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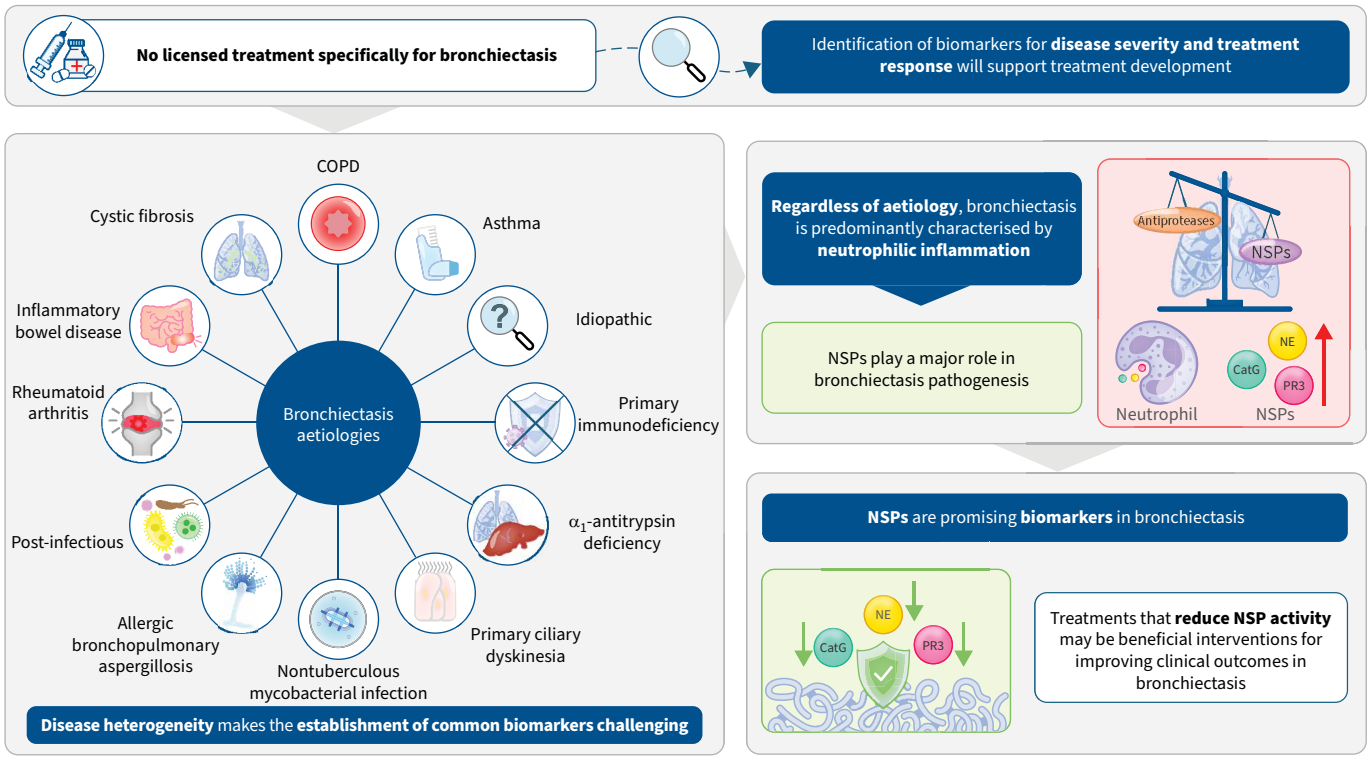

GRAPHICAL ABSTRACT Summary of the review. NSP: neutrophil serine protease; CatG: cathepsin G; NE: neutrophil elastase; PR: proteinase.

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Neutrophil-derived biomarkers in bronchiectasis: identifying a common therapeutic target

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Due to the extensive role of neutrophilic inflammation in the pathogenesis and progression of bronchiectasis, neutrophil serine proteases currently present as promising, treatment-responsive biomarkers in the disease https://bit.ly/4kSqQqE

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Abstract

Bronchiectasis is a chronic respiratory disease that can lead to a substantial decline in lung function, ultimately leading to a significantly increased risk of morbidity and mortality. Despite the increasing global impact of bronchiectasis, no specific (or licensed) treatment for the disease currently exists, with most available therapies, though beneficial, focusing on symptom management and infection control. In part, the lack of specific treatments for bronchiectasis may be due to a lack of established biomarkers for the disease. Because bronchiectasis varies so widely in its clinical presentation and can be caused by various aetiologies, the establishment of validated biomarkers has proven challenging. However, identifying key biomarkers in bronchiectasis is crucial to developing appropriate diagnosis and management plans, as well as to measuring effective responses to treatment. While there is a multitude of potential biomarkers in bronchiectasis, almost all instances of bronchiectasis are underpinned by chronic neutrophilic inflammation. The imbalance in neutrophil serine proteases (NSPs) and their endogenous inhibitors has been strongly linked to the lung destruction, mucosal-related defects, infection and worsening of clinical outcomes that are frequently observed in bronchiectasis. In this review, we discuss the various biomarkers linked to bronchiectasis, with a specific focus on NSPs as the most validated biomarkers in bronchiectasis, given their marked role in the pathogenesis of the disease. Lastly, we touch on potential therapeutic approaches aimed at reducing NSP activity in bronchiectasis, showing that, to date, indirect NSP inhibition appears to be the strategy that most effectively addresses chronic neutrophilic inflammation in bronchiectasis.

Introduction

Bronchiectasis is a chronic respiratory disease that affects the airways and is largely mediated by neutrophilic inflammation [1, 2]. Clinically, bronchiectasis is characterised by chronic cough, excessive sputum production and recurrent pulmonary exacerbations [3–5]. Radiologically, bronchiectasis in adults is evidenced by the often permanent dilatation of the bronchi [1]. However, bronchiectasis has been shown to be reversible in children if diagnosed early and accompanied by optimised treatment [6]. Both the prevalence and incidence of bronchiectasis are increasing worldwide [1, 5], with bronchiectasis reported as the third most common chronic airway disease, termed "an emerging global epidemic" [7, 8]. Its prevalence increases with age [9] and is more common in females [10, 11]. Aetiologies vary across geographical regions [12]. Bronchiectasis has a major impact on quality of life and has placed a significant burden on individual people, caregivers and healthcare systems internationally [4, 13, 14].

Bronchiectasis aetiologies

The clinical phenotype of bronchiectasis is heterogeneous, and people with bronchiectasis can differ in their clinical and radiological presentation, as well as their response to treatment. Irrespective of aetiology, bronchiectasis is typically underpinned by chronic airway inflammation and infection.

Cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) are two well-characterised genetic diseases that severely impair mucociliary clearance and lead to bronchiectasis. In CF, disruptions in mucociliary clearance are primarily caused by mucosal-related defects, with mucus that is dehydrated and has increased viscosity [15, 16]; in PCD, these disruptions are caused by the improper biogenesis, assembly and activity of cilia [17]. Both disorders lead to muco-obstructed airways, which then facilitate an environment that is primed for inflammation and chronic infection. Conversely, other aetiologies of bronchiectasis can be triggered by past infection and chronic inflammation [18], which can indirectly impair mucociliary clearance. In addition to this wide range of associated pathologies, bronchiectasis can also be divided into several infective and inflammatory endotypes (table 1).

To aid in diagnosis and management, it is crucial to identify the underlying pathology, prognosis, risks and treatable traits pertaining to bronchiectasis. The identification of validated biomarkers in bronchiectasis, both during stable state and exacerbations, is crucial to measuring appropriate responses to treatment. However, because of the wide aetiological background of bronchiectasis and its heterogeneity, clinically validated biomarkers that are specific to bronchiectasis are lacking. In this review, we aim to assess the associations between potential or established biomarkers and exacerbation rates and symptoms across various aetiologies, which may also assist in identifying a common therapeutic target in the treatment of bronchiectasis.

The pathogenesis of bronchiectasis and the vicious vortex

Chronic neutrophilic inflammation is a key feature of many chronic inflammatory respiratory diseases, including bronchiectasis, CF, PCD and COPD [19, 20]. In small studies, it has been found that neutrophils are a dominant leukocyte in the airways of people with bronchiectasis [21], whereas neutrophils are low in number in the airways of healthy people [21–23]. In one small study, it was found that the properties and functions of neutrophils were altered even in stable-state bronchiectasis [21], displaying prolonged viability and delayed apoptosis, reducing their functional ability to phagocytose and kill bacteria [21]. Additionally, an increase in airway levels of neutrophil-associated proteins is associated with greater disease severity in bronchiectasis [24, 25]. In bronchiectasis, chronic neutrophilic inflammation occurs in both the presence and absence of active infection [22], and airway neutrophilia is present independent of exacerbations [26], suggesting that active inflammation persists even in clinically stable people with bronchiectasis [26, 27]. Importantly, macrolides, which modulate neutrophilic function [28–31], are effective in reducing exacerbation frequency in people with chronic airway diseases [27, 32, 33], further eliciting the impact of neutrophilic inflammation in bronchiectasis.

The pathogenetic events that initiate bronchiectasis are mostly unclear, but once established, a complex interaction between chronic inflammation, chronic or recurrent infection, impaired mucociliary clearance and progressive structural lung damage in a "vicious vortex" can lead to disease progression, increased exacerbations and declining pulmonary function, with associated mortality [1, 5, 34]. Each component of the vicious vortex conveys an entry point through which it may be initiated and perpetuated [35]. Because chronic neutrophilic inflammation is a central pathogenic feature of most bronchiectasis cases [1, 36, 37], it is likely a key driver of initiating and sustaining the vicious vortex through the effects of neutrophil serine proteases (NSPs) (figure 1).

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TABLE 1 Summary of bronchiectasis aetiologies and associated clinical and pathobiological features

	Clinical and radiological features (where applicable)	Main pathobiological feature or pathway
COPD[#]	Airway obstruction Mucus hypersecretion [158] Emphysema	As with bronchiectasis, COPD is driven by neutrophilic inflammation, therefore upholding the vicious vortex hypothesis [4]
Asthma[#]	Airway obstruction Wheezing Hyperresponsive airways Frequent exacerbations	Largely driven by a Th2 lymphocyte-predominant immune reaction in response to environmental triggers, resulting in atopy and eosinophilic inflammation [159]
AATD	Chronic bronchitis Frequent exacerbations Emphysema Lower-lobe-predominant bronchiectasis on background of emphysema [160]	Genetic disease caused by mutations in the serine protease inhibitor (*SERPINA1*) gene that results in diminished α_1-antiprotease activity, facilitating lung tissue destruction and weakened host defence as a result [161]
PCD	Frequent infections Mucus plugging [162] Middle- and lower-lobe-predominant bronchiectasis, in a tree-bud organisation, often with atelectasis [162]	Genetic disease that affects the structure and function of cilia along the entirety of the airway tracts, causing impaired mucociliary clearance and mucus accumulation, further reducing overall clearance [17]
CF	Frequent infections Airway obstruction [163] Upper- and middle-lobe-predominant bronchiectasis [164]	Genetic disease that results in reduced or complete loss of function in the CFTR chloride channel, resulting in dehydrated, thickened mucus that impairs effective mucociliary clearance [16, 165, 166] Inflammation perpetuated by abhorrently high NSP activity [167]
Primary immunodeficiency	Frequent infections	Increased predisposition to bronchiectasis due to susceptibility to recurrent infections [4]
Rheumatoid arthritis	Subclinical ILD noted on HRCT scans Bronchiectasis noted in ~30% of rheumatoid arthritis cases (on HRCT scans)	Unclear; hypotheses suggest a bidirectional relationship between increased susceptibility to infections in rheumatoid arthritis leading to bronchiectasis, and *vice versa*; or a shared genetic predisposition to either disease [168]
IBD	Large airway involvement (rare) that can involve chronic bronchitis [4] Large sputum volume Bronchiectasis often associated with bronchial inflammation and suppuration [169]	Unclear; hypotheses point towards a "gut–lung axis" and a "shared antigen" state or shared abnormalities in mucus properties, which are based on the common epithelium of the gut and lung, as well as the commonality of lymphoid inflammation in gut and lung disorders [18]. In this regard, inflammation and mucus alterations act in a vicious cycle: it is proposed that inflammation initially alters the viscoelastic properties of mucus, while these changes in mucus then trigger inflammation and gut microbial imbalances that further worsen systemic outcomes [170]
Allergic bronchopulmonary aspergillosis	Sensitivity to fungal infections Wheezing Mucus plugging Upper-lobe-/central-predominant varicose bronchiectasis, high-density mucus plugs [171]	Th2 cells create a hypersensitivity reaction involving elevated IgE levels, eosinophilic inflammation and mast cell degranulation following exposure to the *Aspergillus fumigatus* antigen [172]
Nontuberculous mycobacterial infection	Progressively worse lung function Other symptoms independent of the lung (such as weight loss) Localised bronchiectasis often in the middle lobe and lingula [173], associated with tree-in-bud and scattered nodules [174]	Infections can occur in an opportunistic manner in susceptible people (whose host defence responses are impaired), as is the case in bronchiectasis [175]

AATD: α_1-antitrypsin deficiency; PCD: primary ciliary dyskinesia; CF: cystic fibrosis; IBD: inflammatory bowel disease; Th: T-helper; CFTR: cystic fibrosis transmembrane conductance regulator; NSP: neutrophil serine proteases; ILD: interstitial lung disease; HRCT: high-resolution computed tomography; Ig: immunoglobulin. [#]: there is debate around whether COPD and asthma are true causes of bronchiectasis [18], with a lack of natural history studies and difficulty in differentiating the two diseases making proof of causality difficult. Reproduced and modified from [18] with permission.

