is not currently known if the immunological processes promoting mucus or the composition of mucus in COPD would be similar to that in patients with asthma. Like asthma, MP in COPD correlates with worse symptom control, higher exacerbation risk, decreased pulmonary function and exercise impairment measured by walk distance.[28,30,31] Moreover, it has been associated with increased mortality in patients with severe COPD undergoing lung volume surgery[32] but also with all-cause mortality in mild to very severe disease, even after adjusting for other mortality-risk factors.[33] Notably, individuals with demonstrated mucus impaction may exhibit scarce mucus-suggestive symptoms such as cough and expectoration.[34] In this setting, the sensitivity of symptoms of chronic mucus hypersecretion as per the ATS/WHO and SGRQ questionnaires has been proven as low as 33% and 34%, respectively, with only moderate specificity of 81% and 75%.[28]

Assessment of mucus in clinical practice

Despite the compelling evidence supporting its role in chronic obstructive diseases, intraluminal mucus is not routinely measured

in clinical practice and remains regrettably unacknowledged in the current management guidelines. This could be perhaps attributed to the historical absence of an effective and non-invasive method to assess intraluminal mucus in vivo. Efforts have been made to adequately evaluate the presence and impact of MP in airway diseases. Both MUC5AC and inducible nitric oxide synthase are partly regulated by STAT6 transcription factor, with IL-13 also signalling through STAT6.[35] Consequently, persistently raised FeNO is likely to indicate IL-13-dependent airway mucus in symptomatic asthmatics,[27] although FeNO (and mucus) may not solely depend on IL-13.[36–39]

The use of tomographic techniques along with direct interventional assessment of the airways using bronchoscopy emerged as a promising method to provide in-vivo real-time information on small airway features. This technique, called optical coherence tomography (OCT), uses near-infrared light to generate cross-sectional images of the airway wall layers up to the 9th generation of bronchi to quantify mucosal, submucosal and luminal area.[40,41] OCT parameters have been shown to correlate with histological findings of extracellular matrix protein deposition in the airway wall,[42] suggesting a potential role in estimating airway mucin content through automatic segmentation algorithms.[43] More recently, Dunican *et al.* developed a non-invasive radiographic instrument aimed at estimating the burden of MP in asthma and other respiratory conditions.[15] This radiographic measurement, called *mucus score*, has been validated as a valuable tool to quantify mucus plugging and to correlate it with multiple clinical and functional features and asthma biomarkers.[20,28] Briefly, CT images are examined for the presence or absence of mucus plugs within each of the 20 pulmonary segments, and a score of 1 or 0 is given accordingly to provide a composite mucus score that ranges between 0 and 20 and thus an estimation of the MP througout the lung. Unfortunately, and despite its many clinical advantages (non-invasive, fairly affordable and available, and requiring no extra technologic resources), the measurement of mucus score remains almost exclusively restricted to research and has not been widely incorporated into regular respiratory practice.

Contribution of eosinophils to airway mucus

While the radiologic scoring system to identify mucus plugging has significantly helped to quantify the extent of airway mucus in severe asthma, it does not capture the components or composition of the mucus plugs. In other words, the identification of intraluminal occlusion does not account for the specific content of the plug, which could be not only mucus but also cellular debris, particularly eosinophilic inflammation.[44]

Airway eosinophils are well-recognized contributors to asthma severity, and their role in the pathophysiology of the disease and as a predictor of response to glucocorticosteroids, described as early as the late 50s by Harry Morrow Brown,[45] has been extensively reviewed.[46–50] The association between eosinophils and intraluminal mucus has been consistently reported,[15,27,51] with a linear correlation between the percentage of sputum eosinophils, MP and more severe airflow obstruction[15] and a positive association between changes in sputum eosinophils and mucus scores.[20] Eosinophils can potentially contribute to mucus hyperproduction and tenacity through several mechanisms, including IL-13 release and subsequent upregulation of MUC5AC transcription[11,52] and active cytolysis leading to liberation of danger-associated molecular patterns (DAMPs) such as galactin-10, high mobility group box 1 (HMGB1), eosinophil peroxidase (EPX) and eosinophil-derived extracellular traps (EET).[53,54] Galactin-10, being periplasmic in location, is released upon eosinophil cytolysis[55] and later crystallizes to form CLCs. CLCs and/or the free/soluble galactin-10, along with the DAMPs, act as danger signals to further promote airway inflammation.[53,55] This is particularly relevant in an airway with autoimmune responses with active autoantibody immune complex mediated EETs.[56,57] Eosinophil extracellular traps[58] and the EPX secreted from eosinophil granules might contribute to the formation of abnormally viscous mucus through increased crosslinking of mucin polymers by oxidative mechanisms acting on thiol groups.[59,60] As such, CLCs can drive mucus by multiple mechanisms, including the promotion of goblet-cell metaplasia, acting as danger signals that elicit T2 and non-T2 responses, and through autoimmune phenomena induced by EET and neutrophil extracellular traps (NETs).[61] Alternative non-eosinophilic mechanisms behind mucus impaction may be hypoxia-induced, where hypoxia-inducing factor 1/2 alpha (HIF1a/HIF2a) increases the epithelial Na^+ channels (ENaC) to create hyper-concentrated mucus but increasing Na+ absorption.[62] Hence, MP results due to mucus hypersecretion and altered physicochemical properties caused by hyper-concentrated mucus, autoimmune responses leading to immune complexes, cellular debris, DAMPs, EETs and CLCs, ultimately affecting airway lumen clearance.

Contribution of airway mucus to eosinophilic inflammation

The interaction between mucus and eosinophils could be bidirectional. As eosinophil-mediated mechanisms can increase mucus production and pathogenicity, mucus presence and composition could enhance eosinophilic inflammation. This holds true particularly in asthma airways, where MUC5AC predominance over MUC5B correlates with higher eosinophil counts, suggesting a role of MUC5AC on eosinophil survival.[63] A possible mechanism behind this observation could be decreased eosinophilic apoptosis related to reduced activation of Siglec-8, an inhibitory receptor expressed on mature eosinophils, in the setting of relative MUC5B deficiency in the airway mucus gel, and the presence of the specific sugar moiety in an individual patient's sputum that could bind to the sialic lectin.[12,64] The increased MUC5AC/MUC5B ratio observed in the presence of eosinophil-released IL-13 in murine and in-vitro models[65] supports that this likely is a redundant inflammatory mechanism. Indeed, persistent mucus plugs are associated with changes in mucin relative proportions with increased MUC5AC mRNA levels and MUC5A/MUC5B ratio.[13,63]

Relative importance of eosinophils and mucus attenuation in asthma control outcomes

Approximately 3–10% of asthmatics experience a severe condition that requires high-intensity treatment, usually in the form of high-dose inhaled corticosteroids (ICS) plus additional controller medication.[66,67] Among these individuals, the use of oral corticosteroids (OCS) is unfortunately very frequent,[68] as these effectively block multiple mechanisms involved in asthma pathogenesis.[69] The role of OCS in asthma control has been traditionally linked to reduced eosinophil activity, which led to the development of eosinophil-targeting therapies aimed at improving asthma outcomes.[70]

Clearing eosinophils using anti-IL-5 antagonists has demonstrated a significant impact on several asthma endpoints, including exacerbation rates, OCS requirements and symptom burden.[71] Similarly, previous data has shown that normalizing sputum eosinophils is likely to attenuate mucins expression and mucus plugs.[27,72] In this context, IL-5R inhibition with benralizumab has been demonstrated to reduce mucus both after a single dose and 2.5 years of treatment,[73,74] which again correlates with overall improved asthma control.

IL4/IL-13 blockade with dupilumab has also been shown to reduce mucus[75,76] and sputum eosinophils.[77] Therefore, it is not surprising that dupilumab also has significant clinical benefits, particularly in severe oral corticosteroid-dependent patients.[78,79] The reduction in sputum eosinophils is not by a direct effect on IL-5 but presumably by a reduction in recruitment into the airways by a reduction in eotaxin-3. Although there is no direct head-to-head comparison with an anti-IL5, the effect on eosinophil reduction is likely to be less than that with direct IL-5 inhibition. This remains to be proven. Nevertheless, the magnitude of clinical benefits in the clinical trials in terms of exacerbation reduction and OCS-sparing seems to be comparable.[78,80] It would appear, therefore, that both eosinophil and mucus reduction are important outcomes in severe asthma treatment.

