EUROPEAN RESPIRATORY journal

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Review

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Please cite this article as: Porsbjerg CM, Sverrild A, Lloyd CM, *et al*. Anti-alarmins in asthma: targeting the airway epithelium with next-generation biologics. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.00260-2020).

# Anti-alarmins in asthma: targeting the airway epithelium with next-generation biologics

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**Take-home message**

Blocking epithelial alarmins, upstream mediators triggered early in the asthma inflammatory response that orchestrate broad inflammatory effects, is a promising alternative approach to asthma treatment, which may be effective in a broad patient population.

## ABSTRACT

Monoclonal antibody therapies have significantly improved treatment outcomes for patients with severe asthma; however, a significant disease burden remains. Available biologic treatments, including anti-immunoglobulin (Ig) E, anti-interleukin (IL)-5, anti-IL-5Rα and anti-IL-4Rα, reduce exacerbation rates in study populations by approximately 50% only. Furthermore, there are currently no effective treatments for patients with severe, type 2 (T2)-low asthma. Existing biologics target immunologic pathways that are downstream in the T2 inflammatory cascade, which may explain why exacerbations are only partly abrogated. For example, T2 airway inflammation results from several inflammatory signals in addition to IL-5. Clinically, this can be observed in how fractional exhaled nitric oxide ($Fe_{NO}$), which is driven by IL-13, may remain unchanged during anti-IL-5 treatment despite reduction in eosinophils, and how eosinophils may remain unchanged during anti-IL-4Rα treatment despite reduction in $Fe_{NO}$. The broad inflammatory response involving cytokines including IL-4, IL-5 and IL-13 that ultimately results in the classic features of exacerbations (eosinophilic inflammation, mucus production and bronchospasm) is initiated by release of ‘alarmins’ thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 from the airway epithelium in response to triggers. The central, upstream role of these epithelial cytokines has identified them as strong potential therapeutic targets to prevent exacerbations and improve lung function in patients with T2-high and T2-low asthma. This article describes the effects of alarmins and discusses the potential role of anti-alarmins in the context of existing biologics. Clinical phenotypes of patients who may benefit from these treatments are also discussed, including how biomarkers may help identify potential responders.

**Keywords:** Cytokines; Interleukin-25; Interleukin-33; Thymic stromal lymphopoietin

## Introduction

Asthma is a chronic, inflammatory disease of the airways that affects over 300 million individuals worldwide [1]. Approximately 5–10% of these individuals have severe asthma [2, 3]. Despite a regimen of multiple maintenance medications, patients with severe asthma carry a substantial burden of disease, experiencing frequent exacerbations and having high rates of healthcare resource use, which are associated with substantial costs [4, 5]. These patients also have severely impaired health-related quality of life, with numerous aspects adversely affected, including sleep, work, study, exercise and daily activities. Comorbidities in severe asthma are common and include chronic rhinosinusitis, nasal polyposis and atopic dermatitis [6].

The management of severe asthma is a considerable challenge, particularly in patients with recurrent exacerbations, because these patients are often receiving maximal inhaled therapy and may require regular oral corticosteroid (OCS) treatment [7]. Until relatively recently, there were few alternatives to regular OCS in these patients, but the advent of monoclonal antibody therapies provided physicians with important additional options for exacerbation prevention, potentially enabling tapering or discontinuation of OCS [8]. These biologic therapies target the signalling pathways involved in type 2 (T2) inflammation, mediated by the action of cytokines, such as interleukin (IL)-4, IL-5 and IL-13. These pathways play an important role in the pathogenesis of T2-high asthma, which is characterised by high levels of T2 inflammation, as measured using biomarkers such as blood and sputum eosinophils, and fractional exhaled nitric oxide ($Fe_{NO}$) [9]. However, currently available biologic therapies, comprising anti-immunoglobulin (Ig)E, anti-IL-5, anti-IL-5Rα and anti-IL-4Rα monoclonal antibodies (the latter blocking the IL-4 and IL-13 pathways),

decrease exacerbation rates in study populations by approximately 50% only [10-12]. Furthermore, anti-IgE, anti-IL-5 and anti-IL-4/IL-13 therapies typically produce variable improvements in lung function and symptom scores, although some individuals may experience clinically significant improvements [10-13]. An explanation for this lack of complete efficacy may be that each therapy targets only some of the elements of the pathways that regulate T2 inflammation, leaving other elements of the disease pathophysiology untreated; hence, there is a need for treatments with broader effects on T2 inflammation. Furthermore, we urgently need treatments for patients with T2-low asthma, in whom the disease shows less involvement of T2 pathways [9]. Defining and therefore diagnosing T2-low asthma is made challenging by the lack of reliable biomarkers. Although sputum neutrophilia (in the absence of sputum eosinophilia) may be present in some patients, diagnosis of this asthma subtype is generally inferred from the absence of T2 biomarker elevation [14]. Related to this, it is important to emphasise that the use of corticosteroids, as recommended in patients with severe asthma, reduces eosinophil counts and can therefore confound T2-low classification [15]. For the purposes of this paper, we define T2-low asthma as asthma with low T2 biomarker levels.

## Inflammatory pathways in asthma

Within the T2-high subtype, the major pathways involved in airway inflammation (*i.e.* IL-4, IL-13 and IL-5 signalling) are driven by T helper type 2 (Th2) cells and type 2 innate lymphoid cells (ILC2) [16]. IL-4 signalling is central to B cell class switching and triggers the release of IgE from B cells, resulting in airway hyperresponsiveness among other effects [17]. IL-13 is believed to be a central regulator of IgE synthesis, as well as of mucus hypersecretion, airway hyperresponsiveness and fibrosis (an

element of airway remodelling) [18, 19]. IL-5 is a key effector of eosinophilic inflammation [20], which results in airway remodelling [21, 22].