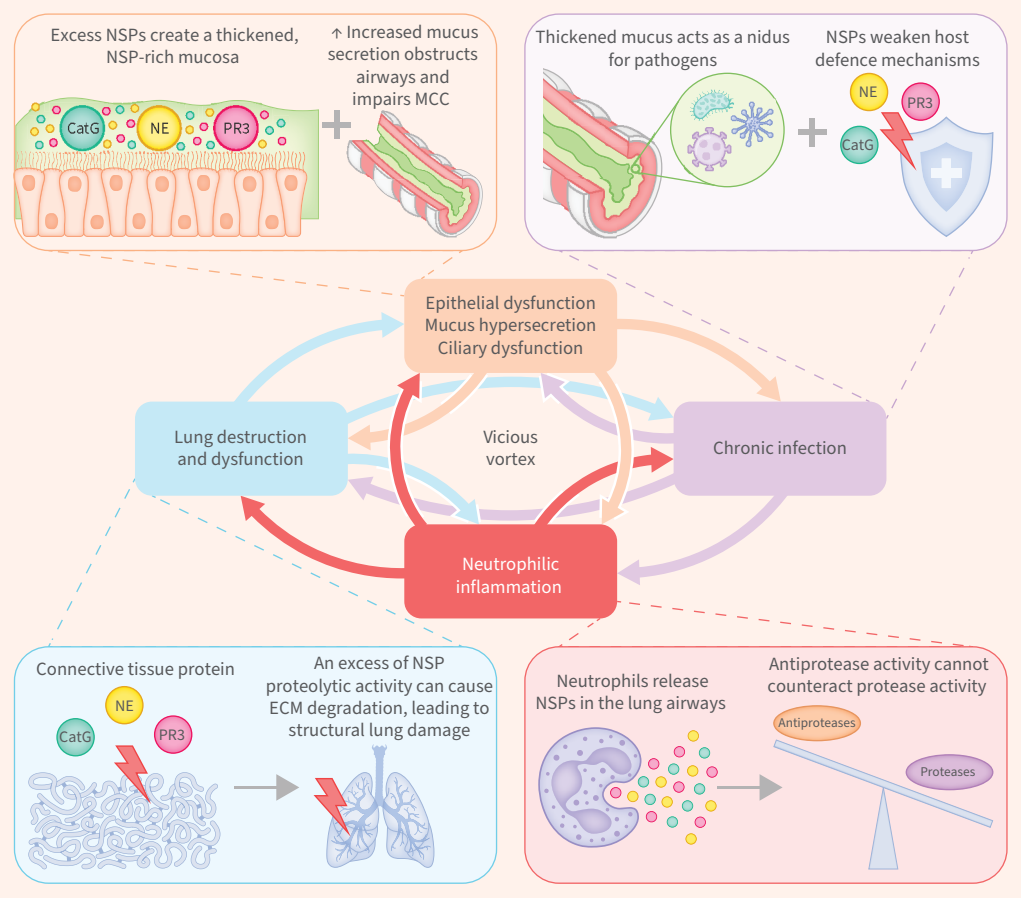

FIGURE 1 Neutrophil serine proteases (NSPs) are involved in all aspects of the vicious vortex in bronchiectasis. CatG: cathepsin G; NE: neutrophil elastase; PR: proteinase; MCC: mucociliary clearance; ECM: extracellular matrix.

In most cases of bronchiectasis, the chronic accumulation and activation of neutrophils lead to the abnormal and excessive activity of NSPs. Excessively high NSP activity can dysregulate the inflammatory process and damage lung tissue [38, 39]. An imbalance between the NSPs neutrophil elastase (NE), proteinase (PR)3 and cathepsin G (CatG) and their antiproteases is central to the pathogenesis of bronchiectasis [1, 40, 41]. This high NSP activity promotes and maintains the pathogenic environment in the diseased lung, affecting every component of the vicious vortex.

The role of NSPs in mucociliary dysfunction in bronchiectasis

Increased NE activity can lead to goblet cell hyperplasia and metaplasia in the airways, with subsequent mucus hypersecretion. Indeed, NE induces expression of MUC5AC, and does so *via* oxidation-related mechanisms [42]. MUC5AC is a key component of airway mucus [42, 43] that, in excess, has been shown to severely impair mucociliary clearance [43, 44]. Furthermore, mucus hypersecretion is diminished in models of NE-knockout mice with CF-like lung disease [45]. PR3 has also been implicated in mechanisms relating to mucus hypersecretion [46].

Hypersecretion of mucins leads to hyperconcentration of airway mucus, with emerging evidence suggesting that hyperconcentrated airway mucus impairs mucociliary clearance [47, 48], feeding into the vicious vortex. Compared with induced sputum from healthy subjects, the sputum of people with bronchiectasis has an increased percentage of solids, total and individual mucin concentrations, osmotic pressure, and elastic and viscous moduli [47]. These biophysical changes in sputum, coupled with increases in mucus concentration, impair mucus clearance and contribute to bronchiectasis pathogenesis, where mucus hyperconcentration strongly correlates with disease severity [47]. Hyperconcentrated mucus on airway surfaces also impairs clearance by cough [49–51]; this causes mucus plugging in both proximal and distal bronchi [52]. Moreover, hyperconcentrated mucus has been shown to correlate with both impaired lung function and the extent of

bronchiectasis [47]. Additionally, NE is reported to promote mucus obstruction by decreasing ciliary beat frequency [40]; however, some literature contrasts with this [53].

