

REVIEW ARTICLE

Mechanisms and therapeutic strategies for non-T2 asthma

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Abstract

Non-T2 asthma is traditionally defined as asthma without features of T2 asthma. The definition is arbitrary and is generally based on the presence of neutrophils in sputum, or the absence (or normal levels) of eosinophils or other T2 markers in sputum (paucigranulocytic), airway biopsies or in blood. This definition may be imprecise as we gain more knowledge from applying transcriptomics and proteomics to blood and airway samples. The prevalence of non-T2 asthma is also difficult to estimate as most studies are cross-sectional and influenced by concomitant treatment with glucocorticosteroids, and by the presence of recognized or unrecognized airway infections. No specific therapies have shown any clinical benefits in patients with asthma that is associated with a non-T2 inflammatory process. It remains to be seen if such an endotype truly exists and to identify treatments to target that endotype. Meanwhile, identifying intense airway neutrophilia as an indicator of airway infection and airway hyperresponsiveness as an indicator of smooth muscle dysfunction, and treating them appropriately, and not increasing glucocorticosteroids in patients who do not have obvious T2 inflammation, seem reasonable.

KEYWORDS

neutrophilic asthma, non-T2 asthma, paucigranulocytic asthma, sputum cell counts, Th17 cells

1 | INTRODUCTION

Asthma is a heterogeneous disease involving diverse pathobiological mechanisms (endotypes) with different clinical presentations (phenotypes). One of the earliest demonstration of this was by Wenzel and colleagues who described eosinophil-positive and eosinophilic-negative inflammation in bronchial mucosal biopsies¹ and provided early indications of their distinct pathologic and clinical features. Since then, there has been substantial research to describe allergic asthma and nonallergic eosinophilic asthma, which has sometimes (incorrectly) been used to be synonymous with Type 2 (or Type 2 high) asthma, which is characterized by Type 2 inflammation involving Type 2 (Th2) lymphocytes (CD4+) that secrete proteins such as interleukin (IL)-4, IL-5, IL-13, and immunoglobulin E (IgE) resulting in the recruitment of cells such as eosinophils, basophils, and mast cells into the airways. The terminology has recently changed from "Th2

high" to "T2 high" reflecting the identification of nonclassic cells other than the CD4+ cells, such as the innate lymphoid cells group 2 (ILC2), which also contribute to this process. T2-high asthma also encompasses allergic asthma, which is mediated by allergen-specific IgE-dependent process. While eosinophils are almost always present in circulation or in the airways in these patients, they may not be the key effector cells in all patients; hence, T2 high asthma is not always synonymous with "allergic asthma" or "nonallergic eosinophilic asthma." On the other hand, "noneosinophilic asthma," also known as Non-Type 2 (non-T2 or T2-low) asthma, is less well characterized, where T helper 1 (Th1) and T helper 17 (Th17) cells, neutrophils and proteins including IL-1 β , IL-6, IL-8, IL-17A/F, IFN- γ , and TNF- α are implicated in its pathobiology. In addition to inflammatory markers, non-T2 asthma may also be driven by structural abnormalities involving airway smooth muscle (ASM) as well as irregular neuronal activation. Non-T2 asthma is further subclassified into neutrophilic and paucigranulocytic, depending on the cellular findings in sputum specimens. Asthma with mixed granulocytic inflammation

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might reflect a transition between eosinophilic and neutrophilic phenotypes which will be further discussed in subsequent sections. However, despite recent advances, inconsistencies still exist regarding various aspects of non-T2 asthma. This article aims to review the definition, mechanisms, current therapeutic strategies, and future potential targets of non-T2 asthma.

2 | DEFINITION AND PREVALENCE OF NON-T2 ASTHMA

There is no universally agreed definition of non-T2 asthma. In general, it is defined as a noneosinophilic asthma without the presence of Type 2 inflammatory markers. Using quantitative sputum cytometry, Simpson et al² showed that airway inflammation patterns in asthma could be categorized into eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic subtypes. However, there is no agreed cut-off for sputum neutrophils proportion to be regarded as neutrophilic asthma, where various cut-offs ranging from >40% to 76% neutrophils had been utilized.^{3,4} These generally do not take into consideration the total cellularity in sputum (ie, the total cell count). Very high total cell count (typically >25 million cells/g with >75% neutrophils) usually indicate an airway infection.⁵ Paucigranulocytic asthma (PGA) is defined as normal eosinophils and neutrophils proportions in sputum. Mixed granulocytic inflammation reflects presence of both neutrophils and eosinophils and may be a transition phenotype. These subtypes will be further elaborated in the following sections. Non-T2 inflammation in the airway lumen is not uncommon and may account for half to two-thirds of asthma patients.^{6,7} Most studies were conducted cross-sectionally but not longitudinally thus yielding only a point prevalence estimate, while sputum cellularity has been shown to change over time.⁷ Analyzing data from Asthma Clinical Research Network (ACRN), McGrath et al⁸ demonstrated that the persistently noneosinophilic asthma prevalence ranged from 47% to 72%, in subjects with and without ICS, respectively. Our experience has been different and has been more consistent with those of Cowan and colleagues who demonstrated that most patients (>90%) considered to have neutrophilic bronchitis may have a re-emergence of sputum eosinophils when their steroid doses are tapered for long enough.⁹ An important reason why eosinophils in sputum may be missed (and therefore misclassified as noneosinophilic) is the under-recognition of free eosinophil granules (in contrast to intact eosinophils)¹⁰ either in the matrix or within the macrophages.¹¹

3 | PHENOTYPES AND CLINICAL CHARACTERISTICS OF NON-T2 ASTHMA

There are a number of clinical characteristics with which non-T2 asthma is associated. These patients usually have late-onset asthma.¹² Indeed, aging has been associated with an increase in sputum neutrophil.^{13,14} Both T2 and non-T2 inflammation can be found in obese patients with asthma. For those obese patients associated

with a noneosinophilic phenotype, it is characterized by late-onset asthma in middle-aged female with high expression of symptoms¹⁵ which has also been identified in cluster analyses.^{16,17} These patients tend to have poorer asthma control and higher risks of exacerbation. As illustrated further in the weight reduction section below, other noninflammatory mechanical factors may also contribute to poor asthma control in obesity. Another common feature seen in non-T2 asthma is a poor response to inhaled and oral corticosteroid.^{8,18} Furthermore, airway neutrophilic inflammation is also associated with smoking,¹⁹ road traffic air pollution,²⁰ inhaled ozone,²¹ and other pollutants including nitrogen dioxide.²² Moreover, intensive exercise and cold environment have also been recognized as inducers of neutrophilic asthma.^{23,24} Additionally, infective asthma exacerbations cause airway neutrophilia.²⁵ *Moraxella*, *Streptococcus* and *Haemophilus* sp colonization in the airway microbiota was associated with higher sputum neutrophilia and IL-8 concentrations in asthmatic patients.²⁶ Bronchiectasis is another confounder for sputum neutrophilia.²⁷ Patients with asthma may have bronchiectasis that may contribute to a neutrophilic or a pleiotropic bronchitis as in allergic bronchopulmonary aspergillosis.