The different T2 pathways have varying prominence across clinical phenotypes, resulting in distinct patterns of inflammatory biomarkers [23]. For instance, patients with early-onset allergic asthma have high serum levels of allergen-specific IgE and total IgE, and elevated $Fe_{NO}$ [23, 24], which is characteristic of inflammation predominantly driven by Th2 cells and/or IL-4/IL-13. In contrast, late/adult-onset eosinophilic asthma is a particularly severe form of the disease characterised by higher levels of sputum eosinophils than early-onset asthma [25], as well as elevated blood eosinophils [24, 26], related predominantly to IL-5-mediated inflammation. Elevated $Fe_{NO}$, driven by IL-13, is also characteristic of this phenotype [27]. In the clinic, it may be observed that $Fe_{NO}$ remains high in these patients during anti-IL-5 treatment, despite the reduction in eosinophils [28, 29]. Similarly, anti-IL-4Rα treatment can reduce $Fe_{NO}$, but transient eosinophilia may be observed [8]; caution should therefore be taken when considering anti-IL-4Rα for patients with a history of hypereosinophilic conditions [30]. Alternatively, mixed granulocytic asthma may be present, in which there are elevated levels of both eosinophils and neutrophils [24]. In adults with non-eosinophilic asthma, within the T2-low subtype, there is airway inflammation with a lack of eosinophils [24]. Instead, the dominant inflammatory cell types in blood and sputum may include neutrophils, or there may be very few inflammatory cells, termed paucigranulocytic inflammation [24, 31, 32].

Our understanding of the role of the airway epithelium in driving asthma exacerbations has advanced considerably in recent years, from a simple passive

barrier function to an immunologically active front-line, initiating early responses to external triggers and orchestrating the resulting inflammatory cascade. As the interface between the external environment and the tissue of the bronchi, the airway epithelium is in constant contact with an array of stimuli, such as infectious agents, environmental allergens and atmospheric pollutants. The epithelium has been shown to mediate complex inflammatory processes in response to these allergic and non-allergic triggers, including the release of a trio of epithelial cytokines, known as 'alarmins' [33]. The alarmins thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 instigate inflammatory responses via numerous downstream pathways, including T2 (IL-4, IL-13 and IL-5) (**figure 1**) and others such as T1 or T17 (IL-17), resulting in various pathophysiologic outcomes that may lead to asthma symptoms and exacerbations [33]. It should be noted here that the term 'alarmin' is not limited to these three epithelial cytokines; other proteins produced in response to microbial invasion or tissue injury may also be referred to as alarmins [34].

## Understanding the pathophysiology of acute exacerbations: a path to better treatments?

To improve management options for patients with asthma, we require a better understanding of the mechanisms driving acute asthma exacerbations. Exacerbations are characterised by increasing symptoms, including dyspnoea, wheezing, cough and sputum, owing to increased inflammation and bronchospasm. They may be triggered by exposure to environmental allergens or pollutants but are most often provoked by viral infection (most commonly with rhinovirus) [35-37]. As with the overall patterns of chronic inflammation in patients with asthma described above, the pattern of inflammatory pathways activated during acute exacerbations is

also heterogeneous, with some patients having eosinophilic inflammation and others having neutrophilic, mixed or paucigranulocytic inflammation [38-40]. In patients with eosinophilic exacerbations, inflammation appears to be driven by multiple, simultaneously activated T2 pathways (*e.g.* both IL-5 and IL-4/IL-13). This can be inferred from human allergen challenge studies that show increases in airway IL-5, IL-4 and IL-13 levels during virus-induced asthma exacerbations [41, 42], as well as proteomic data showing elevated sputum levels of both IL-5 and IL-13 in patients with eosinophilic asthma [43]. With regard to the considerable proportion of patients who have non-eosinophilic, neutrophilic inflammation in relation to an exacerbation [24], less is understood about the pathways involved in non-eosinophilic exacerbations, although they may involve IL-17 and IL-23 [44, 45]. There is also evidence of airway microbiome involvement because subclinical infection by particular bacterial species is associated with neutrophilic, steroid-resistant asthma [46, 47].

Potential associations between an inflammatory asthma phenotype and specific exacerbation triggers are also poorly understood. Virus-induced exacerbations are more common in patients with allergic asthma [35], as well as in patients with elevated $Fe_{NO}$ or blood eosinophils [48], suggesting a link between virus-induced exacerbations and T2 inflammation. Eosinophils, activated by IL-5, drive pathophysiology in exacerbating and stable eosinophilic asthma. Eosinophils have recently been found to play a role in the defence against viruses. However, in patients with asthma, the degree to which eosinophils can carry out this role correlates with disease severity [49], explaining why patients with asthma and high eosinophil counts still experience virus-induced exacerbations. This may also explain

the lack of efficacy of anti-IL-5 therapy in mild asthma. For example, in a virus challenge study in patients with mild asthma, anti-IL-5 altered elements of the immune response, including B lymphocytes, macrophages and neutrophils, as well as attenuated eosinophils, but there were no associated improvements in lung function [50]. Less is known about potential links between asthma phenotypes and other associated exacerbation triggers.

There is a pressing need for better understanding of the drivers of exacerbations. One route towards achieving this is to study the impact of specific biologics on patient exacerbations with respect to their phenotypes and triggers. A retrospective analysis of exacerbations in a trial in patients with severe eosinophilic asthma receiving anti-IL-5 treatment with mepolizumab found that exacerbations occurring during treatment were associated with a lower induced sputum eosinophil count and less wheezing than those occurring in patients receiving placebo. This was particularly apparent in patients who had not started rescue treatment with corticosteroids, suggesting that exacerbations were less severe with mepolizumab [51]. This finding is corroborated by previous observations that non-eosinophilic exacerbations are less severe than eosinophilic exacerbations [52]. Data from ongoing studies designed to explore the nature of exacerbations in patients being treated with biologics, such as anti-IL-5 monoclonal antibodies [53], are keenly anticipated. With our current limited knowledge, without full appreciation of the heterogeneity of inflammation in acute exacerbations, prescription of treatments targeting specific inflammatory pathways may not effectively prevent all exacerbations. Therefore, targeting multiple inflammatory pathways may be a more effective approach.