Cytokine-targeting approach to reduce airway mucus

Mucus plugs can persist despite chronic treatment with corticosteroids and even in well-controlled airway eosinophilia,[39,81] supporting the contribution to other T2 and non-T2 pathways to mucus hypersecretion.[16,26,52,81,82] As such, reduction in airway mucus has become an attractive measurement to evaluate the impact of cytokine-targeting monoclonal antibodies. To date, three prospective clinical trials have reported a significant effect in MP reduction following biologic treatment, which correlated with improvement in diverse clinical and functional outcomes.[74,76,83] As summarized in Table 1, benralizumab (eosinophil-depleting strategy by blocking IL-5 signalling), dupilumab (directly blocking IL-4 and IL-13 signalling) and tezepelumab (by blocking TSLP signalling and thereby attenuating both IL-5 and IL-13 activity and perhaps mast cell activation) can decrease the airway mucus burden measured as the CT-derived mucus score. A clear comparative effect is difficult to estimate given varied study designs, variation in baseline MP and follow-up time, and different reported measures (median vs mean). While a direct comparison between biologics is not yet possible, this data suggests that mucus reduction could be achieved by either targeting eosinophils or other mucus-promoting mechanisms such as IL-13. In this setting, we illustrate the effect of cytokine-targeted treatment on mucus burden in three case scenarios (Table 2A–C), with representative CT and MRI shown in Figure 2–4.

The first case scenario (Table 2A) describes a patient with significant intraluminal defects on CT with intense sputum eosinophilia. He did not respond adequately to the standard approved 100 mg mepolizumab dose, as this was insufficient to clear the eosinophilia in the airway although the blood eosinophil count was normalized. This phenomenon is likely due to the formation of immune complexes between airway IL-5, the anti-IL-5 IgG_1 mepolizumab, and endogenous IgGs in the airway directed against EPX.[84] The immune complexes activate type 2 innate lymphoid cells (ILC2) through danger signals via Death Receptor 3 overexpression.[85] Switching the patient to benralizumab, which depletes both eosinophils and the ILC2 cells expressing IL-5R, led to reduced associated mucus (Fig. 2) and significantly improved pulmonary function and asthma control. The reduction of mucus was likely due to the suppression of IL-13[86] from cellular sources that were IL-5R+.

The second case scenario (Table 2B) illustrates the excellent response to treatment with dupilumab in a patient whose inflammatory and functional parameters indicated a robust IL-4/IL-13-mediated disease. This patient had been previously treated with mepolizumab without optimal response. This is not surprising since the patient had normal sputum eosinophils despite a modestly elevated number of circulating eosinophils. Bronchial thermoplasty was performed since we believed his symptoms were entirely driven by smooth muscle dysfunction, as evidenced by significant bronchodilator reversibility. However, this too did not control his symptoms. ACQ-5 remained over 2.0 with unimproved post-salbutamol change. This, along with elevated FeNO, high sputum levels of IL-4 and IL-13 and significant mucus plugging, predicted response to IL-4R-targeting treatment, which was confirmed by clinical, inflammatory and imaging parameters (Fig. 3).

The third case scenario (Table 2C) represents a non-T2 cytokine-dependent mucus accumulation in the airway. The severe mucus burden associated with moderate sputum eosinophilia and AHR suggested an IL-4/IL-13 mediated mechanism; however, treatment with dupilumab did not result in significant clinical or functional improvement (Fig. 4). Furthermore, the patient continued to deteriorate with worsening FEV_1 and repeated exacerbations in the setting of airway infections. In this context, the sputum cytokines suggested an active infectious component, which along with *Mycobacterium avium* growth in BAL cultures, prompted the initiation of antitubercular treatment. Sputum cytokines measurements showed normal levels of IL-13[87] but very high levels of BAFF, IL-1B, and IL-18, all consistent with an innate immune response to infection or an inflammasome activation. Her remarkable response with normalization of pulmonary function with antitubercular treatment and sputum clearance strategies confirmed the infectious mechanism as the predominant contributor to her airway mucus and not a T2-dependent process, although she had modest airway eosinophilia. This case highlights the profound complexity and interaction of mechanisms behind mucus plugging, where the intraluminal plugs could be driven by a combination of multiple cytokine pathways (either T2 or non-T2), inflammasome, NETs, etc. Identifying the optimal therapeutical strategy could be exceptionally challenging in these scenarios, as complete responses are unlikely to be achieved by targeting a sole inflammatory mechanism.

Table 1
Effectiveness of asthma biologics in reducing mucus plugging.

	Benralizumab[74]	Dupilumab[76]	Tezepelumab[83]
Study design	Longitudinal OL	RCT	RCT
Sample size, n	12	13	37
Follow-up time	2.5 years	16 weeks	28 weeks
Change in CT–MS	−1.0 (95% CI −5.2 to −0.6)	−4 (95% CI −7 to −1)	−1.76 ± 2.6[†]

OL, open label; RCT, randomized clinical trial; CT–MS, computed tomography mucus score.
[†] Reported as mean ± SD.

Evaluation and management of airway mucus: our strategy

Figure 5 illustrates our local strategy to assess mucus in asthma and other respiratory conditions. This approach focuses on investigating the airway immunology, the mucus plugs itself, its composition and physicochemical properties to know (i) if there is excess mucus being produced and why, (ii) if mucus composition is changed, making it too thick to be cleared, or (iii) both. In addition, we explore the structural and functional consequences of mucus impaction through novel lung imaging, as described below.

We first approach the inflammatory component of mucus by collecting sputum samples to evaluate the presence and type of cellular inflammation (eosinophilic, neutrophilic, mixed or paucigranulocytic[88]). Cultures (including bacterial and fungal) are concomitantly requested to complete the infectious workup. Sputum is further analysed for free eosinophil granules, EPX, CLCs and EETs to evaluate cell-activity indices and cytolysis. Processed cell-free supernatant is examined for T2 (IL-4, IL-5, IL-13, IL-33,

Table 2A
Clinical history and indices of response: Patient 1: Mucus driven by IL-5: Response to Benralizumab.

Clinical presentation	58 M, former smoker (35 pack-year history), nonatopic. Severe asthma with moderate airflow obstruction and recurrent eosinophilic exacerbations and 3–4 courses of prednisone per year.		
Relevant investigations	Peak blood eosinophils: 1.0 × 10^9/L; peak sputum eosinophils 26.5%; peak FeNO: 75 ppb; maximal observed FEV_1 reversibility: 0.24 L (15%).		
Prior treatments	Mepolizumab (5 months): recurrent eosinophilic exacerbations (9.8% sputum eosinophils while on treatment).		
Cytokines in sputum	IL-5		
Intervention	Benralizumab		
Interval progress	Parameter	Baseline	Post-treatment[†]
	ACQ-5	3.8	0.8
	AAER	3	0
	FEV_1, L (%)	1.32 (36)	2.76 (76)
	pBDR, L (%)	NA	NA
	Blood eos, ×10^9/L	0.3	0
	Sputum eosinophils, %	26.5	0
	FeNO, ppb	75	33
	HRCT	Multiple centrilobular and tree-in-bud nodules in bilateral lower lobes with peribronchial wall thickening and diffuse mucus plugging. MS = 5	Mild mucous plugging in the bilateral lower lung lobes without significant bronchiectasis. MS = 3

FeNO, Fraction of exhale nitric oxide; FEV1, forced expiratory volume in 1 s; ACQ-5, Asthma control questionnaire; AAER, Annualised asthma exacerbation rate; pBDR, Post-bronchodilator reversibility; HRCT, High-resolution computed tomography; MS, Mucus Score.
[†] 1 year.