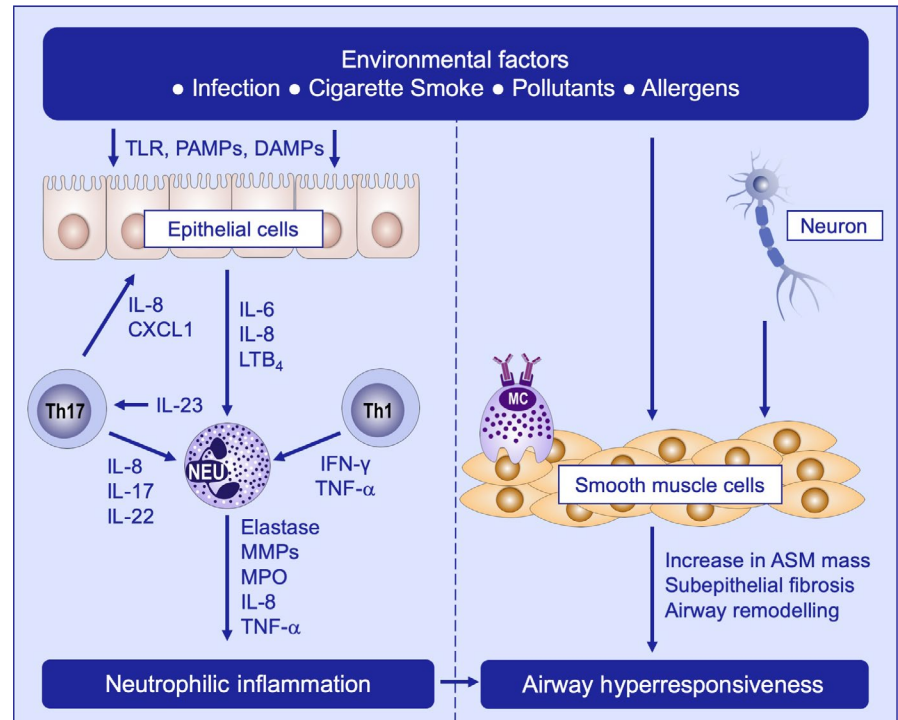
4 | ENDOTYPES AND UNDERLYING MECHANISMS OF NON-T2 ASTHMA

Non-T2 asthma, simplistically referred to as noneosinophilic asthma, encompasses both inflammatory endotypes where non-T2 cytokines are involved in driving asthma pathobiology, as well as noninflammatory endotypes, which including structural abnormalities involving ASM as well as erratic neuronal activation. These endotypes can further be classified according to the nature of underlying airway inflammation, as characterized by sputum cytometry, and airway hyperresponsiveness (AHR). Underlying mechanisms of non-T2 asthma are depicted in Figure 1).

4.1 | Mechanisms underlying neutrophilic asthma

Neutrophils are produced in the bone marrow from myeloid precursors, a process that is regulated by granulocyte colony stimulating factor (G-CSF).²⁸ In lungs, exposure to various environmental factors including ozone, particulate matter, infections, airborne endotoxins, smoking, and occupational chemicals may trigger neutrophilic inflammation. These irritants can cause airway injury and subsequently lead to release of toll-like receptors (TLRs). Indeed, sputum analysis from patients with neutrophilic asthma demonstrates an increase in expression of innate immune receptors, including TLR2, TLR4, and CD14 as well as pro-inflammatory cytokines IL-8 and IL-1 β .²⁷ Activation of TLR has been known to induce a shift toward the T helper 1 (Th1) and T helper 17 (Th17) responses leading to generation of IL-8, IL-1 β , IFN- γ , and TNF- α .²⁹ IL-1 β is a typical innate immune cytokine, and an increase in airway IL-1 β (as a result of previous exacerbations) has been associated with systemic inflammation (noted by an increase in IL-6) and risk of future exacerbations, hence triggering a vicious

FIGURE 1 Mechanisms of non-T2 asthma, including neutrophilic inflammation and airway hyperresponsiveness. ASM, airway smooth muscle; CXCL1, C-X-C Motif chemokine ligand 1; DAMPs, damage-associated molecular patterns; IFN- γ , interferon gamma; IL, interleukin; LTB₄, leukotriene B₄; MMP, metalloproteinase; MPO, myeloperoxidase; PAMPs, pathogen-associated molecular patterns; Th1, T helper 1 cell; Th17, T helper 17 cell; TLR, Toll-like receptor; TNF- α , tissue necrosis factor alpha



cycle.³⁰ Moreover, Gibson and colleague demonstrated that asthmatics with neutrophilic inflammation have a deficiency in soluble form of receptor for advanced glycation end-products (sRAGE).³¹ RAGE is a pattern-recognition receptor that interacts with various endogenous ligands involved in host response to injury, infection, and inflammation, including high mobility group box 1 (HMGB1, which has been noted to promote Th17 polarization³²), advanced glycation end-products (AGEs), serum amyloid A (SAA), amyloid β -peptide and S100 proteins. Interaction of RAGE with these ligands leads to activation of inflammatory responses, induction of oxidative stress and tissue remodeling responses. Soluble form of receptor for advanced glycation end-products serves as a decoy receptor by sequestering RAGE ligands and thus inhibiting RAGE dependent cellular responses.³³ However, further studies are needed to determine whether deficiency in sRAGE is a causative factor for airway neutrophilia or rather a result of neutrophilic inflammation (degradation due to excessive levels of neutrophil-derived enzymes).

Chemokines, including leukotriene B₄ (LTB₄) acting via LTB₄ receptor, and IL-8 (CXCL8), acting via CXCR1 and CXCR2, are principle signals for neutrophil migration.³⁴ However, other chemotactic factors including C5a, CXCL1 (growth-related oncogene- α), CXCL5 (epithelial-cell neutrophil-activating protein-78), and elastase- α 1-antitrypsin complexes may also contribute.^{35,36} Increased levels of neutrophil active mediators, such as IL-8, neutrophil elastase, MMP-9, IL-17A, LTB₄, GM-CSF, and TNF- α have been detected in blood, bronchoalveolar lavage (BAL) fluid and bronchial epithelium in patients with neutrophilic asthma.³⁷ Neutrophil contains three main granules including azurophilic granules (containing myeloperoxidase), specific granules (containing lactoferrin), and gelatinase granules (containing matrix metalloproteinase 9). Neutrophils, once activated,

eliminate pathogens via phagocytosis, degranulation or forming neutrophil extracellular traps (NETs).^{28,38} Additionally, neutrophils may also contribute to pro-inflammatory cytokine production, recruitment of other inflammatory cells (including T cells and APCs), epithelial and endothelial damage, mucous production, angiogenesis, and smooth muscle proliferation.³⁹ Given neutrophils' role in mediating persistent inflammation and causing airway damage, traditionally these cells have been thought of as primitive and indiscriminate killers. Nonetheless, neutrophils may also play a role in tissue repair and healing.⁴⁰⁻⁴² Neutrophils may have immunomodulatory effects through production of anti-inflammatory cytokine, including release of IL-1RA, IL-10, TGF β 1, and TGF β 2, elimination of debris through phagocytosis and efferocytosis, as well as promotion of remodeling through mucus plug removal.⁴³ Thus, neutrophils demonstrate a great deal of plasticity and diverse pleiotropic effects, which is dependent on location of the cells, comorbidities and surrounding inflammatory milieu. Multiple neutrophil phenotypes with distinct morphological and functional characteristics have now been discovered, with capacity to either activate a pro-inflammatory cascade or differentiate into immunomodulatory role.³⁵ A greater understanding of this dichotomy of neutrophil biology as "friend or foe" is crucial in devising treatment strategies, as single faceted treatment may not address underlying pathobiology and may lead to undue adverse effects.