## Epithelial alarmins: early orchestrators of airway inflammation in acute asthma

### *Role of TSLP and IL-33 in T2-high asthma*

There is substantial evidence that the alarmins, TSLP and IL-33, play key roles in driving T2 inflammation in asthma. TSLP levels in bronchial lavage fluid and biopsies are elevated in patients with asthma compared with healthy individuals, and correlate with disease severity (including a negative correlation with lung function defined by forced expiratory volume in 1 second [$FEV_1$]) [54-57]. In addition, genetic studies of TSLP have identified alleles that are associated with asthma [58]. Similarly, expression levels of IL-33 and its receptor ST2 in serum and bronchial biopsy samples are higher in patients with asthma than in healthy individuals, and positively correlate with disease severity [59-61]. Additionally, patients with allergic and eosinophilic asthma phenotypes have higher serum levels of IL-33 than those with non-allergic and non-eosinophilic phenotypes [62]. Furthermore, in patients with allergic asthma, ST2 expression on eosinophils from blood and sputum is significantly upregulated after allergen inhalation challenge [54]. Higher serum soluble ST2 levels are associated with an increased risk of exacerbations [63]. Certain alleles of IL-33 and its receptor are associated with asthma [58], and a rare IL-33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma [64]. Bronchial allergen challenge directly increases airway expression of all three alarmins (TSLP, IL-33 and IL-25) in patients with allergic asthma, to a degree correlating with the degree of airway obstruction [42].

TSLP has been shown to drive various elements of asthma pathophysiology, including airway hyperresponsiveness, mucus overproduction and airway remodelling, via effects triggered downstream. However, it should be noted that not all these elements have been demonstrated conclusively in humans. During T2 inflammatory responses, TSLP potently activates dendritic cells and induces production of the Th2-attracting chemokines thymus and activation-regulated chemokine (TARC; also known as CCL17) and macrophage-derived chemokine (CCL22). TSLP-activated dendritic cells prime naïve Th cells to produce various inflammatory mediators, including IL-4, IL-5 and IL-13 [65]. The IL-5 secreted from polarised Th2 cells mediates eosinophilic inflammation via effects on eosinophil recruitment, maturation and survival [66]. When activated, eosinophils release cysteinyl leukotrienes, which are potent bronchoconstrictors [22]. They also induce airway remodelling through airway smooth muscle cell proliferation [21] and the release of mediators, such as transforming growth factor-β, cationic proteins and cytokines, as well as through interactions with mast cells and epithelial cells [22]. Mast cells also release acute-phase inflammatory mediators, including cysteinyl leukotrienes, histamine and prostaglandins [67]. IL-4 and IL-13 secreted from polarised Th2 cells activate B cells, which produce IgE, resulting in airway hyperresponsiveness [18] and triggering mast cell degranulation, leading to vascular permeability [68]. IL-13 also increases mucus production via goblet cell proliferation, and airway inflammation via increased epithelial production of $Fe_{NO}$ [19]. In addition to Th2 cells, TSLP activates multiple other cell types that release IL-13, including mast cells, basophils and ILC2s [69-71].

Meanwhile, IL-33 is thought to act as a positive regulator of TSLP dendritic cell signalling, initiating and maintaining Th2 cell-mediated inflammatory responses [72]. IL-33 drives airway hyperresponsiveness through IL-13-mediated mast cell–airway smooth muscle crosstalk. Human lung mast cells are stimulated by IL-33 to release histamine and IL-13, resulting in airway constriction [60]. Further roles of IL-33 include promoting mast cell survival and cytokine production [73], eosinophil survival [74], basophil activation, survival and proliferation [71], and increasing IL-13 release from ILC2s [75].

***Role of IL-25 in T2-high asthma***

Of the three alarmins, the actions of IL-25 in patients with asthma are probably the least understood. IL-25 appears to play a key role in allergic inflammation, particularly as a driver of the T2 inflammatory response during virus-induced asthma exacerbations [76]. Elevated plasma levels of IL-25 are associated with the allergic asthma phenotype [77], and IL-25 concentration in sputum correlates with disease severity [78]. Sputum IL-25 is also increased in atopic versus non-atopic asthma patients [78]. In addition, a rare allele of a component of the IL-25 receptor, IL-17RB, is associated with a reduced incidence of asthma [79].

After inhaled allergen exposure, there is increased expression of IL-25 and its receptor in the bronchial mucosa and dermis (collected by skin biopsy) [80], and in circulating eosinophils and airway dendritic cells [81, 82] in patients with allergic asthma. Bronchial epithelial expression of IL-25, but not IL-33 or TSLP, is heterogeneous in patients with asthma. Those expressing high levels of IL-25 have greater airway hyperresponsiveness and remodelling, increased blood and airway

eosinophilia, and higher allergen skin-test reactivity, with elevated IgE, than those expressing low levels of IL-25 [83]. Aside from epithelial cells, which form the principal location of all three alarmins, IL-25 release appears to be prominently localised to eosinophils [42]. IL-25 is hypothesised to play a role in allergen-induced trafficking of eosinophil-lineage committed progenitor cells to the airways, and local differentiation, promoting tissue eosinophilia during asthmatic responses [84].

***Role of TSLP in T2-low asthma***

Much less is known about the role of alarmins in T2-low asthma than in T2-high asthma; most of our understanding comes from animal studies, with relatively few studies having been conducted in humans or human cells. Of the three alarmins, TSLP has the most compelling evidence for an effect in T2-low asthma. Anti-TSLP treatment has been shown to reduce exacerbations in patients with severe, uncontrolled asthma and low levels of blood eosinophils and $Fe_{NO}$ (as discussed later) [85]. TSLP is thought to play a role in neutrophilic, T2-low airway inflammation by activating dendritic cells to induce polarization of naïve T cells towards a Th17 phenotype [86]. Th17 polarization and subsequent IL-17 release promotes neutrophilic inflammation and a non-allergic/non-eosinophilic response [87]. TSLP, but not IL-33 or IL-25, expression in human bronchoalveolar lavage fluid has been shown to correlate most closely with neutrophil infiltration [54]. Nevertheless, future studies are warranted to understand whether there is a broader role for IL-33 in the pathogenesis of T2-low asthma.