As a result of mucus obstruction, hypoxia of the airway epithelium occurs and, in turn, stimulates a metabolic shift that results in the release of interleukin (IL)-1α due to necrotic cell death [54]. IL-1α is a strong trigger for neutrophilic inflammation and mucus hypersecretion [54–58], relaying into the vicious vortex. Indeed, IL-1α has been found to induce the expression of the secreted mucin MUC5B, contributing to additional mucus plugging [55]. In CF bronchial epithelial cells, IL-1β induces MUC5B and MUC5AC protein secretion and mucus hyperconcentration [58]. This characteristic inflammatory response, mediated by IL-1R activation following hypoxic cell necrosis, has been demonstrated across multiple muco-obstructive lung diseases [55, 56]. The accumulation of hyperconcentrated mucus in the airway lumen may contribute to sterile inflammation in bronchiectasis [47, 48, 54, 55]. Additionally, Mall *et al.* [59] found that airway mucus hyperconcentration initiated persistent airway mucus obstruction and neutrophilic airway inflammation in a mouse model of muco-obstructive lung disease. These results indicate that mucus hyperconcentration may play a critical role in the pathogenesis of diseases, for example COPD, which is closely linked to bronchiectasis. Other pre-clinical studies of bronchiectasis models showed that mucus plugs in bronchiectasis are pro-inflammatory, populated by IL-1β-expressing macrophages [52]. High levels of IL-1β in the airways are also associated with microbial dysbiosis, mucus hyperconcentration and bronchiectasis severity [60].

The role of NSPs in infection in bronchiectasis

In parallel, the adhesion of thickened mucus to airway surfaces not only prevents airflow, but also acts as a nidus for chronic infection and inflammation [49]. Bacterial infection is particularly common in muco-obstructive lung diseases and is theorised to compound the production of NE-rich mucus that causes the airway wall damage that is typical of bronchiectasis [48]. Excess NSP activity greatly affects innate host responses to infection. Indeed, NE is known to cleave antimicrobial peptides and degrade surfactant proteins, adversely affecting host defence mechanisms [40]. Further to this, NSPs can also act as chemotactic mediators for various immunocytes, and even regulate the activity of other proteases and antiproteases [40, 61].

The role of NSPs in structural lung damage in bronchiectasis

Due to their proteolytic effects, NSPs degrade the extracellular matrix (ECM) by damaging surrounding tissues *via* the degradation of elastin [1], ultimately causing lung tissue destruction [1, 38, 40, 61, 62]. In animal models of emphysema, connective tissue resynthesis is defective after initial elastin degradation and the resultant elastin components are morphologically defective [63, 64]. In turn, progressive airway loss and worsened lung function are observed [63]; features that are also characteristic of bronchiectasis. Sputum desmosine and isodesmosine levels specifically reflect elastin degradation in the lung [63], and high levels of serum desmosine also correlate with disease severity in people with bronchiectasis [37].

Biomarkers in bronchiectasis, associated symptoms and exacerbations

Classifying clinical and biological markers in people with bronchiectasis may aid in predicting disease severity, disease activity, risk of future exacerbations and disease prognosis. Classifying a patient's risk for pulmonary exacerbations is particularly important in this regard. Across multiple bronchiectasis aetiologies, exacerbations are associated with lung function decline, poor quality of life, and mortality [26, 65–67].

The milieu of the vicious vortex provides an attractive platform for determining biomarkers. Recent work by Johnson *et al.* [24] describes how biomarkers could be grouped according to the four facets of the vicious vortex. Of these facets, the biomarkers that are currently established in bronchiectasis include sputum colour (concerning inflammation), *Pseudomonas aeruginosa* infection or *Aspergillus* sensitisation and infection (indicative of infection), spirometry (pulmonary function testing) and radiological features including bronchial dilatation (structural lung changes) [24]. The heterogeneity of bronchiectasis ideally requires tailored, individualised approaches to therapy that depend on the patient's underlying pathology and symptoms [68], and biomarkers are needed to evaluate risk and, in future, to target therapies. Evidence is beginning to indicate the importance of such exploratory biomarkers and their role in bronchiectasis (table 2).

Biomarkers directly related to neutrophilic inflammation

NSPs in bronchiectasis

Overwhelming evidence shows that most bronchiectasis cases share a common pathophysiological trait of abnormal neutrophil activity, facilitating the excessive release of NSPs [1, 36, 37]. The role of NSPs in the pathobiology of bronchiectasis, including CF-related bronchiectasis [40], is well established, and the extent

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TABLE 2 Biomarkers in bronchiectasis

	Collected and measured *via*	Pathophysiological mechanism	Downstream effects	Associated bronchiectasis symptom(s) and outcomes
NSPs (NE, PR3, CatG)	Blood, sputum, BALF	Neutrophilic inflammation in the airways leads to the excessive release of NSPs and thus aberrantly high activity of NSPs [1, 36, 37]	NSPs are proteolytic by nature. Aberrantly high NSP activity causes direct damage to adjacent tissues, can mediate the inflammatory response, and can disrupt host defence mechanisms due to the inhibition of antiproteases [1, 40]	NE: linked to airway infection, increased risk of exacerbation, disease severity, and all-cause mortality [37] PR3: elevated during pulmonary exacerbations [176] CatG: activity proportional to disease severity; known to injure airway epithelium [83]
NETs and NET-associated proteins	Sputum, BALF, blood	High levels of neutrophilic inflammation invariably lead to NETosis, which then leads to the expulsion of several pro-inflammatory agents [18, 35]	Excess NSP activity feeds into several components of the vicious vortex [1]	Sputum NETs correlate with disease severity [25]
Procalcitonin	Sputum	Procalcitonin is pro-inflammatory in nature and has immunomodulatory effects [107]	Immunomodulatory effects of procalcitonin induce an inflammatory cascade, particularly involving TNF-α [107]	Elevated during exacerbations [107] Linked to increased symptoms and an increased likelihood of antibiotic prescription [108]
Fibrinogen	Serum	Fibrinogen is elevated in response to systemic inflammation [111]. Fibrinogen is termed an "effector" in lung disease because it promotes airway fibrosis [177]	Airway fibrosis may affect mucociliary clearance (impaired ciliary function and changes in mucus qualities) and cause significant changes to lung function and physiology [178, 179]	Increased fibrinogen levels are associated with increased bronchiectasis severity [180] Sustained higher fibrinogen levels are associated with worsened lung function in men [177, 180]
CatS	BALF, *ex vivo* lung tissues	Excess CatS levels promote inflammation by cleaving chemokines such as CX3CL1 and are involved in TGF-β signalling [181]. CatS is also involved in ECM remodelling [112]	Immunomodulatory effects of CatS facilitate leukocyte migration [181]. ECM remodelling can contribute to lung fibrosis [182]	Thus far, no data demonstrating any effect in bronchiectasis have been published [1]
MMPs	Sputum, blood	MMPs can degrade collagen and elastin (remodel tissue), degrade AAT, and are involved in pulmonary immunity *via* cellular signalling [1]	MMPs can be immunomodulatory, sustaining inflammation [183]. Additionally, MMPs are involved in tissue remodelling [98], which may disrupt lung integrity	MMP levels positively correlate with disease severity (worse spirometry measurements and higher BSI scores) [100] MMPs are linked to the airway mucus obstruction and surface dehydration observed in both COPD and emphysema [100, 101]
IL-1β	Sputum	IL-1β is prominent in over-exaggerated inflammatory responses [60], and its release may be mediated by airway neutrophilic inflammation [184]	IL-1β relates to all aspects of the vicious vortex [60]. IL-1β also impacts ciliary beating [60], but more research is needed to determine the strength of this effect in comparison to other cytokines indicating the complexity of determining the impact of a single protease or cytokine in the bronchiectasis airway [60]	Increased airway IL-1β is linked with increased disease severity, airway colonisation, mucus dehydration and hyperconcentration, and ciliary dysfunction [60]