TSLP), T-17 (IL-17, TNFa) and non-T2/non-IL-13 (IL-12p70, IFNγ, BAFF, IL-6) cytokines (Ella™, R&D Systems) and for ANAs and autoimmune markers[56] to identify specific inflammatory signatures. Additional assessments performed through our research laboratory can provide a further understanding of the mucus-specific characteristics. For instance, the biophysical mucus properties, such as critical stress and strain, viscosity, and elasticity, can be assessed by the Rheomuco® rheometer (Rheonova, Gières, France), and MUC5B and MUC5AC expression can also be measured for mucin content and composition. If patients have mucus impaction associated with recurrent airway infections and sputum neutrophilia, as illustrated in the third case scenario, we investigate for immunodeficiencies by performing whole exome sequencing for immunodeficiency genes (including those for cystic fibrosis and primary ciliary dyskinesia). Approximately 6% of our severe asthmatic population and 11% of the sub-group with recurrent airway

Table 2B
Clinical history and indices of response: Patient 2: Mucus driven by IL-13: Response to Dupilumab.

Clinical presentation	40 M, early-onset asthma with progressive worsening in adulthood, former smoker (9 pack-year), non-atopic, pauci granulocytic sputum with isolated episodes of neutrophilic bronchitis, recurrent exacerbations despite daily treatment with fluticasone 1000 mcg, formoterol 20 mcg, tiotropium 5 mcg, prednisone 5 mg and montelukast 10 mg.		
Relevant Investigations	Peak blood eosinophils: 0.8 × 10^9/L, peak sputum eosinophils 2%, peak FeNO; 93 ppb, maximal observed FEV_1 reversibility: 0.9 L (43%).		
Prior treatments	Mepolizumab (3 years): persistent baseline symptoms, recurrent non-eosinophilic exacerbations, persistent post-bronchodilator reversibility Bronchial thermoplasty (3 sessions): initial improvement in baseline symptoms however subsequent frequent exacerbations and persistent post-bronchodilator reversibility.		
Cytokines in sputum	IL-4, IL-18		
Intervention	Dupilumab		
Interval progress	Parameter	Baseline	Post-treatment[†]
	ACQ-5	4.4	1.8
	FEV_1, L(%)	1.54(37)	2.44(59)
	pBDR, L(%)	0.65(42)	0.57(23)
	Blood eos, ×10^9/L	0.3	0.1
	Sputum eosinophils, %	1.5	0.5
	FeNO, ppb	55	13
	HRCT	Lobar to subsegmental wall thickening, and multifocal mucus plugging. MS = 13	Improved bronchial wall thickening and mucus plugging. MS = 3

FeNO, Fraction of exhale nitric oxide; FEV1, forced expiratory volume in 1 s; ACQ-5, Asthma control questionnaire; AAER, annualised asthma exacerbation rate; pBDR, post-bronchodilator reversibility; HRCT, high-resolution computed tomography; MS, Mucus Score.
[†] 16 weeks.

Table 2C
Clinical history and indices of response: Patient 3: Mucus not driven by IL-13, no response to Dupilumab.

Clinical presentation	54F, early-onset asthma, never smoker, allergic rhinitis with aspergillus sensitization (total IgE 217 kU/L, Aspergillus IgE 1.18 kU/L), moderate airway hyperresponsiveness, recurrent mixed eosinophilic and neutrophilic bronchitis (peak sputum eosinophils 3%), mild cylindrical bronchiectasis. Multiple airway infections with demonstrated growth of *Aspergillus fumigatus*, *Moraxella catarrhalis*, and *Pasteurella multocida* in sputum and *Mycobacterium avium* in sputum and BAL.		
Relevant investigations	Peak blood eosinophils: 0.9 × 10^9/L; peak sputum eosinophils 9%; peak FeNO: 10 ppb; PC_{20} methacholine 0.5 mg/ml.		
Prior treatments	Omalizumab: 5 months, discontinued due to uncontrolled symptoms. Dupilumab: 4 months, discontinue due to uncontrolled symptoms and decline in pulmonary function.		
Cytokines in sputum	IL-1B, IL-18, BAFF		
Intervention	Antitubercular treatment (azithromycin, rifampin, and ethambutol) in addition to regular inhalers (ciclesonide 400mcg, olodaterol 5mcg, tiotropium 5mcg), sputum clearance with hypertonic saline and Aerobika positive pressure device		
Interval progress	Parameter	Baseline	Post-treatment[†]
	ACQ-5	2.8	0.6
	FEV_1, L(%)	0.92(33)	2.03(74)
	pBDR, L(%)	NA	NA
	Blood eos, x10^9/L	0.3	0.2
	Sputum total cell count and differential, 10^6/g cells (%neutrophils)	72.7 (97)	17.4 (88.3)
	FeNO, ppb	10	NA
	HRCT	Multiple diffuse bilateral groundglass and nodular opacities with tree-in-bud distribution	Significant improvement in the severity of mucus plugging and tree-in-bud nodularity.
Specialized investigations	Primary immunodeficiency genetic panel revealed an heterozygous mutation in two genes related with agammaglobulinemia (c.419C>A, p.(Thr140Asn)), and ciliary dyskinesia (c.880G>A, p.(Glu294Lys)). Ciliary motility test showed beat frequency reduced to 42% compared to normal.		

BAL, bronchoalveolar lavage; FeNO, Fraction of exhale nitric oxide; FEV1, forced expiratory volume in 1 s; ACQ-5, Asthma control questionnaire; AAER, Annualised asthma exacerbation rate; pBDR, Post-bronchodilator reversibility; HRCT, High-resolution computed tomography; MS, Mucus Score.
[†] 16 months.

infections are heterozygous for gene mutations associated with proteins of the microtubular structure.[89]

To complement these inflammatory and biophysical markers, the structural and functional consequences of mucus impaction can be evaluated by advanced lung imaging. As described above, MP can be quantified by CT to detect segmental and subsegmental mucus plugs and provide a mucus score that helps to estimate the burden of airway mucus. This scoring system does not take into account additional plugs characteristics such as size, density or localization that are likely to have a clinical and functional impact, however it has proven great utility when correlating with asthma inflammatory features and severity.[15,20,28] To address the above-mentioned issues and better characterize the airway mucus and its correlation with asthma severity parameters, a thorough method using whole-lung mucus annotation, segmentation and subsequent 3D-reconstruct mucus plugs has been recently developed.[90] Mucus plugs can thus be characterized in terms of shape, size, volume, density, location and possible contribution to airflow obstruction, providing a more detailed understanding of their impact on airway physiology.

As quantitative CT is able to provide structural and spatial information on mucus plugs, its functional impact may be evaluated by direct visualization of ventilation using hyperpolarized ^{129}Xe MRI[27,91] (as shown in Fig. 3, 4). Briefly, this technique involves delivery of an inhaled contrast agent, most commonly hyperpolarized ^{129}Xe, and images are subsequently obtained under breath-hold conditions (static ventilation imaging) or within the respiratory cycle (dynamic ventilation imaging).[92] Acquired images permit the evaluation of ventilation heterogeneity through the visualization of gas distribution (commonly quantified as the ventilation defect percent, or VDP).[93]

The integration of all these parameters offers a deeper understanding of the specific contribution of mucus to asthma severity, helping in the selection of therapeutic strategies guided by specific molecular, cellular, biophysical, structural and functional markers. This approach has been proven effective in improving overall asthma care, reducing ER visits and hospital admissions due to exacerbations, and ameliorating annual costs compared to standard care.[94]

Integration of sputum eosinophil and airway mucus assessments to choose biologics