***Role of the alarmins in steroid-refractory asthma***

Airway levels of ILC2s are elevated in several phenotypes of asthma, including late-onset eosinophilic asthma [25, 88-90], and may be the key driver of eosinophilic inflammation in these patients. Although individual alarmins do not directly promote proliferation of ILC2s, TSLP promotes the longevity of ILC2s, while ILC2 activation is promoted by IL-33, especially when in combination with TSLP (or IL-2) [70]. In a recent, *in vitro* study of human blood and lung ILC2s from patients with asthma and healthy controls, IL-25- and IL-33-stimulated expression of T2 cytokines by blood ILC2s was inhibited by steroid treatment; however, TSLP-stimulated T2 cytokine expression was not [91]. TSLP levels were elevated in bronchoalveolar lavage fluid in patients with asthma versus healthy controls and correlated with the level of steroid resistance. TSLP was shown to induce steroid resistance in the ILC2s [91]. TSLP-initiated T2 signalling may therefore be particularly important in severe asthma patients with steroid-refractory disease and both Th2- and ILC2-driven inflammation. Non-eosinophilic asthma is also associated with steroid resistance [92], linked to high IL-17 and high numbers of neutrophils in these patients, potentially resulting from TSLP signalling (as described above) [44, 87].

## Targeting the airway alarmins with biologic therapies

The inability of current biologic therapies to prevent all asthma exacerbations and the complexity of the inflammatory pathways involved in asthma, provide a strong rationale for finding alternative, more effective means of reducing airway inflammation and thereby preventing exacerbations. The central, upstream role of alarmins makes them attractive potential therapeutic targets in this regard. Current biologics are not disease modifying, and available evidence suggests that asthma severity returns to pre-treatment levels after stopping treatment with them [93, 94].

Blocking alarmins, however, has the potential to inhibit airway hyperresponsiveness and remodelling [95] and produce sustained reductions in disease activity. Biologics targeting the alarmins are now in development, with anti-TSLP and anti-IL-33 therapies having entered clinical trials. The current evidence for the effects of alarmin blockade in humans is summarised in **table 1** and **figure 2**.

***Anti-TSLP***

Tezepelumab, an anti-TSLP human monoclonal antibody that prevents TSLP interacting with its receptor complex [96], is the anti-alarmin that has reached the furthest stage of clinical development (**table 1**). In a proof-of-concept allergen challenge study in patients with mild allergic asthma, tezepelumab 700 mg was administered every 4 weeks for 12 weeks, with an inhaled allergen challenge at baseline and after 6 and 12 weeks [96]. $FEV_1$ reductions post-allergen challenge at week 6 and week 12 were significantly smaller with tezepelumab than with placebo (34% and 46%, respectively). In addition, patients receiving tezepelumab had significant decreases in levels of blood and sputum eosinophils before and after allergen challenge. Similarly, $Fe_{NO}$ levels dropped throughout the study, and post-allergen $Fe_{NO}$ increases were significantly reduced. These results paved the way for a phase 2b study in patients with moderate-to-severe uncontrolled asthma, who were administered tezepelumab 70 mg every 4 weeks, 210 mg every 4 weeks or 280 mg every 2 weeks, or placebo every 2 weeks, over 52 weeks [85]. Tezepelumab was well tolerated and significantly reduced annualised asthma exacerbation rates in the tezepelumab groups versus placebo (by 61%, 71% and 66%, respectively). Similar results were observed regardless of patients' blood eosinophil counts at enrolment. Pre-bronchodilator $FEV_1$ at week 52 improved within the first month of tezepelumab treatment relative to placebo and persisted throughout the study, as did

improvements in patient-reported outcomes. In an exploratory analysis of the phase 2b data, tezepelumab was found to reduce annualised asthma exacerbation rates irrespective of patients' baseline blood eosinophil count, level of $Fe_{NO}$, or serum levels of IgE, IL-5, IL-13, periostin or TARC [97]. In addition, levels of these proinflammatory biomarkers and cytokines were reduced as early as 4 weeks after treatment initiation; the reductions were then maintained throughout the 52-week treatment period.

Taken together, these findings indicate that tezepelumab has broad inhibitory effects on multiple T2 inflammatory mediators of asthma, suggesting that anti-TSLP treatment may be beneficial in patients with a range of inflammatory phenotypes (**table 2**). As described above, because TSLP is postulated to initiate T2 inflammation via both Th2 and ILC2 cells, blocking TSLP with tezepelumab may effectively reduce inflammation in patients with steroid-refractory asthma. Furthermore, the observation that tezepelumab was efficacious in patients with low blood eosinophils and low $Fe_{NO}$ indicates that it may be effective in the T2-low asthma subtype. By contrast, the existing biologics targeting T2 pathways, such as anti-IL-4R and anti-IL-5, have shown less efficacy in patients with low versus high eosinophil counts [98-100]. Whether tezepelumab is effective in patients with T2-low asthma is yet to be confirmed and will depend on how T2-low asthma is defined in terms of biomarkers in future clinical studies.

Several clinical investigations assessing the efficacy and safety of tezepelumab in different patient populations are ongoing, with results yet to be published (**table 3**). Two phase 2 (NCT02698501 and NCT03688074) and two phase 3 studies are

ongoing: one investigating the efficacy and safety of tezepelumab in reducing OCS use in adults with OCS-dependent asthma (NCT03406078), and one investigating the efficacy and safety of tezepelumab in adults and adolescents with severe, uncontrolled asthma (NCT03347279).

In addition to tezepelumab, CSJ117, an anti-TSLP monoclonal antibody fragment delivered by inhalation, is in phase 1 development (NCT03138811), with results not yet published.