Transcriptomics studies provide further evidence for differentiating noneosinophilic from eosinophilic asthma. Transcriptional asthma phenotype (TAP) associated with increased neutrophils (TAP2) has an increase gene expression corresponding to IL-1 and TNF- α /NF- κ B pathways, including increased gene expression of *IRAK3* and *NFBIZ* in peripheral blood neutrophils and *IRAK3*, *IRAK2*, and *IL1R2* in airways.⁴⁴ Furthermore, using a sputum gene expression signature of

six biomarkers, Baines and colleagues were accurately able to discriminate asthma inflammatory phenotypes, where neutrophilic subtype was identified by increase sputum expression of IL-1 β , alkaline phosphatase tissue-nonspecific isozyme (ALPL) and CXCR2.⁴⁵

4.1.1 | Role of T helper 17 (Th17) and T helper 1 (Th1) cells

Potential inflammatory processes leading to neutrophilic airway inflammation have mainly been linked to activation of Th17 and Th1 cells. At the time of T-cell activation, naïve CD4⁺ T cells are differentiated into Th17 cells in presence of TGF- β , IL-1 β , IL-6, and IL-23. Th17 cells have been shown to secrete IL-17A, IL-17F, and IL-22, which is often resistant to steroids.⁴⁶ In moderate-to-severe asthmatics, increased levels of these cytokines have been found in bronchial biopsies and bronchoalveolar lavage fluid and is associated with increased disease severity.^{47,48} Other cells including $\gamma\delta$ T cells, Innate lymphoid cells (ILCs) and granulocytes are also known to secrete IL-17A, IL-17F, and IL-22.^{49,50} Th17 cytokines increase secretion of epithelial-derived neutrophilic chemokines (namely CXCL1 and CXCL8), hence leading to neutrophil recruitment to the airway.⁵¹ Additionally, these cytokines also induce mucous cell metaplasia and increase ASM mass resulting in airway narrowing.⁵² IL-17A also plays a role in airway remodeling by promoting fibroblast proliferation.⁴⁷

Moreover, Th1 cells may also play a role in driving neutrophilic inflammation. Th1 cell activation is stimulated by IL-12 and leads to production of IFN- γ .⁵⁰ Experimental animal models of severe asthma demonstrate high IFN- γ and low secretory leukocyte protease inhibitor (SLPI) expression, which correlated with high airway resistance and corticosteroid refractoriness.⁵³

Adding to the complexity of asthma endotyping, although two main inflammatory signatures, involving Th2 or Th17 cells and their respective cytokines, have been described as discrete subpopulation, it has been demonstrated that some patients may show both Th17 and Th2 mediated inflammation.⁵⁴ Majority of T helper cells show a degree of plasticity depending on environmental factors and may transdifferentiate into other effector CD4⁺ cells. Th2 cytokines, IL-4 and IL-13, and regulatory cytokine, IL-10 negatively regulate IL-17A and IL-17F expression from Th17 cells.^{55,56} On the other hand, the role of IL-17A and IL-17F on allergic airway inflammation is conflicting and may be dependent on timing. Traditionally, IL-13, which is often co-expressed with IL-4, is associated with T2 inflammation and plays a role in goblet cell hyperplasia, ASM cell hypertrophy, and subepithelial fibrosis. Gallo and colleagues demonstrated IL-13 production by Th1 and Th17 cells, which are distinct from classical IL-4⁺ Th2 cells.⁵⁷

4.1.2 | Key cytokines involved in non-T2 asthma: IL-17, IL-8, and IL-6

IL-17 promotes neutrophil migration by inducing IL-6 and IL-8 release from bronchial epithelial cells, a process that is dependent on p38 mitogen-activated protein (MAP) and extracellular-signal regulated kinase (ERK) kinases.⁵⁸ Increased levels of IL-17 have been demonstrated in sputum and serum of severe asthmatics and are noted to be an independent risk factor for severe asthma.^{59,60} The release of IL-17A is in turn controlled by IL-23 which is released from tissue-resident macrophages and dendritic cells.²⁸

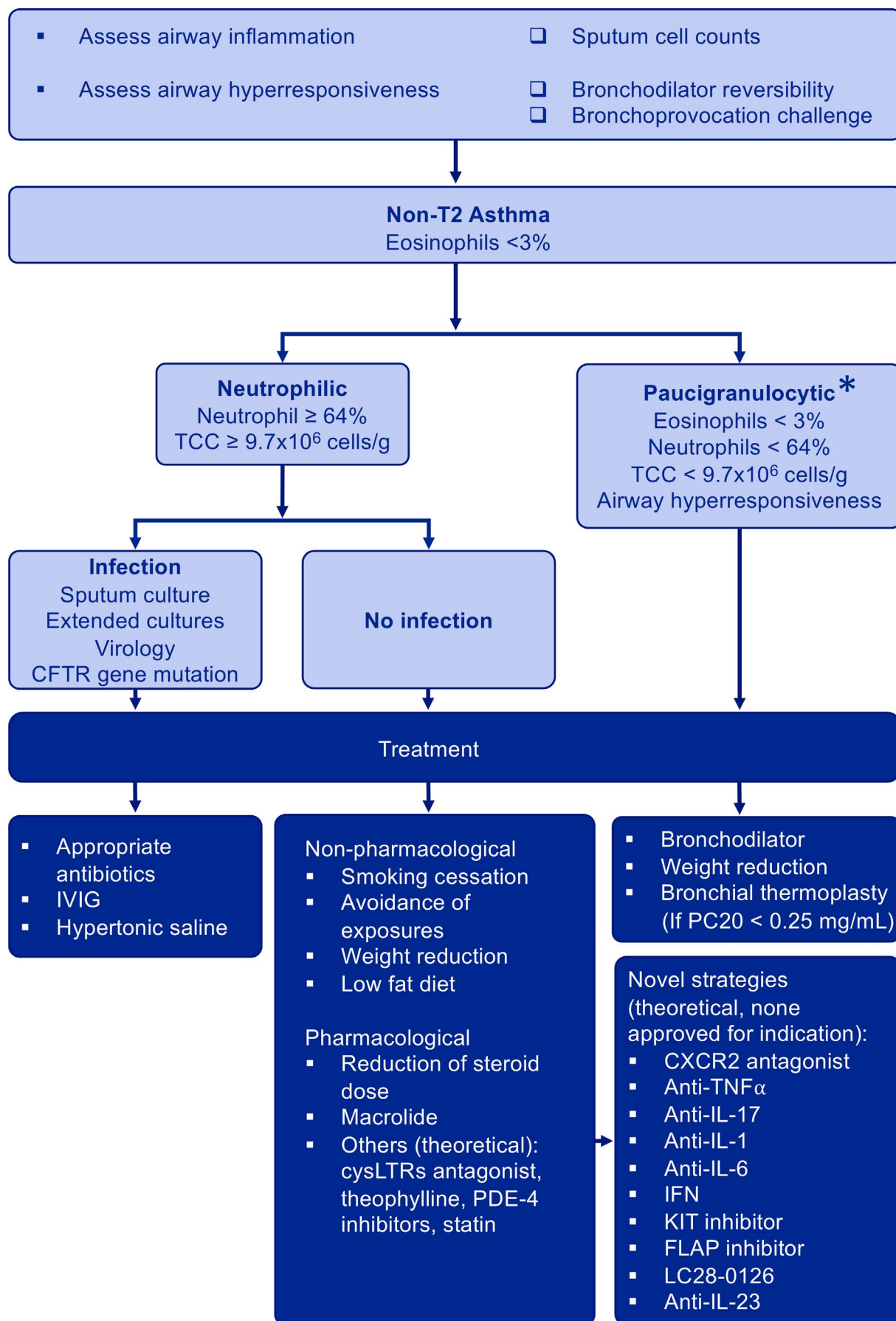
IL-8 (CXCL8), secreted by airway epithelial cells, T cells, and macrophages, is the most potent chemoattractant in the lung.⁶¹ Indeed, increased levels of IL-8 were observed in subjects with noneosinophilic asthma as compared with subjects with eosinophilic asthma and controls, which correlated with both increased neutrophil percentage and absolute neutrophil counts.⁶² Moreover, neutrophils have also been demonstrated to secrete IL-8 creating a positive feedback loop that promotes further neutrophilic inflammation.