NSP: neutrophil serine protease; NE: neutrophil elastase; PR: proteinase; Cat: cathepsin; NET: neutrophil elastase trap; MMP: matrix metalloprotease; IL: interleukin; BALF: bronchoalveolar lavage fluid; TNF: tumour necrosis factor; TGF: transforming growth factor; ECM: extracellular matrix; AAT: α_1-antitrypsin; BSI: Bronchiectasis Severity Index.

of NSP activity has been correlated with disease severity in multiple instances. The key role of NSPs is illustrated by the genetic condition α_1-antitrypsin (AAT) deficiency, which is associated with the development of bronchiectasis [1]. AAT is a key antiprotease that irreversibly inhibits NSPs [69] and is thus a key regulator of NSP activity. AAT deficiency has also been associated with bronchiectasis extension, disease activity and radiological severity [70].

Neutrophil elastase

NE, a serine protease expressed in neutrophils, accounts for ~80% of the total protease hydrolysis activity in the human body, promoting inflammation, bacterial infection progression and the hypersecretion of mucus [71]. In adults with bronchiectasis, sputum NE activity correlates with disease severity; a decline in forced expiratory volume in 1 s (FEV_1) over time; an increased risk and frequency of exacerbations, and a shortened time to next exacerbation; infections; and hospitalisations [37]. Longitudinal analyses in CF have revealed that NE is associated with a decline in FEV_1, yielding as much as a 2.9% decline in FEV_1 per 1-log increase in NE (independent of bacterial colonisation) [72]. These observations have also been made in children with bronchiectasis, in whom sputum NE correlated with exacerbations and disease severity [73]. Similarly, in early life, NE activity in bronchoalveolar lavage fluid (BALF) has been associated with early bronchiectasis development in children with CF [74]. In addition to free NE activity, membrane-bound NE on neutrophils has also been linked to more severe disease in adults with CF lung disease [75], and an association between NE exocytosis and early lung damage is present in children with CF [76]. Additionally, in the CF lung, both secretory leukocyte protease inhibitor (SLPI) and elafin (two potent NSP inhibitors) are cleaved and inactivated by NE [65, 77]. These effects on both SLPI and elafin are promoted by the presence of *P. aeruginosa* and, interestingly, studies have shown that only direct inhibition of NE can prevent elafin degradation [65, 77, 78]. The proteolytic effects of NE on antiproteases may be a core aspect in the continuation of the vicious vortex, given that these antiproteases also play a pivotal role in host defence. This supports recent work describing that people with CF who commonly experience pulmonary exacerbations have a diminished host protein defence in their airways [65]. Interestingly, it is suggested that NE can block the release of SLPI from airway epithelial cells, which is independent of NE's proteolytic activity [79]. Finally, low sputum SLPI levels have been linked with shorter intervals between exacerbations [79]. These data point toward a strong association between NE and bronchiectasis. Despite the evident validity of NE as a biomarker for bronchiectasis, its utility in a clinical setting is currently limited. The availability of point-of-care tests for NE may facilitate its use as a clinical biomarker and assist in therapeutic decision-making in the clinic [80].

Proteinase 3 and cathepsin G

PR3 and CatG are serine proteases stored in neutrophilic granules (both PR3 and CatG) and on the surface of secretory vesicles (PR3 only) [81]. Less is known about the role of PR3 and CatG in bronchiectasis and CF; however, both contribute to the inflammatory process. Sputum PR3 is raised during pulmonary exacerbations in bronchiectasis [82], and PR3 during bronchiectasis exacerbation is associated with the isolation of bacteria and viruses [82]. CatG has been implicated in ciliary dysfunction and the resultant destruction of airway epithelium. Increasing CatG activity is also linked to increasing disease severity [81]. Finally, CatG has been implicated in CF and COPD, with some research suggesting that this biomarker may be directly involved in the pathogenesis of these conditions [83]. These results suggest that PR3 and CatG may be valuable biomarkers in bronchiectasis, where PR3 has been proposed as an equally important protease as NE, if not more so, in the pathogenesis of chronic lung diseases [81].

NSPs as biomarkers of CF-associated bronchiectasis in the era of CF transmembrane conductance regulator modulator therapy

Although the initiating mechanisms for CF- and non-CF-related bronchiectasis are different, it is well established that neutrophilic inflammation is a shared feature of both diseases [84]. The restoration of protease–antiprotease balances is thus expected to benefit both people with CF- or non-CF-related bronchiectasis [84].

With the advent of CF transmembrane conductance regulator (CFTR) modulator therapy, restoration of CFTR channel function has yielded significant improvements in CF and CF lung disease. For the ~90% of people with CF with eligible mutations who can tolerate CFTR modulator therapy [85], CFTR modulator therapy is increasing life expectancy, resulting in a steadily increasing population of older people with CF [86] who are in a disease state that has many similarities with non-CF bronchiectasis.

While some evidence suggests that CFTR modulator therapy partially reverses bronchiectasis (based on small subgroups of adults with CF) [87], recent studies demonstrated that airway inflammation persists in people with CF treated with CFTR modulator therapy, though at a lower level [88, 89]. In a study of adults

with CF, 3 months of treatment with elexacaftor/tezacaftor/ivacaftor (ETI) led to decreased concentrations of NSPs, IL-1β and IL-8 in sputum, along with decreased *P. aeruginosa* burden and restored SLPI levels. These beneficial effects were sustained to 1 year of therapy. All airway inflammatory markers that were examined in the study were reduced to levels that matched those of people with non-CF bronchiectasis used as controls, indicating marked improvements in the cohort [88]. In another study, after observing no alterations in protease levels following the administration of CFTR modulator therapy, Maher *et al.* [90] recently postulated that mechanisms independent of CFTR protein function (such as infection or structural lung damage) may contribute to the incomplete resolution of neutrophilic inflammation with the use of CFTR modulator therapy. Rheological analyses have also shown that restoration of CFTR function by ETI improves sputum viscoelastic properties in people with CF [89]; however, this improvement did not restore sputum viscoelastic properties to those observed in healthy controls [89], and instead resembled abnormalities in sputum rheology observed in people with bronchiectasis [47, 89]. Additionally, treatment with even the most effective CFTR modulator therapies does not completely eradicate pathogenic organisms present in CF airways [89, 91]. This is a notable finding, given that *P. aeruginosa* is present in ~25% of people with non-CF bronchiectasis, as reported from people from the EMBARC registry who had available sputum or BALF samples [14]. Even in stable, nonexacerbating states, *P. aeruginosa* remained in up to 22% of people with bronchiectasis [14], emphasising that infection persists. *P. aeruginosa* and other bacterial pathogens are strong triggers for neutrophilic inflammation, and these studies suggest that they perpetuate the vicious vortex and contribute to higher exacerbation rates in people with non-CF- and CF-related bronchiectasis.