***Anti-IL-33***

Monoclonal antibodies targeting IL-33 or ST2 are in clinical development, with several in phase 2 trials (**table 3**), but results from these studies have not yet been published in peer-reviewed journals. AMG282 (RG6149/MSTT1041A) is being evaluated in a 12-month phase 2b study in patients with uncontrolled, severe asthma, with the primary outcome being the rate of exacerbations (NCT02918019). REGN3500 (SAR440340) was recently trialled alone and in combination with dupilumab in a 12-week phase 2a study in patients with moderate-to-severe asthma (NCT03387852). Top-line findings from this study are available but are yet to be published in a peer-reviewed journal. Although the anti-IL-33 antibody met its primary endpoint of reducing the incidence of 'loss of asthma control' events compared with placebo, outcomes were no better than those seen with dupilumab monotherapy. In addition, combined REGN3500 and dupilumab did not demonstrate increased benefit compared with dupilumab alone [101]. Two earlier phase 1 studies of REGN3500 have also been completed (NCT03112577, NCT02999711). Etokimab (ANB020) has recently been evaluated in a proof-of-concept phase 2a study in

adults with severe eosinophilic asthma, including a primary outcome of change in blood eosinophil count (NCT03469934). GSK3772847 (CNTO7160) has recently completed a phase 2a study in patients with moderately severe asthma, with the primary outcome being loss of asthma control events at week 16 (NCT03207243). A 28-week phase 2b study in patients with moderate-to-severe asthma with allergic fungal airway disease is ongoing, with primary outcomes of change from baseline in blood eosinophil count and $Fe_{NO}$ levels at week 13 (NCT03393806).

***Anti-IL-25***

Although IL-25 appears to play a key role in allergic inflammation, particularly during virus-induced asthma exacerbations (as described above), no clinical studies of anti-IL-25 antibodies are in progress.

***Patients who may benefit from anti-alarmin treatment***

From a clinical perspective, there are several groups of patients who may benefit from the potentially broad anti-inflammatory effects of anti-alarmins, such as those with multiple activated T2 pathways. For example, in patients with allergic eosinophilic asthma, it may be difficult to discern whether exacerbations are primarily driven by allergen exposure or are related to eosinophilic inflammation. The ability to target pathways driving both IgE and eosinophils with a single treatment may be a more attractive option than anti-IgE or anti-IL-5. A second example is those patients with non-allergic eosinophilic asthma who have very high $Fe_{NO}$, indicating concomitant activation of both IL-5 and IL-13, perhaps through activation of ILC2 cells. Although there is presently no evidence available to inform us whether these patients could achieve sufficient control on either anti-IL-5 or anti-IL-4/13, it appears

plausible that some need a broader suppression of the T2 pathways. Thirdly, patients with severe eosinophilic asthma and comorbidities, such as nasal polyposis or atopic dermatitis, that do not improve with current biologic therapies may also have co-activation of cells releasing both IL-5 and IL-4/IL-13. This suggests that these patients may benefit from an upstream-targeted treatment. Other patients who may benefit from anti-alarmin therapy could include those with predominantly mast cell-driven disease or those with no clear pattern of T2 inflammation.

***Outstanding questions regarding anti-alarmins***

Although tezepelumab has thus far been found to be well tolerated in clinical studies, questions remain regarding the long-term safety of blocking the alarmins and the benefit–risk of anti-alarmin treatment compared with that of cumulative OCS exposure, for which the side effects are well-known and can lead to significant morbidity [102]. One area for potential exploration is the antimicrobial activity of TSLP, in particular the short form of TSLP (sfTSLP), which has been shown to exert potent antimicrobial activity [103]. It is therefore conceivable that blocking sfTSLP could result in a greater risk of infection.

Upregulation of alarmins has been implicated in a number of human cancers, although their role is controversial; in some neoplasms, TSLP appears to play a pro-tumorigenic role, while in others, TSLP seems to be protective [104]. As such, further studies are required to determine the precise role of TSLP in human cancer. Similarly, further studies are warranted to determine the effect of blocking IL-33 on cardiovascular disease, because IL-33 is thought to be cardioprotective [105].

An additional and important question is whether suppressing one alarmin alone is sufficient to suppress all inflammatory pathways induced by the alarmins. In this context, it is clearly important to understand the potential interactions between the alarmins and the relative importance of each alarmin in inflammatory responses. Using chronic models of helminth infection and T2 cytokine-driven lung inflammation, targeting all three alarmins (TSLP, IL-25 and IL-33) was found to be more efficacious than blocking a single alarmin alone [106]. Furthermore, disruption of all three mediators in a model of chronic house dust mite-induced allergic lung inflammation resulted in reduced inflammation, mucus production and lung remodelling. The authors concluded that the results of these studies suggest redundant roles for TSLP, IL-25 and IL-33 in the maintenance of T2 pathology and that these alarmins interact to potentiate their individual effects. An additional study found that both IL-33 and TSLP were required for epithelial cell IL-25 expression, mucus metaplasia and ILC2 expansion after early-life rhinovirus infection in mice [107]. The authors concluded that generation of mucus metaplasia involves a complex interplay among IL-25, IL-33 and TSLP. Nevertheless, blocking the TSLP pathway alone appears sufficient to suppress inflammation, as shown so far in the tezepelumab studies, albeit other anti-TSLP biologics such as RG7258 were not successfully developed into asthma therapies [108]. To date, no clinical studies have investigated the potential interactions between alarmins in patients with asthma, but it would be of importance to understand such interactions, both in terms of effects on the efficacy and safety of these treatments.

Of further interest is the mechanism by which anti-alarmins prevent exacerbations. It has been postulated that omalizumab exerts its beneficial effects through restoration

of innate antiviral immunity in plasmacytoid dendritic cells and through increasing the release of interferon-α on rhinovirus exposure, which is deficient in the presence of high IgE [109]. It is unknown whether anti-alarmins have an effect on antiviral immunity, and it could be speculated that such a mechanism may result in treatment benefit for patients with both T2-high and T2-low asthma. Mechanistic studies are required to determine how these new treatments work.

## Conclusions and future perspective

Monoclonal antibody therapies represent a new treatment era in severe asthma, offering patients who are exacerbating despite high-dose anti-inflammatory treatment a safer and more effective alternative option. However, because blocking specific T2 pathways with currently available biologics fails to prevent all exacerbations, a significant burden of disease remains. This limitation of current biologics may be a consequence of blocking pathways downstream in the immunological cascade, leaving others still active, as the partial suppression of T2 pathways may be insufficient to abrogate exacerbations in some patients. Blocking alarmins, upstream mediators triggered early in the inflammatory response that orchestrate broad T2 inflammatory effects, is a promising alternative approach that may be effective in a broader patient population.