There are no clinical trials that have evaluated the initial choice of biologics in patients with severe asthma with predominant eosinophilic disease versus muco-obstructive lung disease (MOLD). As we have discussed above, targeting both IL-5 and IL-4R reduces both eosinophils and mucus. Our current strategy, based on clinical experience of treating over 200 severe prednisone-dependent patients with asthma, is illustrated in Figure 6. Central to this clinical algorithm is determining the predominant luminal contributor to airflow obstruction and asthma symptoms. If the dominant pathology is eosinophils (more than 15–20% in sputum), we use an anti-IL-5 (ligand or receptor) as first-line drug. These patients contribute to approximately 40% of our clinic population. If the predominant contributor to obstruction is mucus (assessed by airway CT mucus scores), even when associated with sputum eosinophils (less than 15%), we now use dupilumab as the first line biologic since we have demonstrated that dupilumab can also decrease sputum eosinophils.[77] Some of them may also respond to anti-TSLP biologic (Tezepelumab), however this requires further investigation. A small proportion of patients (approximately 5%) require concurrent treatment with anti-IL-5 and anti-IL-4R.[80] This algorithm needs to be tested prospectively in clinical trials. There are two additional groups of patients who are the most difficult to treat. The first group includes patients with multifactorial causes of mucus (e.g., associated with eosinophils, neutrophils degranulation and debris in the setting of recurrent airway infections). In this

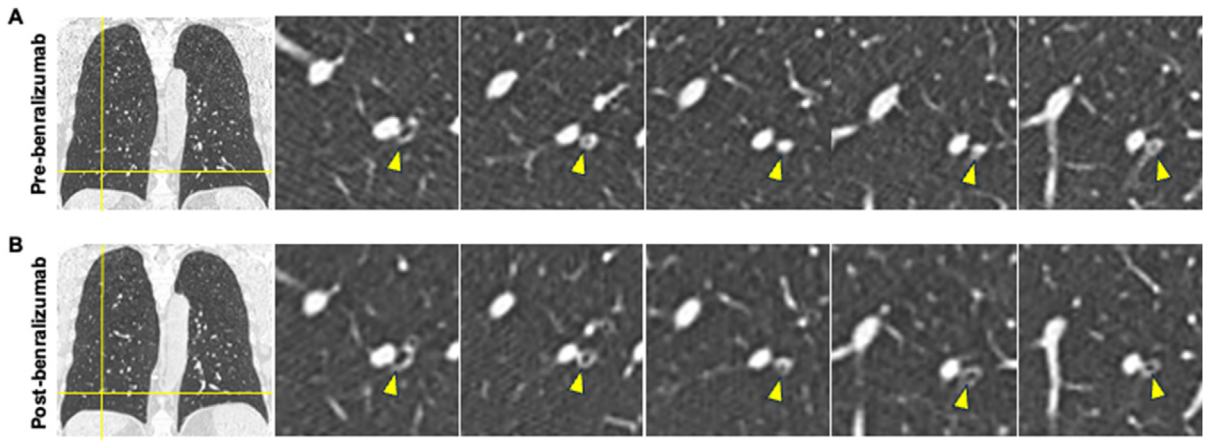

Fig. 2. CT images of mucus before and after treatment with benralizumab. **Panels A** and **B** show sequential axial images through the left lower lobe of a patient before and after treatment with benralizumab. In **Panel A**, the yellow arrows indicate complete opacification of a subsegmental airway developed in sequential imaging of the RB9 segment. In **Panel B**, the yellow arrows correspond to the same images as in **A**, revealing a complete resolution of the mucus plug at 1-year follow-up imaging.

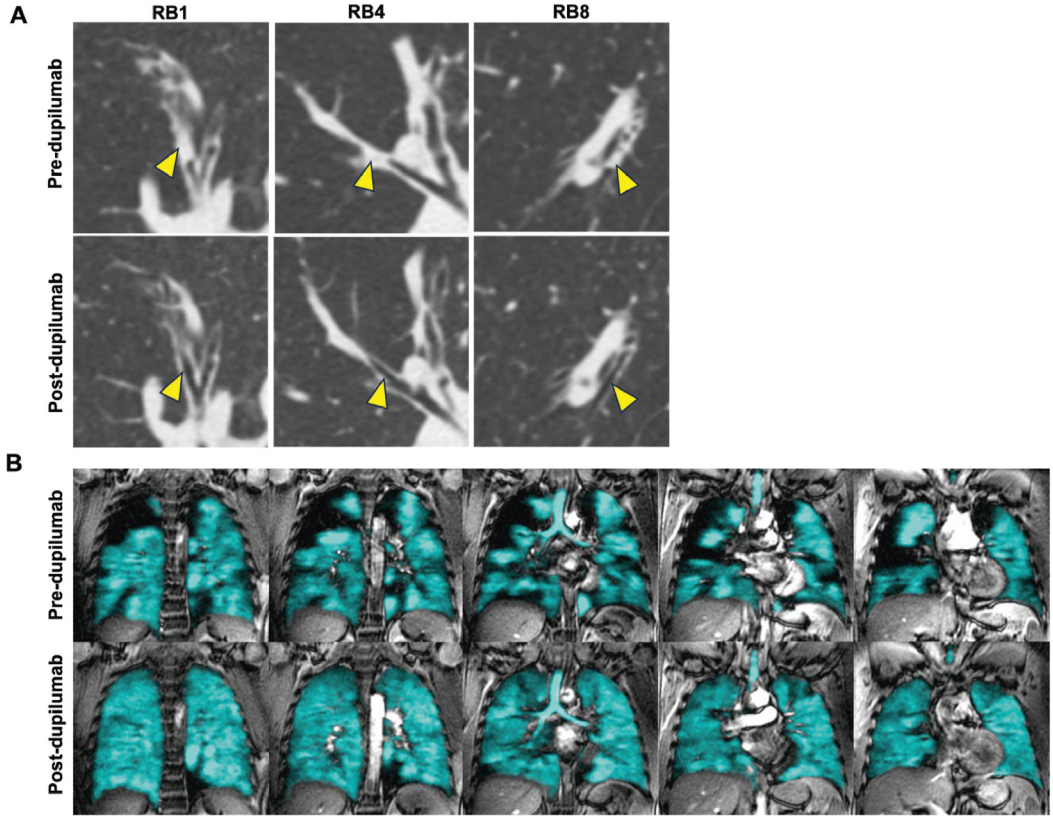

Fig. 3. CT images of mucus and Xe^{129} MRI images of ventilation before and after treatment with dupilumab. **Panel A** shows the change in mucus plugs in coronal (RB1 and RB8) and axial (RB4) planes of CT imaging performed in a 40-year-old male between baseline and after 16 weeks of treatment with dupilumab. The yellow arrows in the superior panel indicate complete intraluminal occlusion corresponding with mucus plugs, which were resolved after the administration of dupilumab (inferior panel). **Panel B** shows the change in ^{129}Xe ventilation MRI (cyan), with a significant reduction in ventilation defect percentage (VDP) from 13% (superior panel) to 3% (inferior panel), matching with the remarkable reduction in mucus burden (CT mucus score from 13 to 3).

scenario, identifying the reasons for susceptibility to infections, including primary and secondary immunodeficiency disorders, is crucial before considering a biologic. The second refers to patients whose asthma is driven entirely by severe airway hyperresponsiveness not associated with luminal cells or mucus impaction. In these subjects, we consider treatment with bronchial thermoplasty.