Neutrophil extracellular traps, their associated proteins and other extracellular traps in bronchiectasis

Neutrophilic inflammation in the vicious vortex of bronchiectasis is also characterised by the formation of neutrophil extracellular traps (NETs). NETs are web-like structures containing DNA and enzymes such as NSPs, released during NETosis, that typically function as host defence mechanisms; however, the high concentrations of proteases, antimicrobial proteins and DNA released from NETs lead to increased airway inflammation and the degradation of surrounding tissues [18, 35]. Indeed, airway NETs and NETosis have been linked with greater disease severity, increased exacerbations and overall worse disease outcomes in multiple airway diseases [35, 92, 93]. The level of NETosis at which the host defence is breached and tissue destruction occurs, as well as the cause of this deregulation, is unclear. NET-associated proteins appear to be the most abundant in the sputum of people with bronchiectasis, and have been the proteins most strongly associated with disease severity in bronchiectasis [25]. In CF, one study showed that NETosis-related DNA structures correlated with airflow obstruction in people with CF and a mouse model of CF-like lung disease [94]. The implication of NETosis in this regard is evident, given that chronic airflow limitation is a major determinant of morbidity and mortality in people with CF [94]. A variety of biomarkers including NSPs, resistin and calprotectin are released in NETs and can be measured as indirect biomarkers of NETosis [35]. Recently, in addition to NETs, other extracellular traps were also found in the BALF of children with bronchiectasis [95]; of the cohort of examined children, NETs were found in only 33% of BALF samples, while macrophage extracellular traps (METs) presided, found in over half of all samples [95]. Interestingly, MET formation may be induced by NE, suggesting that MET formation can occur in response to neutrophilic inflammation [96]. Though the implications of these other extracellular traps are unknown, it is possible that extracellular traps may be used as a biomarker to better understand respiratory endotypes in bronchiectasis [95].

Matrix metalloproteases

Matrix metalloproteases (MMPs) are zinc-dependent proteases that act on inflammatory effectors [1]. They are able to degrade the ECM (resulting in tissue remodelling and structural lung damage) [97, 98], and MMP-12 is known to inactivate the antiprotease AAT [81]. Neutrophils produce and secrete MMP-8 and MMP-9 [99]. Compared with healthy controls, sputum levels of MMP-8 and MMP-9 are elevated in people with bronchiectasis. Additionally, elevated MMP levels are positively correlated with poorer spirometry test outcomes and higher Bronchiectasis Severity Index (BSI) scores [100].

Airway mucus obstruction due to impaired mucociliary clearance has been shown to induce emphysema in a mouse model of muco-obstructive lung disease, even in the absence of cigarette smoke [59, 101]. MMP-12 was established as the driver of this phenomenon, which was further confirmed by the genetic deletion of MMP-12 being associated with better lung function [101]. These results indicate that MMP-12 may serve as a biomarker for emphysema in muco-obstructive lung diseases [101].

However, in CF, the utility of MMPs as a biomarker of lung disease is unclear. Though MMP levels are upregulated in the airways of people with CF [98], a 2022 study showed that MMP-9, for example, does not seem involved in the *in vivo* pathogenesis of muco-obstructive lung disease in mice. However, the

authors did note that genetic deletion of MMP-9 may be compensated for by other proteases, and that mice and humans may have differences in the relative effects of proteases, leading to this surprising result [102]. In contrast, another study in bronchiectasis demonstrated that the level of activated MMP-9 and the MMP-9 to tissue inhibitor of metalloproteinase ratio were associated with NE activity and bronchiectasis progression, providing evidence that MMP-9 may be relevant in bronchiectasis [103].

Biomarkers unrelated to neutrophilic inflammation

Eosinophilic bronchiectasis and other inflammatory endotypes

Although neutrophilic inflammation is the predominant feature of bronchiectasis, ∼20% of people with bronchiectasis may exhibit concurrent eosinophilic inflammation [104]. Alone, elevated blood eosinophil count is not associated with exacerbations; however, when controlled for other variables such as *P. aeruginosa* infection, elevated blood eosinophil count may predict and contribute to exacerbation risk in bronchiectasis [104]. Furthermore, a study by Choi *et al*. [105] revealed that a combination of eosinophilic and neutrophilic inflammation may also predict exacerbation risk in bronchiectasis. It is commonly misunderstood that because 20% of patients show eosinophilic inflammation, the remaining 80% are "neutrophilic"; however, the data by Choi *et al*. [105] indicate that, most often, people with bronchiectasis exhibit mixed neutrophilic and eosinophilic inflammation. In the same study, it was also found that some people with bronchiectasis also experience eosinophilic inflammation with mixed-epithelial inflammation; however, this endotype did not appear associated with an increased exacerbation risk [105]. These studies may indicate that distinct inflammatory endotypes in bronchiectasis are associated with future exacerbation risk. The key implication of the eosinophilic endotype is to identify people who may respond to targeted therapies such as inhaled corticosteroids or biologic drugs used in severe asthma, especially since these treatments are not recommended for bronchiectasis [24]. Data on efficacy in this subgroup are still lacking.

Sputum procalcitonin

Procalcitonin, a pro-hormone for calcitonin secreted from the thyroid, is typically known for its correlation with sepsis [106]. However, during bacterial infection, procalcitonin gene expression and release have been identified in many different tissues, including the lungs [107]. The pro-inflammatory action of procalcitonin increases surface markers CD16 and CD14 on neutrophils and lymphocytes [107]. In sputum, elevated procalcitonin levels have been identified in people with bronchiectasis requiring hospitalisation for an infective exacerbation (in contrast to serum, where procalcitonin levels are characteristically low during these events) [107]. Sputum procalcitonin levels are also higher in people with stable bronchiectasis than in healthy controls [107]. These data suggest the potential of procalcitonin as a biomarker to guide antibiotic treatment in bronchiectasis [107]. However, in a separate study, though higher levels of procalcitonin were associated with increased symptoms and an increased likelihood of antibiotic prescription in outpatients with bronchiectasis, the authors concluded that procalcitonin was not suitable for guiding treatment of an exacerbation in bronchiectasis, due to generally low levels in both outpatients and inpatients [108]. In children, serum procalcitonin levels were also not significantly elevated during bronchiectasis exacerbations [109]. The reliability of procalcitonin as a biomarker in bronchiectasis is thus uncertain.