Further research into the role of alarmins in asthma pathogenesis will improve our understanding of disease severity and the heterogeneity in response to current treatments. This research should include studies of differences between the roles of the alarmins in the different clinical phenotypes of severe asthma, such as late-onset eosinophilic asthma versus early-onset allergic asthma, as well as their relationship

with comorbidities such as atopic dermatitis and nasal polyposis. Understanding the clinical effect of each alarmin in patients with specific phenotypes, and in patients with a combination of asthma, atopic dermatitis and nasal polyposis, will be crucial not only for informing our ability to correctly select the appropriate anti-alarmin as a treatment, but also to our understanding of disease mechanisms in asthma. In addition to this, more studies in which exacerbations are phenotyped are also required to help to identify which types of exacerbation are reduced or otherwise by a given biologic treatment. Finally, mechanistic studies in humans are required to improve our understanding of how next-generation biologics work. This will help clinicians to select the most appropriate treatments for their patients.

## Acknowledgements

The authors thank Richard Claes, PhD of PharmaGenesis London, London, UK for providing medical writing support, which has been funded by AstraZeneca, Cambridge, UK, in accordance with Good Publication Practice 3 (GPP3) guidelines (http://www.ismpp.org/gpp3).

## Conflict of interest

CMP has received unrestricted research grants and honoraria for scientific presentations and consultancies from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Icepharma, Janssen, Nigaard, Nopharma, Novartis, Pharmaxis, Roche, Sandoz, Sanofi and Teva. AS has attended advisory board for AstraZeneca and Sanofi Genzyme and has received speaker fees from AstraZeneca and Novartis. CML is a Wellcome Senior Fellow in Basic Biomedical Science

(107059/15/Z). AMG has attended advisory boards for AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Novartis, Sanofi and Teva, and has received speaker fees from AstraZeneca, Boehringer Ingelheim, Novartis, Roche, Teva and Vectura. He has participated in research with AstraZeneca for which his institution has been remunerated and has attended international conferences with Teva. He has had consultancy agreements with AstraZeneca, Sanofi and Vectura. EB has received personal fees from AstraZeneca, Chiesi, GlaxoSmithKline, Novartis, Sanofi, Sterna and Teva.

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## TABLE 1 Clinical evidence of the effects of TSLP blockade in humans*

| Reference | Setting | Outcomes |
|---|---|---|
| [85] | Patients with uncontrolled moderate-to-severe asthma (phase 2b study) | • Reduced exacerbation rate irrespective of baseline blood eosinophil count<br>• Increased $FEV_1$<br>• Reduced blood eosinophils, $Fe_{NO}$, serum IgE throughout the 52-week treatment period |
| [110] | Patients with uncontrolled moderate-to-severe asthma (phase 2b study) | • Reduced blood eosinophils, $Fe_{NO}$, serum IgE, IL-5, IL-13, periostin and TARC throughout the 52-week treatment period<br>• Reduced exacerbation rate irrespective of baseline blood eosinophils, $Fe_{NO}$, serum IgE, IL-5, IL-13, periostin, TARC |
| [96] | Patients with mild allergic asthma (phase 1/2a study) after allergen challenge | • Reduced airway hyperresponsiveness<br>• Reduced bronchoconstriction<br>• Reduced blood and sputum eosinophils<br>• Reduced $Fe_{NO}$<br>• Reduced Th2:Th1 cell ratio in blood |
| [111] | Patients with mild allergic asthma (phase 1/2a study) after allergen challenge | • No change in T regulatory cell frequency |

$FE_{NO}$: fractional exhaled nitric oxide; $FEV_1$: forced expiratory volume in 1 second; IgE: immunoglobulin E; IL: interleukin; TARC: thymus and activation-regulated chemokine; Th: T helper; TSLP: thymic stromal lymphopoietin. *All data are from studies of anti-TSLP human monoclonal antibody treatment. No clinical data evaluating anti-IL-33 or anti-IL-25 treatment have yet been published in peer-reviewed journals.

**TABLE 2 Effects on T2 inflammatory biomarkers, and OCS-sparing potential, of anti-TSLP and approved biologic therapies for asthma**

| Biologic | Effect on inflammatory biomarker | | | | OCS-sparing data |
|---|---|---|---|---|---|
| | $Fe_{NO}$ | IgE | Blood eosinophils | Sputum eosinophils | |
| Tezepelumab (anti-TSLP) | ↓ [85, 96, 110] | ↓ [85, 96, 110] | ↓ [85, 96, 110] | ↓ [96] | Data pending (NCT03406078) |
| Dupilumab (anti-IL-4/IL-13) | ↓ [100] | ↓ [100] | ↑ [100] | Insufficient data [112] | ~70% reduction in daily OCS dosage (vs ~42% with placebo); 52% of patients achieved complete OCS weaning (placebo, 29%) [113] |
| Omalizumab (anti-IgE) | Minimal effects [114] | ↓ [115] | Minimal effects [116] | ↓ [117] | No RCT data; real-world data indicates reduction in OCS use [118] |
| Mepolizumab (anti-IL-5) | No effect [119] | No effect* | ↓ [119, 120] | ↓ [119, 120] | ~50% reduction in daily OCS dosage (vs 0% with placebo); 14% of patients achieved complete OCS weaning (placebo, 8%) [121] |
| Reslizumab (anti-IL-5) | No effect† | No effect* | ↓ [98, 122] | ↓ [123] | No RCT data; real-world data indicates reduction in OCS use [124] |
| Benralizumab (anti-IL-5Rα) | No effect† | No effect* | ↓ [99, 125] | ↓ [125] | ~75% reduction in daily OCS dosage (vs ~25% with placebo); 52–56% of patients achieved complete OCS weaning (placebo, 19%) [126] |

$Fe_{NO}$: fractional exhaled nitric oxide; IgE: immunoglobulin E; IL: interleukin; OCS, oral corticosteroid; RCT, randomized controlled trial; T2: type 2; TSLP: thymic stromal lymphopoietin. *Observed in clinical practice. †Expected based on observations with other anti-IL-5 biologics.