Unmet needs and future research

While tremendous progress has been made in the understanding of mucus biology and production mechanisms, therapies to clear mucus in asthma and COPD have shown only limited effectiveness so far. Among conventional non-specific treatments, mucus hydration, anticholinergics, mechano-physical clearance

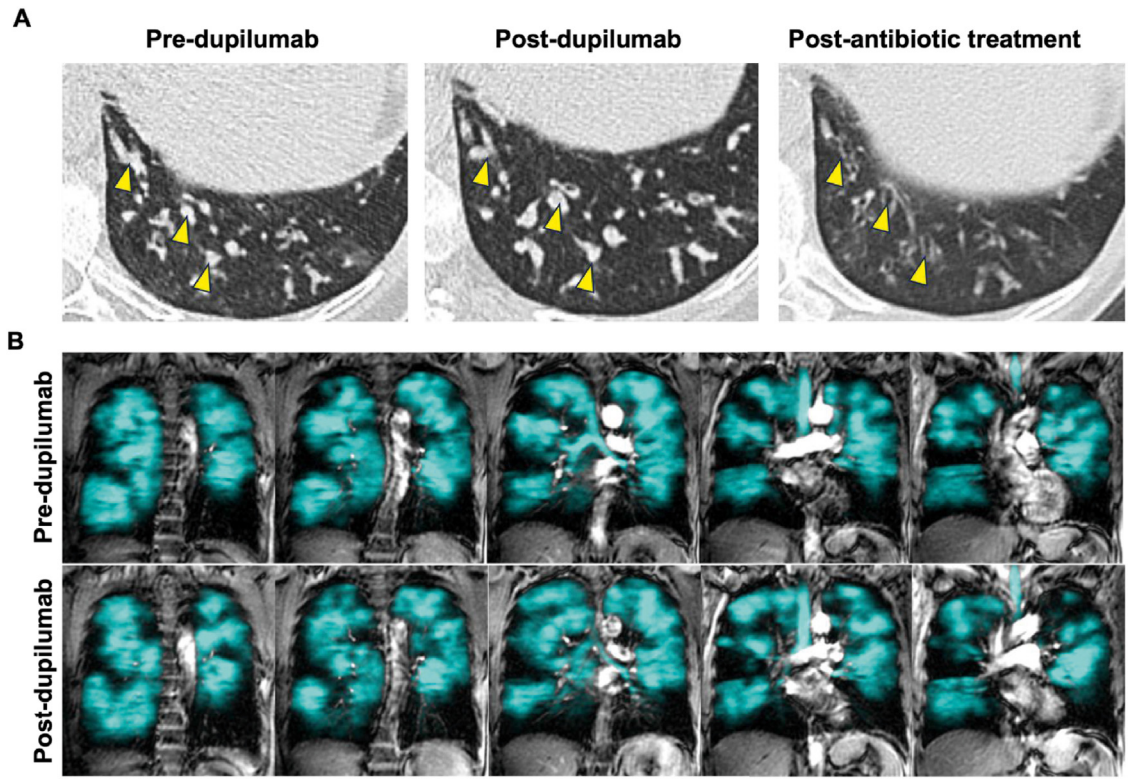

Fig. 4. CT and MRI images of a patient with airway mucus who did not respond to dupilumab. **Panel A** shows three CT axial planes acquired before and after treatment with dupilumab (first and second images from left to right), and after prolonged antibiotic treatment (image on the right). The yellow arrows indicate the persistence of severe mucus plugging despite administration of an anti-IL4/IL-13-targeting biologic, suggesting that the significant burden of airway mucus was not driven by this mechanism. This finding correlated with the severe ventilation defects observed by ^{129}Xe MRI (**Panel B**), which were similarly unchanged between baseline and the end of treatment with dupilumab. A follow-up lung MRI correlating with the marked improvement in CT-mucus scores has yet to be acquired.

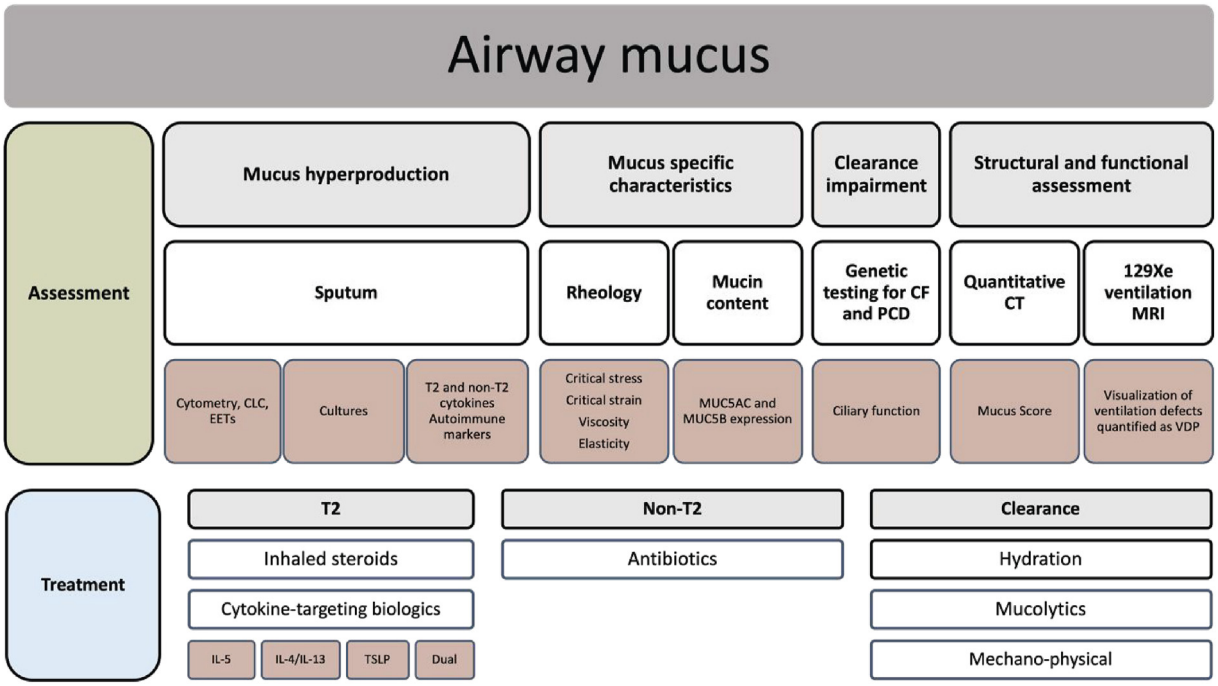

Fig. 5. Clinical algorithm to assess and treat airway mucus. Summary of our local strategy that includes a thorough assessment of inflammatory components in the sputum (i.e., cellular predominance, predominant cytokine signature and autoimmune responses), as well as mucus characterization (such as mucins relative content and rheology parameters), assessment for causes of clearance impairment and structural and functional evaluation through novel lung imaging. The selection of therapeutic strategies, such as cytokine-targeting monoclonal antibodies or optimization of mucus clearance, is subsequently based on this evaluation.

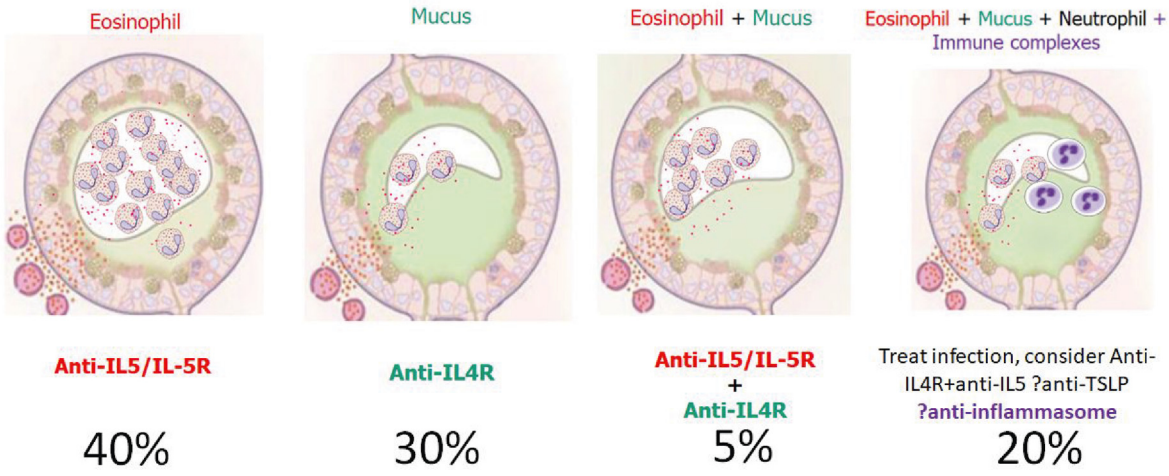

Fig. 6. A scheme to choose biologics depending on predominant luminal inflammatory process (eosinophils, neutrophils or mucus).