Fibrinogen

Fibrinogen is an acute-phase reactant in response to inflammation that modulates tissue injury [110]. In a 2022 study, serum fibrinogen was deemed as a potential biomarker for assessing both disease severity and exacerbations in people with bronchiectasis [111]. Interestingly, the study showed that fibrinogen was independently associated with BSI and FACED (FEV_1, age, chronic colonisation, extension and dyspnoea) scores. People with high fibrinogen levels were more than twice as likely to experience a future pulmonary exacerbation during the study period than those with low fibrinogen levels [111]. Fibrinogen is already an established biomarker in other muco-obstructive lung diseases such as COPD [111]. Thus, fibrinogen may be an important biomarker for bronchiectasis, given that both bronchiectasis and COPD are characterised by chronic neutrophilic inflammation [111].

Cathepsin S

Cathepsin S (CatS) is a cysteine protease that is localised in the lysosomal/endosomal compartments of antigen-presenting cells, but can be produced by neutrophils [112]. Although no specific data relating to bronchiectasis have been published [1], CatS has been linked to various inflammatory airway diseases. In COPD, elevated CatS levels appear proportional to increased disease severity [113]; however, this effect has only been found in current smokers. Some studies suggest that elevated CatS may be an initial trigger in the development of COPD [112]. Indeed, polymorphisms in the *CatS* gene are linked to the increased risk of developing COPD in certain populations [114]. In CF, elevated CatS expression in lower airway tracts is a feature of stable CF and paediatric CF [112, 115]. In paediatric CF, increased levels of CatS in

BALF, produced by epithelial cells, negatively correlate with lung function [112, 115]. A study in the early 2000s concluded that elastolytic cathepsins (cathepsins S, B, L) in BALF can inactivate the antimicrobial properties of human β-defensins, an effect that could be completely prevented *via* use of a cathepsin inhibitor [116]. A more recent study demonstrated that CatS inhibition was able to reduce muco-obstructive lung disease in mice [117]. Here, both genetic ablation and pharmacological inhibition of CatS in BALF resulted in less pulmonary inflammation, mucus obstruction, mucus plugging and structural lung damage when compared with control mice [117]. CatS could also be a potential therapeutic target, albeit with a short window in which it may be most effective in treating early CF lung disease [118]. The above data suggest that CatS may act as a biomarker for disease progression in inflammatory lung diseases such as bronchiectasis.

In general, biomarkers may aid in identifying people who may have the greatest clinical benefit from targeted therapies in clinical trials. Taken together, published data suggest a strong association between NSP activity, exacerbations and lung function in bronchiectasis. Because recent trials for novel anti-inflammatory drugs have specifically assessed end-points relating to exacerbations and lung function, the case that NSPs are promising biomarkers for measuring the efficacy of novel anti-inflammatory treatments in bronchiectasis is further strengthened.

Treatments in bronchiectasis

The central role of neutrophilic inflammation in the pathogenesis and progression of bronchiectasis makes this an attractive therapeutic target. However, there are currently no licensed drugs available for the management of neutrophilic inflammation in bronchiectasis [119, 120]. The heterogeneity of bronchiectasis is one of the most challenging aspects of its management [121]. Existing bronchiectasis treatments are largely based on controlling symptoms, treating the underlying cause of bronchiectasis, promoting the rehydration and thus clearance of excess and aberrant mucus, or managing infection [68, 122]. Certain airway clearance techniques have been shown to effectively increase sputum production from their very first day of implementation, with the long-term benefits of these clearance techniques producing significant reductions in exacerbations, increases in quality of life, and reductions in cough impact [123]. Although other benefits, such as improvements in lung function, have not always been observed [123], research shows that adherence to airway physiotherapy is both justified and constructive for individuals with the disease, and may be more effective when used in conjunction with other therapies [121]. Macrolides are a highly effective therapy to reduce exacerbations and have anti-inflammatory qualities [28, 124, 125], including a modulatory effect on neutrophil function. Macrolides reduce exacerbation frequency and prolong time to first exacerbation in people with bronchiectasis [124], PCD [126] and CF [127]. In a meta-analysis involving >3000 people with bronchiectasis, it was concluded that inhaled antibiotics significantly reduced exacerbations by ∼20%, with severe exacerbations being reduced by ∼50% [128]. The benefit of antibiotic therapies on exacerbation reduction is coupled with improvements in quality of life, as well as effective symptom improvement, particularly in people experiencing symptoms such as cough and sputum production [128, 129].

Despite the well-known role of neutrophilic inflammation and the protease–antiprotease imbalance in bronchiectasis [1, 26, 36], previous methods of controlling neutrophilic inflammation have not always been successful. To directly restore protease–antiprotease balance, there are two principal strategies that could be implemented: pharmacological protease inhibition or antiprotease augmentation [1, 40].

The use of protease inhibitor therapy has primarily focused on NE inhibition, due to the general acceptance that NE has a major role in lung disease [40]. NE inhibition has been tested across many clinical trials for muco-obstructive lung diseases, including those of CF, COPD and bronchiectasis [41]. However, there has been varying success in these phase II trials of NE inhibitors. An example of this is AZD9668, which yielded increases in pulmonary function and decreases in inflammatory biomarkers in tested subjects [130]. This is in contrast to BAY 85-8501, which yielded no improvement in participants' pulmonary function, potentially due to the short treatment period of 4 weeks [131].

It has been postulated that AAT supplementation may prove beneficial in diseases marked by neutrophilic inflammation, such as bronchiectasis [132]. Mechanistically, the effectiveness of antiprotease-based therapies in reducing airway inflammation has been shown in people with CF [133]; however, therapies that augment AAT are currently not approved for CF.

In recent years, an indirect approach to NSP inhibition has proven to be more promising. Rather than aiming to reduce the excess activity of individual NSPs, the target is the enzyme responsible for the activation of all three [1]. The cysteine protease cathepsin C (CatC, also known as dipeptidyl peptidase 1)

activates all NSPs (NE, PR3 and CatG) during neutrophil maturation in the bone marrow [35, 134, 135]. The inhibition of CatC during neutrophil development is expected to reduce levels of active NSPs that could be secreted, thereby improving the protease–antiprotease balance and making CatC an attractive target for reducing NSP activity [119]. However, the level of CatC inhibition required to see a clinical response is still unclear.