## TABLE 3 Ongoing and recently completed clinical studies of anti-alarmin treatments in patients with asthma

| Drug | ClinicalTrials.gov identifier | Phase | Population | Primary endpoint |
|---|---|---|---|---|
| ***Anti-TSLP*** | | | | |
| Tezepelumab | NCT02698501 | 2 | Asthma, taking daily ICS | Decrease in airway hyperresponsiveness to mannitol |
| | NCT03688074 | 2 | Uncontrolled asthma | Change from baseline in the number of airway submucosal inflammatory cells |
| | NCT03406078 | 3 | Severe, uncontrolled asthma | Reduction in OCS dose while not losing asthma control |
| | NCT03347279 | 3 | Severe, uncontrolled asthma | Annualised asthma exacerbation rate |
| CSJ117 | NCT03138811 | 1 | Mild, atopic asthma | Number of adverse events and serious adverse events |
| ***Anti-IL-33*** | | | | |
| REGN3500 (SAR440340) | NCT02999711 | 1 | Mild-to-moderate asthma | Number of treatment-emergent adverse events |
| | NCT03112577 | 1 | Mild, allergic asthma | Sputum inflammatory biomarkers |
| AMG282 (RG6149/MSTT1041A) | NCT02918019 | 2b | Severe, uncontrolled asthma | Rate of exacerbations |
| Etokimab (ANB020) | NCT03469934 | 2a | Severe, eosinophilic asthma | Change in blood eosinophil count |
| GSK3772847 (CNTO7160) | NCT03207243 | 2a | Moderately severe asthma | Loss of asthma control events at week 16 |

| | | | | |
|---|---|---|---|---|
| | NCT03393806 | 2b | Moderate-to-severe asthma with allergic fungal airway disease | Blood eosinophil count and $Fe_{NO}$ levels at week 13 |

$Fe_{NO}$: fractional exhaled nitric oxide; ICS: inhaled corticosteroids; IL: interleukin; OCS: oral corticosteroids; TSLP: thymic stromal lymphopoietin.