techniques and even mucolytics to break down the disulfide bonds that held the mucin glycopolymers have demonstrated only modest efficacy or clinical benefits.[95–97] Similarly, targeting mucin production mechanisms alone (either with anti-IL13, anti-TSLP or other cytokine antagonists) as a single therapeutic strategy is likely insufficient to achieve complete mucus clearance. There might be patients who require simultaneous targeting of two cytokines, for example, TSLP and IL-13, using new nanobodies that are being developed.[98] Development of reliable assays (whether at a protein level or a gene level) to indicate the dominant cytokine or cellular pathways (e.g. TSLP or mast cell) would be welcome to guide the appropriate therapy. Similarly, better mucolytics with high disulfide disruption capacity without requiring high concentrations causing secondary effects such as bronchospasm might represent an attractive option to treat mucus. Blocking excessive hypoxia-induced ENaC activity by delivering antagonists by the aerosolized route would be another strategy to improve hydration[99] while facilitating increased chloride transport into the airway through the CFTR modulators.[100] Thus far, the effect of such molecules has been reported only in preclinical scenarios or for the indication of CF, and their utility in other chronic airway diseases remains to be seen.[101,102]

Conclusions

Mucus has been long recognized as a crucial feature in asthma and other chronic respiratory conditions and contributes to disease severity through worsening airflow obstruction that is associated with adverse clinical outcomes and increased mortality. Unfortunately, its clinical and functional consequences remain largely unappreciated and untreated in guideline-based respiratory practice. Eosinophils, among other inflammatory agents, contribute to mucus hyperproduction through IL-13 and non-IL-13 mediated pathways, with a complex bidirectional interaction where mucus is, in turn, able to promote eosinophilic survival and inflammation. A comprehensive and multimodal approach to mucus hyperproduction and mucus clearance impairment is fundamental in order to reduce mucus burden in asthmatic patients. Several strategies, including those targeting specific inflammatory components of mucus, have been developed to address and treat mucus plugging. In this review, we propose an algorithm for evaluating and managing airway mucus in severe asthma, with an emphasis on a thorough assessment of the responsible inflammatory mechanisms, mucus characterization, and evaluation of functional consequences. Further research is required to better understand the mechanisms behind mucus dysfunction in asthma and other respiratory conditions and identify effective therapeutic strategies for mucus attenuation in these patients.

Conflict of interest

CVG reports personal fees from AstraZeneca and GlaxoSmithKline, outside the submitted work. PN reports grants from AstraZeneca, Teva, Roche, Novartis, Sanofi, Cyclomedica, and Foresee, and personal fees from AstraZeneca, Teva, Roche, GlaxoSmithKline, Novartis, Arrowhead Pharmaceuticals, Merck and Equillium, outside the submitted work. MM reports grants from CIHR, CAAIF, Methapharm Specialty Pharmaceuticals, Sanofi, and personal fees from AstraZeneca, Novartis, JP Innova, and GlaxoSmithKline, outside the submitted work. SS reports grants from Cyclomedica and personal fees from AstraZeneca, Novartis, Polarean, and Arrowhead Pharmaceuticals, outside the submitted work.

References

1. Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med* 2010;**363**:2233–47.
2. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009;**61**:75–85.
3. Thornton DJ, Rousseau K, McGuckin MA. Structure and function of the polymeric mucins in airways mucus. *Annu Rev Physiol* 2008;**70**:459–86.
4. Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 2006;**86**:245–78.
5. Hill DB, Button B, Rubinstein M, Boucher RC. Physiology and pathophysiology of human airway mucus. *Physiol Rev* 2022;**102**:1757–836.
6. Benam KH, Vladar EK, Janssen WJ, Evans CM. Mucociliary defense: emerging cellular, molecular, and animal models. *Ann Am Thorac Soc* 2018;**15**(Suppl 3): S210–5.
7. Adler KB, Li Y. Airway epithelium and mucus: intracellular signaling pathways for gene expression and secretion. *Am J Respir Cell Mol Biol* 2001;**25**: 397–400.
8. Button B, Okada SF, Frederick CB, Thelin WR, Boucher RC. Mechanosensitive ATP release maintains proper mucus hydration of airways. *Sci Signal* 2013;**6**: ra46.
9. Okuda K, Chen G, Subramani DB, Wolf M, Gilmore RC, Kato T, et al. Localization of secretory mucins MUC5AC and MUC5B in normal/healthy human airways. *Am J Respir Crit Care Med* 2019;**199**:715–27.
10. Evans CM, Williams OW, Tuvim MJ, Nigam R, Mixides GP, Blackburn MR, et al. Mucin is produced by clara cells in the proximal airways of antigen-challenged mice. *Am J Respir Cell Mol Biol* 2004;**31**:382–94.
11. Kanoh S, Tanabe T, Rubin BK. IL-13-induced MUC5AC production and goblet cell differentiation is steroid resistant in human airway cells. *Clin Exp Allergy* 2011;**41**:1747–56.
12. Kiwamoto T, Katoh T, Evans CM, Janssen WJ, Brummet ME, Hudson SA, et al. Endogenous airway mucins carry glycans that bind Siglec-F and induce eosinophil apoptosis. *J Allergy Clin Immunol* 2015;**135**:1329–40.e9.
13. Bonser LR, Erle DJ. Airway mucus and asthma: the role of MUC5AC and MUC5B. *J Clin Med* 2017;**6**:112.
14. Dunnill MS. The pathology of asthma, with special reference to changes in the bronchial mucosa. *J Clin Pathol* 1960;**13**:27–33.