IL-6 is a pro-inflammatory cytokine, secreted by a host of cells including (innate immune system, B cells, and CD4 effector Th cells, and nonleukocytes such as endothelial, epithelial and fibroblasts) and have been linked to pathogenesis in many inflammatory diseases.⁶³ In asthmatics, increased levels of IL-6 have also been demonstrated in serum,⁶⁴ BAL fluid,⁶⁵ and sputum⁶⁶ and are inversely correlated with FEV₁.⁶⁷ Additionally, Fahy and colleague demonstrated in two asthma cohorts—nonsevere asthmatics recruited at the University of California San Francisco (UCSF) and severe asthmatics recruited through Severe Asthma Research Program (SARP)—that “IL6-high” subjects had significantly higher body mass index and metabolic disease. This was associated with a lower FEV1 and increased exacerbations.⁶⁸ Increased IL-6 levels were also noted to be associated with mixed eosinophilic-neutrophilic exacerbations of asthma and correlated with worse lung function as compared to other inflammatory endotypes.⁶⁹

4.1.3 | Other mechanisms contributing to neutrophilic inflammation

In addition to the above Th17 and Th1 cells, several other factors may also contribute to neutrophilic inflammation. Corticosteroids, while they promote apoptosis of eosinophils have been demonstrated to inhibit neutrophil apoptosis,⁷⁰ where use of corticosteroids (ICS⁷¹ and OCS⁷²) has been shown to contribute to sputum neutrophilia. This was supported by findings from Cowan et al⁹ who showed that inhaled corticosteroid use was associated with an increase in airway

FIGURE 2 Approach to Non-T2 asthma. *A subset of patients characterized as paucigranulocytic and airway hyperresponsive may in fact have T2 inflammation for the following reasons: missing mast cell mediated disease¹⁷⁵, protein crystal driven inflammation¹⁷⁶, or missing intact eosinophils with eosinophilia being reflected by eosinophilic free granules¹⁰, either in matrix or within macrophages.¹¹ TCC, total cell count; CFT R, cystic fibrosis transmembrane conductance regulator; IVIG, intravenous immunoglobulin; cysLT Rs, cysteinyl leukotriene receptors; PDE-4, phosphodiesterase-4; PC20, provocation concentration causing a 20% drop in FEV1; CXCR2, C-X-C motif chemokine receptor 2; TNF- α , tissue necrosis factor alpha; IL, interleukin; IFN, interferon; FLAP, 5-lipoxygenase-activating protein



neutrophilia and a reduction in eosinophilia. Sputum neutrophil count was higher in patients receiving moderate-to-high dose ICS than those receiving low-dose ICS.³ Another study further demonstrated increased airway submucosal neutrophils with oral corticosteroid use.⁷³ Other mechanisms that may also result in neutrophilic inflammation include delayed apoptosis as a result of epithelial-cell-driven neutrophil chemotactic and anti-apoptotic activity, impaired macrophage efferocytosis and altered airway microbiome.⁴ Neutrophilia may not always indicate active infection and may be due to delayed resolution of an inflammatory process triggered by bacterial products, either through intravascular chemokine gradient directed neutrophil migration leading to sterile inflammation,⁷⁴ or leukotriene A₄ hydrolase (LTA₄H) mediated degradation of neutrophil chemoattractant proline-glycine-proline (PGP),⁷⁵ or due to resolvins and protectins induced apoptosis.⁷⁶ Moreover, ILCs have been linked to pathogenesis of asthma, where specifically ILC2s have been well recognized in development of eosinophilic asthma.⁷⁷ ILC3 cells have been linked to obesity-associated AHR, where Kim and colleague⁷⁸ demonstrated that in obese mice, IL-17 producing ILC3, were responsible for development of AHR, a process regulated by lung macrophages derived IL-1 β . Role of Type 1 ILCs is less clear, but activation of these cells has been postulated to result in paucigranulocytic inflammation from excessive IFN- γ production, leading to reduced eosinophils and neutrophils.^{53,79}

4.2 | Mechanisms underlying paucigranulocytic asthma

Paucigranulocytic asthma encompasses patients with absence of airway inflammation (eosinophilia and neutrophilia) with persistent symptoms and evidence of AHR. Underlying mechanisms for this endotype may be due to changes in ASM or airway inflammation not reflected in the lumen or detected by sputum cytometry.

PGA might represent a distinct corticosteroid-resistant asthma phenotype where there is discordance between airway obstruction and inflammation.⁸⁰ Studies have demonstrated that change in ASM contractility plays a key role in development of AHR.⁸¹ Numerous stimuli, including inflammatory cytokines, pollutants, and mechanical strains, can alter calcium signaling and thus predispose ASM to become nonspecifically hyper-responsive.⁸¹ Altered neuronal control of ASM contractility may result in a noninflammatory endotype of asthma, where there is evidence of AHR independent of inflammation. Indeed, animal studies demonstrate that mice models treated with nerve growth factor (NGF) induced AHR to the same degree as allergen-sensitized mice.⁸² However, there is also evidence that suggests NGF may be involved in activation of inflammatory cells and airway remodeling, hence highlighting a potential neuro-immune cross-link.⁸³ Acetylcholine is the primary parasympathetic neurotransmitter involved in inducing ASM contraction, specifically via M₃ receptors; however, it also plays a role in airway inflammation and remodeling, thought to be due to mechanical stress as a result of bronchoconstriction as well as direct effect on airway cells.⁸⁴ Moreover, dysregulation of critical signaling molecules, including

G protein-coupled receptors, transmembrane proteins, and growth transcriptional factors, are thought to be possible mechanisms promoting AHR independent of airway inflammation. In other animal studies, overexpression of susceptible asthma genes present in chromosome 17q21, including gasdermin B (GSDMB) and human orosomucoid-like 3 (ORMDL3), have been identified which may lead to airway remodeling, AHR, increase in ASM mass, subepithelial fibrosis, and mucous production in absence of AI.⁸⁰

Although PGA is typically regarded as absence of both eosinophilic and neutrophilic inflammation, Demarche et al⁸⁵ demonstrated evidence of inflammation, where PGA had higher sputum eosinophil counts, blood leukocytes and blood eosinophils than healthy subjects. Further, mast cell infiltration in ASM has been postulated to play a role in the pathogenesis of this asthma phenotype, where secretion of mast cell mediators could lead to bronchial obstruction, airway remodeling and AHR.⁸⁶ Mast cells may migrate toward Th2-stimulated smooth muscle bundles in asthmatic patients⁸⁷ and may get activated by a number of factors including IL-33 that directly promotes mast cell activation and indirectly promotes ASM contraction via upregulation of mast cell-derived IL-13.⁸⁸

5 | MECHANISTIC CONFIRMATION FROM CLUSTER ANALYSES

Various cluster analyses, including one via an unbiased approach, provide new insights into current understanding of asthma phenotypes. In a subset of SARP, it was found that neutrophilic and mixed granulocytic inflammation were associated with more severe asthma phenotypes compared with minimal or eosinophilic predominant inflammation.⁸⁹ Through analysis of sputum transcriptomics in a subset of U-BIOPRED, three transcriptome-associated clusters were identified using hierarchical clustering of differentially expressed genes.⁹⁰ Sputum eosinophilia was most prominent in the first cluster with highest IL-13/ Th2 cells and ILC2 enrichment signatures. Neutrophilia was most prominent in the second cluster with highest ILC1, neutrophil activation and inflammasome enrichment signatures, along with high IFN- γ and TNF- α . Paucigranulocytic inflammation was most prominent in the third cluster, which was characterized by signatures including ILC3 and Th17 cells, genes of metabolic pathways, ubiquitination, and mitochondrial function. Moreover, the subset study of U-BIOPRED also suggested that mixed granulocytic inflammation might be a transition between neutrophilic and eosinophilic phenotypes. This echoed findings from a previous study,⁴⁴ which postulated that the transition from one to another phenotype could result from a number of external stimuli, including glucocorticosteroid use.