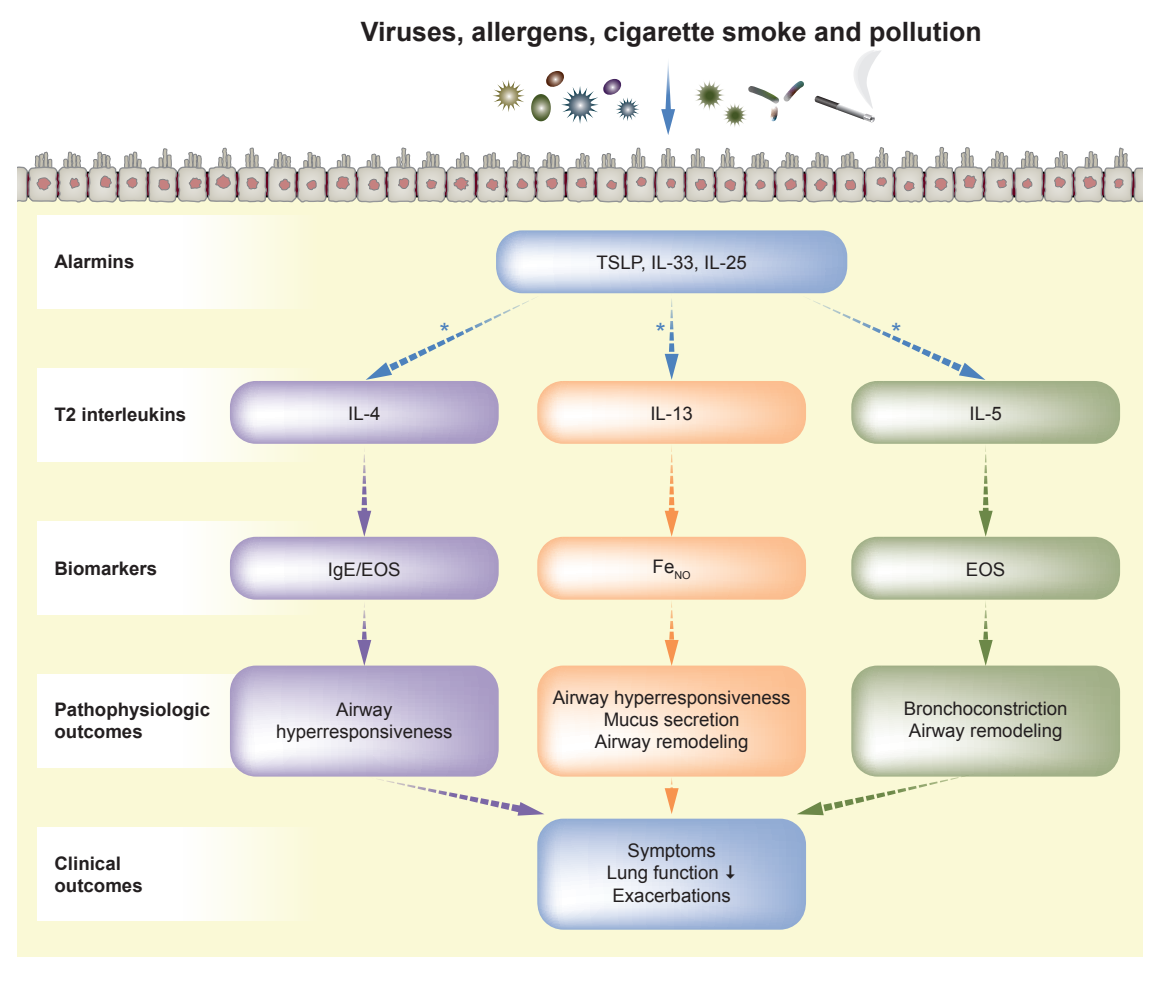
## FIGURE LEGENDS

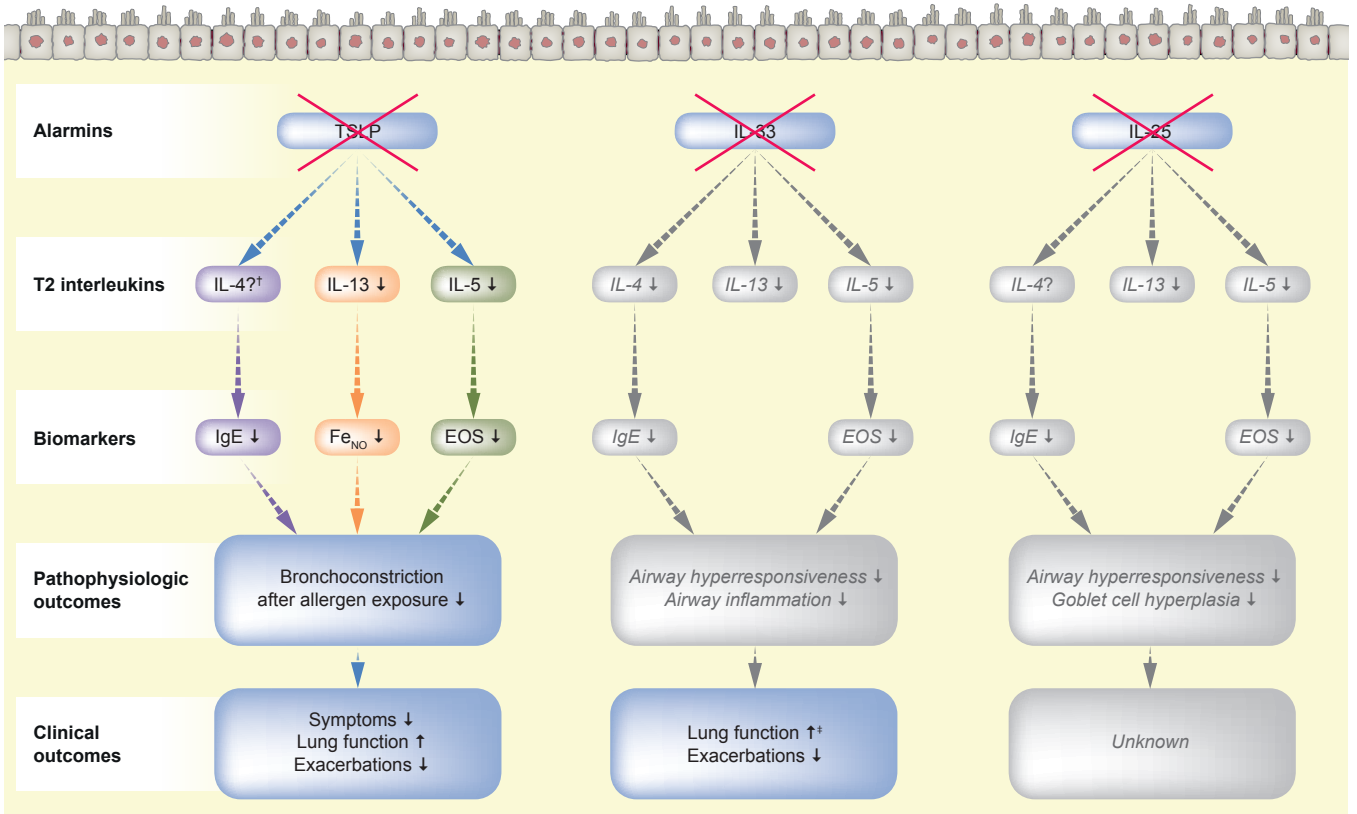
### FIGURE 1 Role of the alarmins in driving T2 inflammation, biomarkers and clinical outcomes in asthma.

EOS: eosinophils; $Fe_{NO}$: fractional exhaled nitric oxide; IgE: immunoglobulin E; IL: interleukin; ILC2: type 2 innate lymphoid cell; T2: type 2; Th2: type 2 T helper cell; TSLP: thymic stromal lymphopoietin. *Released from Th2 cells and ILC2s.

### FIGURE 2 Effects of blocking the alarmins on biomarkers and clinical outcomes in asthma.

EOS: eosinophils; $Fe_{NO}$: fractional exhaled nitric oxide; IgE: immunoglobulin E; IL: interleukin; T2: type 2; TSLP: thymic stromal lymphopoietin. Grey boxes represent findings from studies in mice, which are yet to be confirmed in humans [127, 128]. †Serum IL-4 was not measured in the tezepelumab phase 2b study. ‡Top-line findings reported from a phase 2a study of anti-IL-33 [101], which are yet to be published in a peer-reviewed journal.


Viruses, allergens, cigarette smoke and pollution
Alarmins
TSLP, IL-33, IL-25
*
*
*
T2 interleukins
IL-4
IL-13
IL-5
Biomarkers
IgE/EOS
Fe$_{NO}$
EOS
Pathophysiologic outcomes
Airway hyperresponsiveness
Airway hyperresponsiveness
Mucus secretion
Airway remodeling
Bronchoconstriction
Airway remodeling
Clinical outcomes
Symptoms
Lung function ↓
Exacerbations


Alarmins
TSLP
IL-33
IL-25
T2 interleukins
IL-4?†
IL-13 ↓
IL-5 ↓
IL-4 ↓
IL-13 ↓
IL-5 ↓
IL-4?
IL-13 ↓
IL-5 ↓
Biomarkers
IgE ↓
$Fe_{NO}$ ↓
EOS ↓
IgE ↓
EOS ↓
IgE ↓
EOS ↓
Pathophysiologic outcomes
Bronchoconstriction after allergen exposure ↓
Airway hyperresponsiveness ↓
Airway inflammation ↓
Airway hyperresponsiveness ↓
Goblet cell hyperplasia ↓
Clinical outcomes
Symptoms ↓
Lung function ↑
Exacerbations ↓
Lung function ↑‡
Exacerbations ↓
Unknown