15. Dunican EM, Elicker BM, Gierada DS, Nagle SK, Schiebler ML, Newell JD, et al. Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. *J Clin Investig* 2018;**128**:997–1009.
16. Chan R, Duraikannu C, Lipworth B. Clinical associations of mucus plugging in moderate to severe asthma. *J Allergy Clin Immunol Pract* 2023;**11**:195–199.e2.
17. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;**282**:2258–61.
18. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003;**278**:17036–43.
19. Gorrieri G, Scudieri P, Caci E, Schiavon M, Tomati V, Sirci F, et al. Goblet cell hyperplasia requires high bicarbonate transport to support mucin release. *Sci Rep* 2016;**6**:36016.
20. Tang M, Elicker BM, Henry T, Gierada DS, Schiebler ML, Huang BK, et al. Mucus plugs persist in asthma, and changes in mucus plugs associate with changes in airflow over time. *Am J Respir Crit Care Med* 2022;**205**:1036–45.
21. Whittaker L, Niu N, Temann UA, Stoddard A, Flavell RA, Ray A, et al. Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and interleukin-9. *Am J Respir Cell Mol Biol* 2002;**27**:593–602.
22. Salter B, Lacy P, Mukherjee M. Biologics in asthma: a molecular perspective to precision medicine. *Front Pharmacol* 2022;**12**:793409.
23. Reid LM. The presence or absence of bronchial mucus in fatal asthma. *J Allergy Clin Immunol* 1987;**80**(3 Pt 2):415–6.
24. Hays SR, Fahy JV. The role of mucus in fatal asthma. *Am J Med* 2003;**115**:68–9.
25. Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D. Chest radiographic and computed tomographic manifestations in allergic bronchopulmonary aspergillosis. *World J Radiol* 2012;**4**:141–50.
26. Mummy DG, Dunican EM, Carey KJ, Evans MD, Elicker BM, Newell JD, et al. Mucus plugs in asthma at CT associated with regional ventilation defects at ^{3}He MRI. *Radiology* 2022;**303**:184–90.
27. Svenningsen S, Haider E, Boylan C, Mukherjee M, Eddy RL, Capaldi DPI, et al. CT and functional MRI to evaluate airway mucus in severe asthma. *Chest* 2019;**155**:1178–89.
28. Dunican EM, Elicker BM, Henry T, Gierada DS, Schiebler ML, Anderson W, et al. Mucus plugs and emphysema in the pathophysiology of airflow obstruction and hypoxemia in smokers. *Am J Respir Crit Care Med* 2021;**203**:957–68.
29. Okajima Y, Come CE, Nardelli P, Sonavane SK, Yen A, Nath HP, et al. Luminal plugging on chest CT scan: association with lung function, quality of life, and COPD clinical phenotypes. *Chest* 2020;**158**:121–30.
30. Mettler SK, Nath HP, Grumley S, Orejas JL, Dolliver WR, Nardelli P, et al. Silent airway mucus plugs in COPD and clinical Implications. *Chest* 2023. https://doi.org/10.1016/j.chest.2023.11.033.
31. Kim V, Dolliver WR, Nath HP, Grumley SA, Terry N, Ahmed A, et al. Mucus plugging on computed tomography and chronic bronchitis in chronic obstructive pulmonary disease. *Respir Res* 2021;**22**:110.
32. Hogg JC, Chu FS, Tan WC, Sin DD, Patel SA, Pare PD, et al. Survival after lung volume reduction in chronic obstructive pulmonary disease: insights from small airway pathology. *Am Respir Crit Care Med* 2007 Sep 1;**176**(5):454–9.
33. Diaz AA, Orejas JL, Grumley S, Nath HP, Wang W, Dolliver WR, et al. Airway-occluding mucus plugs and mortality in patients with chronic obstructive pulmonary disease. *JAMA* 2023;**329**:1832–9.
34. Burgel PR, Martin C. Mucus hypersecretion in COPD: should we only rely on symptoms? *Eur Respir Rev* 2010;**19**:94–6.
35. Cohn L. Mucus in chronic airway diseases: sorting out the sticky details. *J Clin Investig* 2006;**116**:306–8.
36. Theofani E, Semitekolou M, Morianos I, Samitas K, Xanthou G. Targeting NLRP3 inflammasome activation in severe asthma. *J Clin Med* 2019;**8**:1615.
37. Hu X, Shen Y, Zhao Y, Wang J, Zhang X, Tu W, et al. Epithelial aryl hydrocarbon receptor protects from mucus production by inhibiting ROS-triggered NLRP3 inflammasome in asthma. *Front Immunol* 2021;**12**:767508.
38. Shao MX, Nadel JA. Neutrophil elastase induces MUC5AC mucin production in human airway epithelial cells via a cascade involving protein kinase C, reactive oxygen species, and TNF-alpha-converting enzyme. *J Immunol* 2005;**175**:4009–16.
39. Mukherjee M, Huang C, Venegas-Garrido C, Zhang K, Bhalla A, Ju X, et al. Benralizumab normalizes sputum eosinophilia in severe asthma uncontrolled by anti-IL-5 antibodies: a single-blind, placebo-controlled clinical trial. *Am J Respir Crit Care Med* 2023;**208**:1330–5.
40. d'Hooghe JNS, Goorsenberg AWM, de Bruin DM, Roelofs JJTH, Annema JT, Bonta PI. Optical coherence tomography for identification and quantification of human airway wall layers. *PLoS One* 2017;**12**:e0184145.
41. Ding M, Chen Y, Guan WJ, Zhong CH, Jiang M, Luo WZ, et al. Measuring airway remodeling in patients with different COPD staging using endobronchial optical coherence tomography. *Chest* 2016;**150**:1281–90.
42. Carpaij OA, Goorsenberg AWM, d'Hooghe JNS, de Bruin DM, van den Elzen RM, Nawijn MC, et al. Optical coherence tomography intensity correlates with extracellular matrix components in the airway wall. *Am J Respir Crit Care Med* 2020;**202**:762–6.
43. Adams DC, Pahlevaninezhad H, Szabari MV, Cho JL, Hamilos DL, Kesimer M, et al. Automated segmentation and quantification of airway mucus with endobronchial optical coherence tomography. *Biomed Opt Express* 2017;**8**:4729–41.
44. Svenningsen S, Nair P. Persistent airway plugs: a call for clinical recognition and novel therapies. *Am J Respir Crit Care Med* 2022;**205**:977–8.
45. Brown HM. Treatment of chronic asthma with prednisolone; significance of eosinophils in the sputum. *Lancet* 1958;**2**:1245–7.
46. Hargreave FE, Nair P. The definition and diagnosis of asthma. *Clin Exp Allergy* 2009;**39**:1652–8.
47. Schleich FN, Louis R. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014;**44**:1098–9.
48. Lambrecht BN, Hammad H. The immunology of asthma. *Nat Immunol* 2015;**16**:45–56.
49. Aleman F, Lim HF, Nair P. Eosinophilic endotype of asthma. *Immunol Allergy Clin N Am* 2016;**36**:559–68.
50. McBrien CN, Menzies-Gow A. The biology of eosinophils and their role in asthma. *Front Med* 2017;**4**:93.
51. Hearn AP, Mak MS, Budaj I, Qurashi N, Snell O, Dhariwal J, et al. The prevalence of mucus plugging in severe eosinophilic asthma and its relationship to clinical efficacy of anti-IL-5R treatment. *J Allergy Clin Immunol Pract* 2022;**10**:1102–3.e1.
52. Yu H, Li Q, Kolosov VP, Perelman JM, Zhou X. Interleukin-13 induces mucin 5AC production involving STAT6/SPDEF in human airway epithelial cells. *Cell Commun Adhes* 2010;**17**:83–92.
53. Ueki S, Miyabe Y, Yamamoto Y, Fukuchi M, Hirokawa M, Spencer LA, et al. Charcot-leyden crystals in eosinophilic inflammation: active cytolysis leads to crystal formation. *Curr Allergy Asthma Rep* 2019;**19**:35.
54. Persson C, Uller L. Theirs but to die and do: primary lysis of eosinophils and free eosinophil granules in asthma. *Am J Respir Crit Care Med* 2014;**189**:628–33.
55. Melo RCN, Wang H, Silva TP, Imoto Y, Fujieda S, Fukuchi M, et al. Galectin-10, the protein that forms Charcot-Leyden crystals, is not stored in granules but resides in the peripheral cytoplasm of human eosinophils. *J Leukoc Biol* 2020;**108**:139–49.
56. Mukherjee M, Bulir DC, Radford K, Kjarsgaard M, Huang CM, Jacobsen EA, et al. Sputum autoantibodies in patients with severe eosinophilic asthma. *J Allergy Clin Immunol* 2018;**141**:1269–79.
57. Mukherjee M, Lacy P, Ueki S. Eosinophil extracellular traps and inflammatory pathologies-untangling the web. *Front Immunol* 2018;**9**:2763.
58. Omokawa A, Ueki S, Kikuchi Y, Takeda M, Asano M, Sato K, et al. Mucus plugging in allergic bronchopulmonary aspergillosis: implication of the eosinophil DNA traps. *Allergol Intl* 2018;**67**:280–2.
59. Arlandson M, Decker T, Roongta VA, Bonilla L, Mayo KH, MacPherson JC, et al. Eosinophil peroxidase oxidation of thiocyanate. Characterization of major reaction products and a potential sulfhydryl-targeted cytotoxicity system. *J Biol Chem* 2001;**276**:215–24.
60. Yuan S, Hollinger M, Lachowicz-Scroggins ME, Kerr SC, Dunican EM, Daniel BM, et al. Oxidation increases mucin polymer cross-links to stiffen airway mucus gels. *Sci Transl Med* 2015;**7**:276ra27.
61. Persson EK, Verstraete K, Heyndrickx I, Gevaert E, Aegerter H, Percier JM, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science* 2019 May;**364**(6442):eaaw4295.
62. Mikami Y, Grubb BR, Rogers TD, Dang H, Kota P, Gilmore RC, et al. Airway obstruction produces hypoxia-dependent sodium absortion in human airway epithelial cells. *J Cyst Fibros* 2021;**20**:S173–4.
63. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, et al. Abnormalities in MUC5AC and MUC5B protein in airway mucus in asthma. *Am J Respir Crit Care Med* 2016;**194**:1296–9.
64. Youngblood BA, Brock EC, Leung J, Falahati R, Bryce PJ, Bright J, et al. AK002, a humanized sialic acid-binding immunoglobulin-like lectin-8 antibody that induces antibody-dependent cell-mediated cytotoxicity against human eosinophils and inhibits mast cell-mediated anaphylaxis in mice. *Int Arch Allergy Immunol* 2019;**180**:91–102.
65. Evans CM, Raclawska DS, Ttofali F, Liptzin DR, Fletcher AA, Harper DN, et al. The polymeric mucin Muc5ac is required for allergic airway hyperreactivity. *Nat Commun* 2015;**6**:6281.
66. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43**:343–73.
67. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015;**135**:896–902.
68. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;**119**:405–13.
69. Barnes PJ, Adcock IM. How do corticosteroids work in asthma? *Ann Intern Med* 2003;**139**(5 Pt 1):359–70.
70. Principe S, Porsbjerg C, Bolm Ditlev S, Kjaersgaard Klein D, Golebski K, Dyhre-Petersen N, et al. Treating severe asthma: targeting the IL-5 pathway. *Clin Exp Allergy* 2021;**51**:992–1005.
71. McGregor MC, Krings JG, Nair P, Castro M. Role of biologics in asthma. *Am J Respir Crit Care Med* 2019;**199**:433–45.
72. Chakir J, Loubaki L, Laviolette M, Milot J, Biardel S, Jayaram L, et al. Monitoring sputum eosinophils in mucosal inflammation and remodelling: a pilot study. *Eur Respir J* 2010;**35**:48–53.
73. McIntosh MJ, Kooner HK, Eddy RL, Jeimy S, Licskai C, Mackenzie CA, et al. Asthma control, airway mucus, and (129)Xe MRI ventilation after a single benralizumab dose. *Chest* 2022;**162**:520–33.