6 | MANAGEMENT OF NON-T2 ASTHMA

It is important to confirm the non-T2 nature of asthma by measuring AHR and the absence of evidence of T2 inflammation (eg, normal blood or sputum eosinophils, serum IgE or FeNO) or high

sputum neutrophil. Currently, there are limited number of clinically available and validated measures of non-T2 inflammation. Exhaled volatile organic compounds measurements provide a promising surrogate marker for neutrophilic inflammation.⁹¹ Perhaps plasma IL-6 concentrations may be helpful. Recognizing the non-T2 nature of asthma in patients is important as most of these patients (particularly the patients with intense neutrophilia) may not benefit from increasing the doses of their maintenance corticosteroids. Nevertheless, there are small subset of patients who may respond to corticosteroids despite having a non-T2 inflammatory subtype on sputum cell counts, for instance, those with a mast cell-mediated disease. Further, since a neutrophilic bronchitis may mask an underlying eosinophilia in about a third of patients,⁹² it is important to recheck the cell counts after the neutrophilia has resolved. With new information emerging about characterizing both transcriptome^{90,93} and proteome in sputum⁹⁴ and in blood, we may soon have better ways of precisely characterizing non-T2 component of asthma and to identify new effective interventions.

6.1 | Nonpharmacological treatment

6.1.1 | Smoking cessation

One study found that IL-17A and neutrophils were significantly more abundant in endobronchial biopsies among asthmatic smokers compared with asthmatic nonsmokers,⁹⁵ which provided implications of smoking in mechanisms of neutrophilic asthma. Chaudhuri et al⁹⁶ demonstrated that smoking cessation was associated with significant improvement in FEV₁ and reduction in sputum neutrophils in asthmatics compared with those who continued to smoke. Smoking cessation ought to be incorporated in clinical management of every patient.

6.1.2 | Low-fat diet

A recent study identified several genes (eg, S100P and S100A16) whose expressions were increased in asthmatics after high-calorie and high-fat meal.⁹⁷ These genes were believed to mediate recruitment of neutrophils in the airways. High-fat diet, when compared with low-fat diet, was demonstrated to be associated with not only an increase in sputum neutrophils and increased expression of Toll-like receptor 4 mRNA,⁹⁸ which signified increased airway inflammation, but also a blunted bronchodilator response (FEV₁ and FEV₁/FVC ratio). Further studies in low-fat diet are needed to look into other important clinical outcomes.

6.1.3 | Weight reduction in obesity

Current evidence suggests that the underlying pathobiological mechanisms contributing to symptoms in asthmatics who are obese may not be associated with a T2 airway inflammation.⁹⁹ Indeed, sputum cell counts were reported to be normal in obese as compared to nonobese asthmatics,¹⁰⁰ although there is some evidence that there may be an airway mucosal eosinophilic inflammation that may

not be reflected in the lumen.¹⁰¹ It remains to be seen if this is significantly contributing to symptoms. The vast majority of evidence suggests that symptoms in obese patients may be related to underlying AHR.^{102,103} This may be related to mechanical factors related to increase expiratory reserve volumes, inability to take deep inspiration,^{104,105} and smooth muscle actin-myosin cross-linking^{106,107} that may be associated with adipose tissue accumulation^{106,108,109} rather than related to an inflammatory process.

In a randomized study involving obese patients with severe asthma, weight reduction program (by a combination of low calorie diet, sibutramine, and orlistat) at 6 months was associated with significant improvement in asthma control and forced vital capacity (FVC) as well as reduction in symptom days, rescue-medication use and emergency room visits as compared to no weight reduction strategies, albeit there were no significant changes in sputum eosinophils or neutrophils between the two groups.¹¹⁰ Bariatric surgery has also been shown to significantly reduce systemic inflammatory markers including high-sensitivity C-reactive protein and leptin in patients with and without asthma,¹¹¹ although there were no changes in IL-6, IL-8, TNF- α , and GM-CSF. Nevertheless, asthma patients with bariatric surgery had significantly reduced mast cells in bronchial biopsies, even though there was no change in submucosal eosinophil, neutrophil, and macrophage. In another large self-controlled case series study of 2261 obese patients with asthma, bariatric surgery was associated with fewer emergency department visits or hospitalizations for asthma exacerbations in the first year postsurgery and the benefit maintained in the second year.¹¹² Thus, the improvements in AHR may be related to reduction in the mast cell numbers in the airways and possibly improvement in smooth muscle mechanics and hyperresponsiveness. Regardless of the underlying mechanism, it is reasonable to consider bariatric surgery or other effective weight reduction strategies in asthmatics with morbid obesity.

6.1.4 | Bronchial thermoplasty

The exact mechanism of effectiveness of bronchial thermoplasty (BT) is not known. Since ASM mass seems to be consistently attenuated through delivery of localized thermal energy,^{113,114} this seems to be one of the mechanisms by which this intervention is effective. Additionally, BT has also been shown to result in a significant reduction in nerve fibers in epithelium and in ASM, and thus, attenuation of neural reflexes may also contribute to beneficial effects of BT.¹¹⁵ This intervention has been demonstrated to improve asthma control, peak expiratory flow, scores on quality of life questionnaires, percent of symptom-free days as well as a decrease in rescue medication use,^{116,117} severe exacerbations, emergency department visits and days missed from work/school.¹¹⁸ Moreover, the beneficial effects of BT in terms of asthma control (maintained reduction in severe exacerbations and ED visits) and safety were demonstrated over long term.¹¹⁹ Experience from our center suggests BT should be reserved for uncontrolled asthmatics with persistent symptoms, frequent exacerbations, and severe AHR (demonstrated by PC₂₀ < 0.25 mg/ml) despite absent or controlled airway inflammation.¹²⁰

6.2 | Pharmacological treatment

6.2.1 | Reduction of corticosteroid dose

Asthmatics with eosinophilic bronchitis have a predictable response to corticosteroids and have been cornerstone of treatment to maintain asthma control and reduce exacerbations.¹²¹⁻¹²³ On the other hand, asthmatics with noneosinophilic inflammation, especially neutrophilic endotype, generally have a poor response to corticosteroids.¹²⁴ As mentioned above, airway neutrophilia has been observed with high dose steroid.^{71,72} Demarche et al¹²⁵ demonstrated that ICS could be discontinued or reduced in two thirds of noneosinophilic asthma patients (sputum eosinophil < 3% and blood eosinophil < 400/ μ L) with no worsening of asthma control or exacerbation. At least in clinical practice, in a patient with neutrophilic bronchitis, the dose of corticosteroids could perhaps not be increased.