There have been some concerns regarding the safety of CatC inhibition, due to a rare genetic disease known as Papillon–Lefèvre syndrome (PLS) that is caused by mutations in the *CatC* gene [136], affecting 1–4 per 1 000 000 people [137]. The loss of CatC gives rise to palmoplantar keratosis and periodontal disease [136]. However, although NSPs contribute to host immune defence under normal conditions, they do not seem to be critical for other neutrophil functions in this respect. This hypothesis is supported by research into PLS, which shows that people with PLS do not incur major infections [136, 138] and neutrophils are able to effectively kill pathogens [136]. Additionally, NE inhibition does affect neutrophil viability [139].

CatC inhibition has already been examined in several pre-clinical, phase I and phase II studies [138, 140–144]. Thus far, the trialled CatC inhibitors include brensocatib, BI 1291583 and HSK31858. In the context of adverse events, CatC inhibitors appear to neither increase the risk of dental-related adverse events, nor result in a higher incidence of bacterial infections. Small numerical increases in skin-related effects have been observed in trials of CatC inhibitors, with a small number of hyperkeratosis cases reported [138, 141, 144]. In general, these skin effects were low in number and severity, with no need for treatment discontinuation in most cases [138, 141, 144]. Moreover, CatC inhibitors are not expected to completely inhibit CatC, and thereby only reduce (do not completely abolish) NSP activity, mitigating potential adverse events. Pre-clinical studies have confirmed that CatC inhibition does not interfere with normal inflammatory processes, again supporting the view that CatC inhibition is unlikely to interrupt conventional host defence mechanisms or increase the risk of infections [145].

In pre-clinical studies, both BI 1291583 and brensocatib showed a high selectivity for CatC over other cathepsins [139, 146], with BI 1291583 inhibiting NE in a dose-dependent manner [139] and brensocatib inhibiting NE in a concentration-dependent manner [146]. Pre-clinical data on BI 1291583 have also shown that BI 1291583 distributes preferentially to bone marrow [139]; in contrast, brensocatib distributes near equally between bone marrow and plasma [139]. At the time of the development of this review, no pre-clinical data were available for the HSK31858 compound.

In terms of efficacy, the first CatC inhibitor to be tested in people with bronchiectasis was brensocatib, examined in the phase II WILLOW study [141]. After 24 weeks, brensocatib significantly improved clinical outcomes, prolonging the time to first exacerbation and reducing the risk of exacerbation compared with placebo [141]. In this study, brensocatib also reduced all three NSPs of interest (NE, PR3 and CatG) in sputum [147]. Furthermore, treatment with brensocatib resulted in significant decreases in MUC5AC levels and increases in SLPI levels [148], showing the downstream impact of CatC inhibition on aspects of the vicious vortex. *Via* increases in SLPI, CatC inhibition with brensocatib aids in restoring the protease–antiprotease balance in the bronchiectasis lung; similarly, *via* reductions in MUC5AC, it is possible that mucociliary clearance may stabilise following CatC inhibition [148].

BI 1291583 is being investigated in phase II trials in people with bronchiectasis and CF-related bronchiectasis [144, 149–151]. The dose-finding AIRLEAF trial in people with bronchiectasis demonstrated a significant dose-dependent benefit of BI 1291583 over placebo based on the time to first exacerbation, and the safety profile of BI 1291583 was similar to placebo [144]. Numerical improvements were also observed in exacerbation-, quality-of-life- and pulmonary function-related parameters [144]. Additionally, the CLAIRAFLY trial, investigating the safety, tolerability, pharmacokinetics and pharmacodynamics of BI 1291583 in people with CF-related bronchiectasis [150] is complete, and the CLAIRLEAF rollover trial, assessing long-term safety and efficacy in people who participated in parent trials (AIRLEAF and CLAIRAFLY) [151], is ongoing. The large phase III AIRTIVITY trial is also planned to commence in 2025 [144].

HSK31858 also led to reductions in the activity of NE, PR3 and CatG in a phase II study [143]. This was accompanied by a markedly reduced exacerbation frequency and a longer time to first exacerbation compared with placebo [143]. The results from the phase II trials of brensocatib (WILLOW), BI 1291583 (AIRLEAF) and HSK31858 underscore the importance of reducing collective NSP activity (and not just focusing on single NSP inhibition) in providing adequate responses to therapy in the context of bronchiectasis.

Brensocatib has now completed phase III development and results from the ASPEN trial support previous findings [152]. In ASPEN, brensocatib treatment significantly reduced the annualised exacerbation rate, prolonged the time to first exacerbation, and reduced lung function decline at the 25 mg dose [152], further validating CatC inhibition as an appropriate therapeutic target to counteract the effects of neutrophilic inflammation in bronchiectasis. Noting the promising results with brensocatib, and similar results with other CatC inhibitors at earlier phases of development, the field of bronchiectasis may anticipate improvements in individual quality of life and reductions in the economic burden of bronchiectasis on healthcare systems. Effect sizes across the various phase II/III studies varied from a ∼20–60% reduction in exacerbation frequency compared with placebo [141, 143, 144, 152]. Exacerbation rates changed during the coronavirus disease 2019 pandemic [153, 154], and response to treatments may have been impacted as a result (as reported in the PROMIS trials of inhaled colistimethate sodium) [155]. Because the AIRLEAF and ASPEN trials were partially conducted during the pandemic [156, 157], whether there are true clinically meaningful differences between subgroups or compounds remains to be determined. Though high and low responders were identified in these trials, further research is needed to understand the underlying differences in responsiveness to therapy. To date, there are no head-to-head trials planned between the three CatC inhibitors that are in development.

Conclusions

In summary, bronchiectasis is a heterogeneous disease with multiple aetiologies, resulting in a significant clinical burden in both adults and children. The establishment of appropriate biomarkers across bronchiectasis aetiologies is crucial to identifying people at high risk of disease progression and calls for the implementation of tailored clinical management plans according to people's needs. A commonality across all bronchiectasis aetiologies is the association between disease severity and high, uncontrolled NSPs, of which NE is a widely validated biomarker. CatC inhibition, to inhibit NSP activity, appears to be a viable therapeutic approach to treat bronchiectasis. CatC inhibition may reduce mortality and the risk of exacerbations, improve quality of life and lung function, and lessen the economic burden on healthcare systems. The identification of NSPs as relevant, promising biomarkers in bronchiectasis provides a reference point from which to assess the efficacy and relevance of novel therapeutic agents undergoing testing in clinical trials for bronchiectasis.

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