74. McIntosh MJ, Kooner HK, Eddy RL, Wilson A, Serajeddini H, Bhalla A, et al. CT mucus score and (129)Xe MRI ventilation defects after 2.5 Years' anti-IL-5Rα in eosinophilic asthma. *Chest* 2023;**164**:27–38.
75. Svenningsen S, Haider EA, Eddy RL, Parraga G, Nair P. Normalisation of MRI ventilation heterogeneity in severe asthma by dupilumab. *Thorax* 2019;**74**:1087–8.
76. Svenningsen S, Kjarsgaard M, Haider E, Venegas C, Konyer N, Friedlander Y, et al. Effects of dupilumab on mucus plugging and ventilation defects in patients with moderate-to-severe asthma: a randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* 2023;**208**:995–7.
77. Svenningsen S, Kjarsgaard M, Zhang K, Serajeddini H, Garrido CV, Bhalla A, et al. Effect of dupilumab on sputum eosinophils in patients with moderate-to-severe asthma. *Allergy* 2024;**79**:509–13.
78. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, et al. Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. *N Engl J Med* 2018;**378**:2475–85.
79. Sher LD, Wechsler ME, Rabe KF, Maspero JF, Daizadeh N, Mao X, et al. Dupilumab reduces oral corticosteroid use in patients with corticosteroid-dependent severe asthma: an analysis of the phase 3, open-label extension TRAVERSE trial. *Chest* 2022;**162**:46–55.
80. Nair P, Wenzel S, Rabe KF, Bourdin A, Lugogo NL, Kuna P, et al. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. *N Engl J Med* 2017;**376**:2448–58.
81. Venegas Garrido C, Mukherjee M, Bhalla A, Nair P. Airway autoimmunity, asthma exacerbations, and response to biologics. *Clin Exp Allergy* 2022;**52**:1365–78.
82. Serajeddini H, Venegas Garrido C, Bhalla A, Kjarsgaard M, Huang C, LaVigne N, et al. A case series of dual biologics therapy for severe asthma. *J Allergy Clin Immunol Pract* 2022;**2023**:335–40.e2.
83. Nordenmark LH, Å Hellqvist, Emson C, Diver S, Porsbjerg C, Griffiths JM, et al. Tezepelumab and mucus plugs in patients with moderate-to-severe asthma. *NEJM Evid* 2023;**2**:EVIDoa2300135.
84. Mukherjee M, Lim HF, Thomas S, Miller D, Kjarsgaard M, Tan B, et al. Airway autoimmune responses in severe eosinophilic asthma following low-dose mepolizumab therapy. *Allergy Asthma Clin Immunol* 2017;**13**:2.
85. Machida K, Aw M, Salter BMA, Ju X, Mukherjee M, Gauvreau GM, et al. The role of the TL1A/DR3 Axis in the activation of group 2 innate lymphoid cells in subjects with eosinophilic asthma. *Am J Respir Crit Care Med* 2020;**202**:1105–14.
86. Mukherjee M, Huynh A, Jegatheeswaran M, Venegas Garrido C, Nair A, Tan N, et al. Sputum mucus rheology, inflammatory profile and CT mucus scores in severe asthma. Presented at American Thoracic Society Congress 2024.
87. Hekking PP, Zhang K, Garrido CPV, Lopez-Rodriguez R, Kjarsgaard M, Mukherjee M, et al. Sputum cytokines associated with raised FeNO after anti-IL5 biologic therapy in severe asthma. *Allergy* 2024. https://doi.org/10.1111/all.16011.
88. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000;**161**(2 Pt 1):475–8.
89. Thawanaphong S, Gonzalez Bravo L, Radford K, Venegas Garrido C, Kjarsgaard M, Adatia A, et al. Unrecognized ciliary motility disorders in neutrophilic severe asthma exacerbations. Presented at American Thoracic Society Conference 2024.
90. Huang BK, Elicker BM, Henry TS, Kallianos KG, Hahn LD, Tang M, et al. Persistent mucus plugs in proximal airways are consequential for airflow limitation in asthma. *JCI Insight* 2024;**9**:e174124.
91. Svenningsen S, Eddy RL, Lim HF, Cox PG, Nair P, Parraga G. Sputum eosinophilia and magnetic resonance imaging ventilation heterogeneity in severe asthma. *Am J Respir Crit Care Med* 2018;**197**:876–84.
92. Foo CT, Langton D, Thompson BR, Thien F. Functional lung imaging using novel and emerging MRI techniques. *Front Med* 2023;**10**:1060940.
93. He M, Driehuys B, Que LG, Huang YT. Using hyperpolarized. *Acad Radiol* 2016;**23**:1521–31.
94. Treleaven O, Lauks S, Nair P, Ho T, Kjarsgaard M, Huang C, et al. Effectiveness and cost of a comprehensive severe asthma management strategy guided by airway inflammometry. *Eur Respir J* 2023;**62**(Suppl 67):PA4125.
95. Rogers DF, Barnes PJ. Treatment of airway mucus hypersecretion. *Ann Med* 2006;**38**:116–25.
96. Ehre C, Rushton ZL, Wang B, Hothem LN, Morrison CB, Fontana NC, et al. An improved inhaled mucolytic to treat airway muco-obstructive diseases. *Am J Respir Crit Care Med* 2019;**199**:171–80.
97. Dickey BF, Evans CM. Towards a better mucolytic. *Eur Respir J* 2023;**61**:2300619.
98. Deiteren A, Krupka E, Imberdis K, Patel N, Staudinger H, Bas S, et al. Late breaking abstract – early improvement in asthma small airway dysfunction after one dose of SAR443765, a novel bispecific anti-thymic stromal lymphopoietin/anti-IL-13 nanobody molecule. *Eur Respir J* 2023;**62**(Suppl 67):OA4296.
99. Mackie A, Rascher J, Schmid M, Endriss V, Brand T, Seibold W. First clinical trials of the inhaled epithelial sodium channel inhibitor BI 1265162 in healthy volunteers. *ERJ Open Res* 2021;**7**. 00447-2020.
100. Mall MA. ENaC inhibition in cystic fibrosis: potential role in the new era of CFTR modulator therapies. *Eur Respir J* 2020;**56**:2000946.
101. Marrades RM, Roca J, Barberà JA, de Jover L, MacNee W, Rodriguez-Roisin R. Nebulized glutathione induces bronchoconstriction in patients with mild asthma. *Am J Respir Crit Care Med* 1997;**156**(2 Pt 1):425–30.
102. Addante A, Raymond W, Gitlin I, Charbit A, Orain X, Scheffler AW, et al. A novel thiol-saccharide mucolytic for the treatment of muco-obstructive lung diseases. *Eur Respir J* 2023;**61**:2202022.