6.2.2 | Bronchodilator

Bronchodilators, including addition of long-acting muscarinic antagonist (LAMA) and long-acting beta-agonist (LABA) to ICS, have also shown to be effective in increasing time to first exacerbation, improving lung function and symptoms,^{126,127} an effect which is independent of T2 inflammation.¹²⁸ Although there are a few studies that demonstrate effect of LABA in decreasing sputum neutrophil numbers and IL-8 in mild asthmatics,^{129,130} however, a subsequent meta-analysis demonstrated that LABA did not have any clinically significant anti-inflammatory or pro-inflammatory effects.¹³¹

6.2.3 | Treating infections

In patients with infective neutrophilia, identification of causative pathogen may help direct antibiotic choice. In addition, molecular microbiology and extended cultures, including 16s deep sequencing, may be considered in patients with recurrent infective exacerbations.¹³² Identification of cystic fibrosis transmembrane conductance regulator (CFTR) gene abnormalities and use of nebulized hypertonic saline in the management of recurrent neutrophilic bronchitis in carefully selected asthmatics may also be beneficial.¹³³ Experience from our center suggests that hypertonic saline can be safely used in asthmatics who do not have significant drop in FEV₁ during sputum induction. However, there is insufficient evidence of hypertonic saline use in asthma and its efficacy will need to be assessed in randomized clinical trials. Recurrent infections in asthmatic patients should also prompt to check for immunoglobulin deficiency in which immunoglobulin replacement may improve asthma control by reducing recurrent infective exacerbation.¹³⁴

6.2.4 | Macrolides

Nonantibiotic doses of macrolides are believed to possess an anti-inflammatory effect and are showed to have clinical benefits in bronchiectasis and COPD. Earlier evidence from Simpson et al¹³⁵ showed

that clarithromycin in severe refractory asthma reduced sputum IL-8 and neutrophils, yet no benefit in lung function or asthma control was observed. The research interest was later shifted to azithromycin. In the Azithromycin for prevention of exacerbations in severe asthma (AZISAST) trial, severe asthma patients on high-dose ICS plus LABA with history of severe exacerbations were randomized into either receiving add-on azithromycin (250 mg daily three times per week) or placebo.¹³⁶ In a pre-defined subgroup of severe noneosinophilic asthma (defined by normal FeNO and normal blood eosinophil levels) azithromycin was significantly associated with fewer severe asthma exacerbations during 26-week period. Subsequently, in the AMAZES trial which compared add-on azithromycin (500 mg three times per week) with placebo for 48 weeks in patients with symptomatic asthma despite medium-to-high dose of ICS and LABA, it was found that azithromycin significantly reduced the incidence of total (moderate and severe) exacerbations and was associated with an improvement in Asthma Quality of Life Questionnaire (AQLQ) score.¹³⁷ Moreover, the benefits of azithromycin were demonstrated in both eosinophilic and noneosinophilic asthma phenotypes. Azithromycin also significantly reduced respiratory tract infections and the rate of use of antibiotics for respiratory infections compared with placebo. There were no significant adverse events including hearing loss and prolonged QTc in subjects receiving azithromycin. While the underlying mechanism accounting for the benefits observed in these studies is not presently clear, Taylor et al¹³⁸ showed that azithromycin was not associated with a decrease in the total bacterial load in the AMAZES trial, which suggested that it was unlikely due to antibiotic effect. More studies are needed to examine immunomodulatory effects (if any) of long term low-dose macrolide.

6.2.5 | Other therapeutic agents

Cysteinyl leukotrienes receptors (CysLTR1) are widely expressed on range of inflammatory and structural cells and have been linked to eosinophilic inflammation, bronchoconstriction, bronchial hyperresponsiveness, mucous hypersecretion, and airway remodeling.¹³⁹ Thus, leukotriene receptor antagonist may play a therapeutic role in multiple asthma endotypes. Selective antagonists of cysLTRs have been studied in treatment of allergic rhinitis, exercise-induced asthma, aspirin-induced asthma, and as an add-on therapy for uncontrolled asthma. Specifically, in neutrophilic inflammation, leukotriene receptor antagonist has been demonstrated, in a dose-dependent fashion, to inhibit superoxide generation, production of LTB₄, and release of elastase by activated neutrophils *in vitro*.¹⁴⁰

Theophylline is another drug that has shown to promote apoptosis in neutrophils and eosinophils *in vitro* via reduction in the antiapoptotic protein Bcl-2.^{141,142} Theophylline has also been noted to inhibit neutrophils from generating reactive oxygen species¹⁴³ and to cause a decline in neutrophil chemotaxis.¹⁴⁴

Additionally, roflumilast, a phosphodiesterase-4 inhibitor, was noted to attenuate allergen-induced bronchoconstriction and airway inflammation, including reduction in both eosinophilic and neutrophilic counts as well as reduction in TNF- α levels.¹⁴⁵ It was

shown that roflumilast improved FEV₁ in mild to moderate asthmatics,¹⁴⁶ and the improvement of lung function was also observed when adding roflumilast to ICS.¹⁴⁷ Furthermore, statins have been demonstrated to enhance the anti-inflammatory effects of inhaled corticosteroids in asthma; this effect was mediated through induction of indoleamine 2,3-dioxygenase, a tryptophan-degrading enzyme, which has been hypothesized to be a T-cell immunomodulator.¹⁴⁸ A 12-year cohort study conducted in Taiwan involving more than 110 000 adult asthma patients showed that statin use was associated with fewer asthma-related emergency department visits or hospitalizations, in a dose-response relationship.¹⁴⁹ Further studies are warranted for identifying specific asthma endotypes that will benefit from these additional therapies.

6.3 | Novel pharmacological agents

6.3.1 | C-X-C motif chemokine receptor 2 (CXCR2) antagonist

CXC chemokine receptors bind IL-8 and play an important role in neutrophils migration to inflammatory sites. In a double-blind, randomized, placebo-controlled trial, Nair et al¹⁵⁰ found that a selective CXCR2 antagonist, SCH527123, significantly reduced sputum and blood neutrophilia in patients with severe neutrophilic asthma (sputum neutrophil > 40%). SCH527123 was associated with significantly less mild exacerbations, despite no improvement in lung function (FEV₁). Subsequently in a larger, multicenter, dose-finding trial, O'Byrne et al¹⁵¹ investigated AZD5069, a selective CXCR2 antagonist, in patients with uncontrolled persistent asthma, with blood neutrophil counts $\geq 2.7 \times 10^9/L$, on medium-to-high dose of inhaled corticosteroids (ICS) and long-acting beta-2 agonists (LABA). No significant benefits were observed in rates of severe exacerbations, FEV₁ and ACQ-5 scores. Of note, sputum differentials were not used in this study as in the trial with SCH527123. It is important to note that sputum neutrophil is poorly predicted by blood neutrophil count.^{152,153} As such, the proportion of patients in the AZD5059 study who indeed had neutrophilic asthma as defined by quantitative sputum cytometry was unclear. Thus, the results of this study may not be generalizable to patients with neutrophilic asthma identified by sputum induction techniques. At present, there is no sufficient evidence to show that CXCR2 antagonist is an effective adjunct in the treatment of neutrophilic asthma.

6.3.2 | Anti-TNF- α

It was shown that inhaled tumor necrosis factor- α could induce AHR with elevated sputum neutrophilia in normal healthy individuals.¹⁵⁴ Golimumab, a human monoclonal antibody against TNF- α , was investigated in a randomized, double-blind, placebo-controlled, multicenter trial in patients with severe uncontrolled asthma.¹⁵⁵ No significant differences between treatment groups and the placebo group were found in the co-primary endpoints of FEV₁ and number of severe asthma exacerbations, although post hoc analysis

demonstrated that in patients with $\geq 12\%$ baseline FEV₁ reversibility, golimumab was associated with fewer severe asthma exacerbations through week 24. However, the risks associated with the treatment significantly outweighed benefits, where one patient developed tuberculosis, one died of septic shock and eight developed malignancies in the golimumab group during the study period. Etanercept is a recombinant fusion protein of TNF receptor linked to Fc portion of IgG1 and blocks TNF by binding them. A small study conducted by Berry et al¹⁵⁶ demonstrated that not only refractory asthma was associated with an upregulation of TNF receptors on the membrane surface of peripheral blood monocytes, but also etanercept treatment significantly improved AHR, FEV₁, and symptoms. Another study demonstrated a small benefit of etanercept in ACQ score in patients with severe steroid-refractory asthmatics,¹⁵⁷ and the treatment appeared to be safe and well-tolerated.¹⁵⁸ Nevertheless, further clinical trials over longer study periods are needed to investigate the role of anti-TNF- α in asthma and to identify patient characteristics to select those who may benefit from it the most.

6.3.3 | Anti-IL-17

IL-17 is implicated in recruitment of neutrophils and their activation in the airways.¹⁵⁹ Hence, targeting IL-17 has been investigated for its potential therapeutic role in neutrophilic asthma. Brodalumab, a monoclonal antibody against IL-17 receptor, was investigated in a randomized placebo-controlled phase IIa study by Busse et al¹⁶⁰ in patients with moderate-to-severe asthma. ICS was permitted but subjects were required to stop long-acting β -agonists (LABA) during the study. At week 12, brodalumab did not result in any statistically significant benefit in terms of ACQ scores, FEV₁, or SABA use. There was an improvement in ACQ score noted with 210 mg dose, but not 280 mg, only in the high-reversibility (postbronchodilator FEV₁ $\geq 20\%$ increase) subgroup of patients. The clinical significance of this finding is uncertain. Currently, there is another ongoing clinical trial (ClinicalTrials.gov NCT03299686)¹⁶¹ evaluating CJM112, an anti-IL-17A antibody, in patients with moderate-to-severe asthma with low IgE and low blood eosinophil levels. The results of this study are awaited. It remains to be seen if certain subpopulation of asthmatics would benefit from anti-IL-17 through better patient characterization.

6.3.4 | Anti-IL1

Postganglionic M2 muscarinic receptors of parasympathetic nerve fibers inhibit acetylcholine release via negative feedback mechanism and limit bronchoconstriction.¹⁶² Pretreatment with anakinra, an IL-1 receptor antagonist, in guinea pigs, prevented respiratory viral infections induced loss of M2 receptor mRNA expression and receptor dysfunction and prevented in vitro tracheal segment hyperresponsiveness.¹⁶³ It remains to be seen whether or not inhibition of IL-1 could prevent virus-induced asthma exacerbations. In another study involving healthy volunteers, short-term pretreatment with anakinra compared with placebo significantly reduced

sputum neutrophilia and also reduced the rise of sputum IL-1 β , IL-6, and IL-8 levels, after challenge by inhaled lipopolysaccharide as an endotoxin.¹⁵³ In murine models, severe steroid-resistant allergic airway disease was induced by ovalbumin, and *Chlamydia* and *Haemophilus* respiratory infections, which mimicked severe steroid-resistant neutrophilic asthma in human subjects,¹⁶⁴ treatment with neutralizing anti-IL-1 β antibody, MCC950 (NLRP3 inhibitor), and Ac-YVAD-cho (caspase-1 inhibitor) reduced IL-1 β levels, airway neutrophilia, and AHR. Reducing IL-1 β production via targeting infection-induced NLRP3 inflammasome might be a potential therapeutic option in severe steroid-resistant asthma and warrants further studies.¹⁶⁴

6.3.5 | Anti-IL-6

At the time of exacerbation, IL-6 levels in sputum supernatant have been demonstrated to be highest in patients with mixed granulocytic asthma compared with eosinophilic, neutrophilic, or paucigranulocytic asthmatics.⁶⁹ Moreover, mixed granulocytic inflammation was also associated with worst airflow obstruction. The investigators found that lung IL-6 levels were higher for mice exposed to house dust mite intranasally than those exposed to saline. House dust mite provoked inflammatory responses in the airway were blunted in IL-6 knockout mice and wild-type mice treated with anti-IL-6 antibody, with all airway and tissue cell types including neutrophils and eosinophils significantly reduced.⁶⁹ Peters et al⁶⁸ demonstrated that high plasma IL-6 levels were associated with metabolic syndrome (hypertension, diabetes, and higher BMI), worse lung function, and higher risk of exacerbations. The study also showed that IL-6 levels were also independent of Type 2 inflammation (eg, blood eosinophil, IgE, and sputum eosinophil). These findings suggest that high plasma IL-6 could be a marker of a subset of non-T2 asthma, and blocking IL-6 might be a potential therapeutic target in these patients, thus necessitating further clinical trials. Presently, there are no clinical trial data available in the public domain of an anti-IL6 intervention in asthma.

6.3.6 | IFN

Several studies had been conducted in examining effects of various IFN treatment on asthma. A small pilot trial demonstrated that nebulized recombinant IFN- γ significantly reduced BAL eosinophils but not neutrophils.¹⁶⁵ Subcutaneous IFN- α was also shown to improve FEV1, reduce blood eosinophils, reduce rescue medications use, emergency visits, and allow oral glucocorticoid dosage tapering in patients with severe persistent uncontrolled asthma.¹⁶⁶ Nebulized IFN- β treatment at the onset of viral upper respiratory tract infections shown to improve morning peak flow, increase in serum CXCR10 and reduced sputum CCL4 concentrations, which suggested this treatment may improve outcomes of URTI induced asthma exacerbations.¹⁶⁷ More studies of larger scale are required to investigate which phenotypes of asthma would benefit best from IFN treatment.

6.3.7 | KIT inhibitor

Imatinib inhibits tyrosine kinase of KIT, induces mast cell apoptosis, reduces bone marrow mast cell burden and is used to treat certain hematological malignancies. It was demonstrated in a recent trial that imatinib improved AHR and reduced airway mast cell burden in severe asthma.¹⁶⁸ Increase in FEV1 was found to be significantly correlated with baseline BAL neutrophil count which suggested a pathobiologic role of mast cell in non-T2 asthma. The study also demonstrated targeting mast cell as a potential therapeutic option in the future.

6.3.8 | 5-lipoxygenase-activating protein (FLAP) inhibitor

Leukotriene B4 (LTB4) is one of the many inflammatory mediators secreted by neutrophils, where 5-lipoxygenase-activating protein (FLAP) plays a key role in its formation. In a small study, Chaudhuri et al¹⁶⁹ investigated the short-term treatment of a FLAP inhibitor, GSK2190915, in asthma patients. Although GSK2190915 was associated with a significant reduction in sputum LTB4, there was no significant difference in sputum neutrophil counts or percentages compared with placebo.

6.3.9 | LC28-0126

LC28-0126 is a mitochondria-targeted reactive oxygen species scavenger with anti-inflammatory and anti-necrotic properties.¹⁷⁰ Treatment with LC28-0126 not only significantly reduced total cell counts, neutrophil counts, IL-4, IL-5, IL-13, and IL-17 in bronchoalveolar lavage fluids of ovalbumin-lipopolysaccharide-ovalbumin-induced mice,¹⁷¹ but also improved airway responsiveness measured by methacholine. Nevertheless, there are currently no human studies evaluating the effects of LC28-0126 in asthmatics.

6.3.10 | IL-23

Interleukin-23 (IL-23) is a heterodimeric cytokine, secreted primarily by dendritic cells and macrophages and also from eosinophils and is composed of an IL12B (IL-12p40) subunit (ie, shared with IL12) and the IL23A (IL-23p19) subunit.¹⁷² A functional receptor for IL-23 (the IL-23 receptor) has been identified and is composed of IL-12R β 1 and IL-23R. IL-23 is critically involved in the development of Th17 cells, and the activity of IL-17A which is critically involved in the recruitment of neutrophils into the airway. Risankizumab (BI 655066) is an IgG1 Mab that is directed against the p19 subunit of IL-23. In a 24-week, placebo-controlled clinical trial in moderate-severe asthma, risankizumab did not improve asthma control or asthma exacerbation frequency (ClinicalTrials.gov NCT02443298).¹⁷³

6.4 | Endotype-guided management strategy in Hamilton

In all severe asthmatics who are referred to our program, we examine sputum cell counts and assess AHR to methacholine if the

baseline FEV₁ > 65% of predicted. If sputum cytometry reveals no eosinophils, we gradually reduce inhaled corticosteroids and re-check sputum cell counts every 6–8 weeks until steroid is weaned off or eosinophilia resurfaces. If the patient has persistent neutrophilia (TCC > 25 million cells, 80% neutrophils), we treat with the appropriate antibiotics guided by extended cultures (16S sequencing if culture negative) and re-check the sputum to examine if eosinophilia is unmasked. Additionally, we investigate the cause for persistent and recurrent neutrophilia, where the two most frequent aetiologies in our severe asthma program are low serum (or sputum) immunoglobulins and single copy mutations of a disease-defining CFTR gene abnormality. We treat these patients with intravenous immunoglobulins every 4 weeks and hypertonic saline inhalation (if the PC20 methacholine is >4 mg/ml). If sputum persistently reveals no eosinophils or neutrophils (paucigranulocytic inflammation), we optimize bronchodilation (with long-acting anti-cholinergics and long-acting beta-agonists) and consider BT if the patient's symptoms are driven by severe AHR (PC20 methacholine <0.25 mg/ml). In select patients, novel imaging techniques, such as hyperpolarized gas magnetic resonance imaging (MRI), are performed to identify fixed areas of ventilation defects that may be amenable to targeted therapy.¹⁷⁴ If the patient has severe AHR and a BMI > 40, we refer the patient for bariatric surgery after normalizing the sputum cell counts with medical therapy. We do not routinely use any other treatments such as leukotriene receptor inhibitors or phosphodiesterase inhibitors. We recognize that the nature of bronchitis may change over time in the same patient and therefore, we re-check sputum at every exacerbation. An algorithm detailing our approach is summarized in Figure 2.

7 | CONCLUSION

Over the past decade, our understanding of asthma pathobiology has undergone a paradigm shift with greater recognition of asthma heterogeneity and distinct asthma endotypes. There have been numerous breakthroughs in T2 asthma and development of several targeted monoclonal antibodies. There is still uncertainty regarding the existence and definition of non-T2 asthma, which encompasses a range of asthma subtypes, including neutrophilic, mixed granulocytic, or PGA, that are characterized by normal eosinophils and low expression of T2 inflammatory markers. The terminology "low-T2 asthma" may therefore be more appropriate than "non-T2 asthma". Nonetheless, recent studies have shed light on complexity of this endotype, which involve various interconnected mechanisms as detailed in this review, that lead to physiological consequence of smooth muscle reactivity and airway inflammation. Although no targeted therapies have shown any clinical benefit (which strengthens the speculation if a non-T2 asthma endotype truly exists or not), appropriately endotyping patient and treating individual mechanistic components (either neutrophilic inflammation or AHR) is key to management. Ongoing trials involving sophisticated transcriptomics, proteomics and imaging radiomics present exciting possibilities to advance our understanding of this endotype, which will assist us in better characterizing patients and delivering precision therapeutic

choices. Further studies evaluating Th17 and Th1 inflammatory mediators, kinases signaling molecules and smooth muscle regulation pathways may provide additional therapeutic targets.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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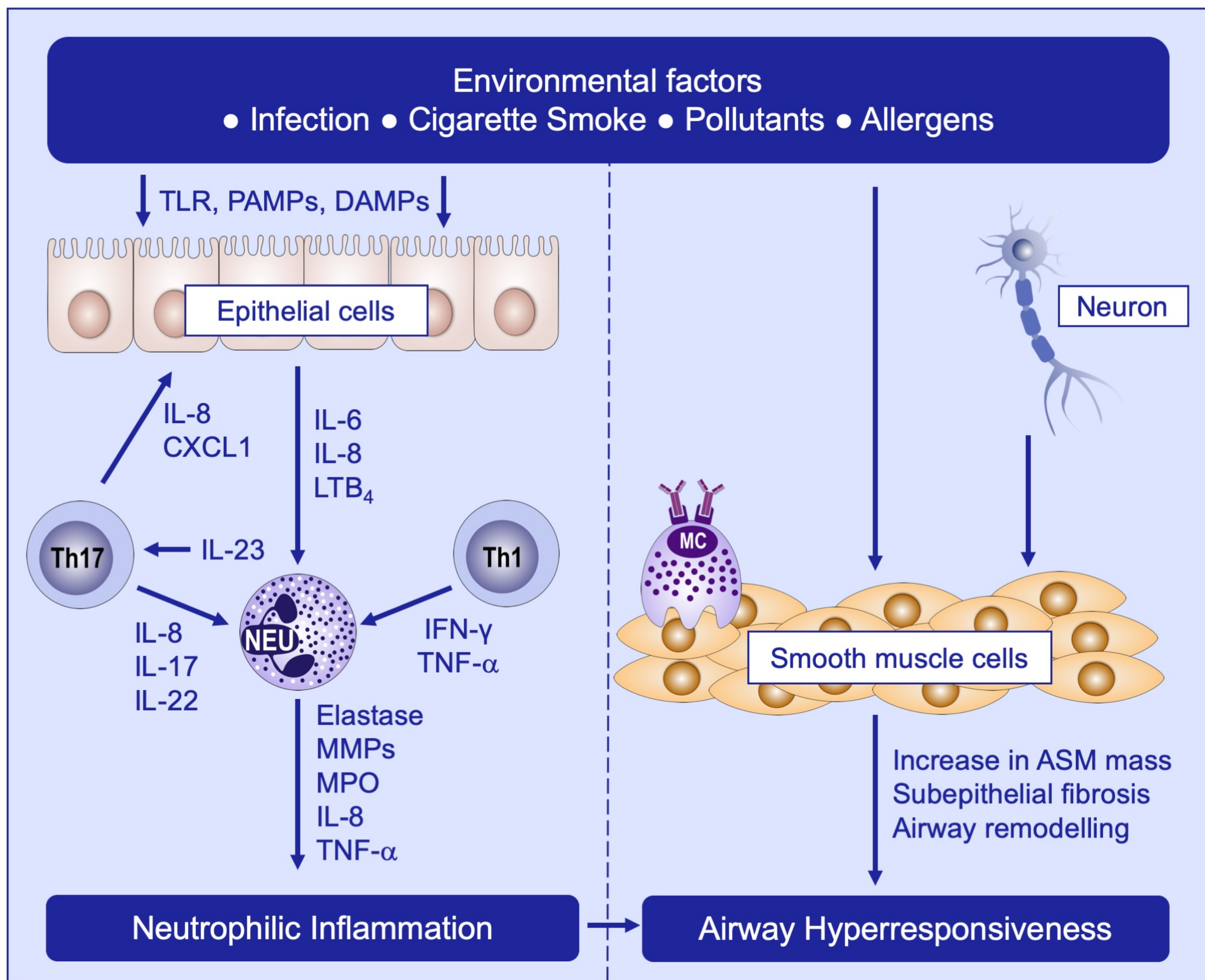


Fig.1_Parameswaran_ALL-2019-00368.R1

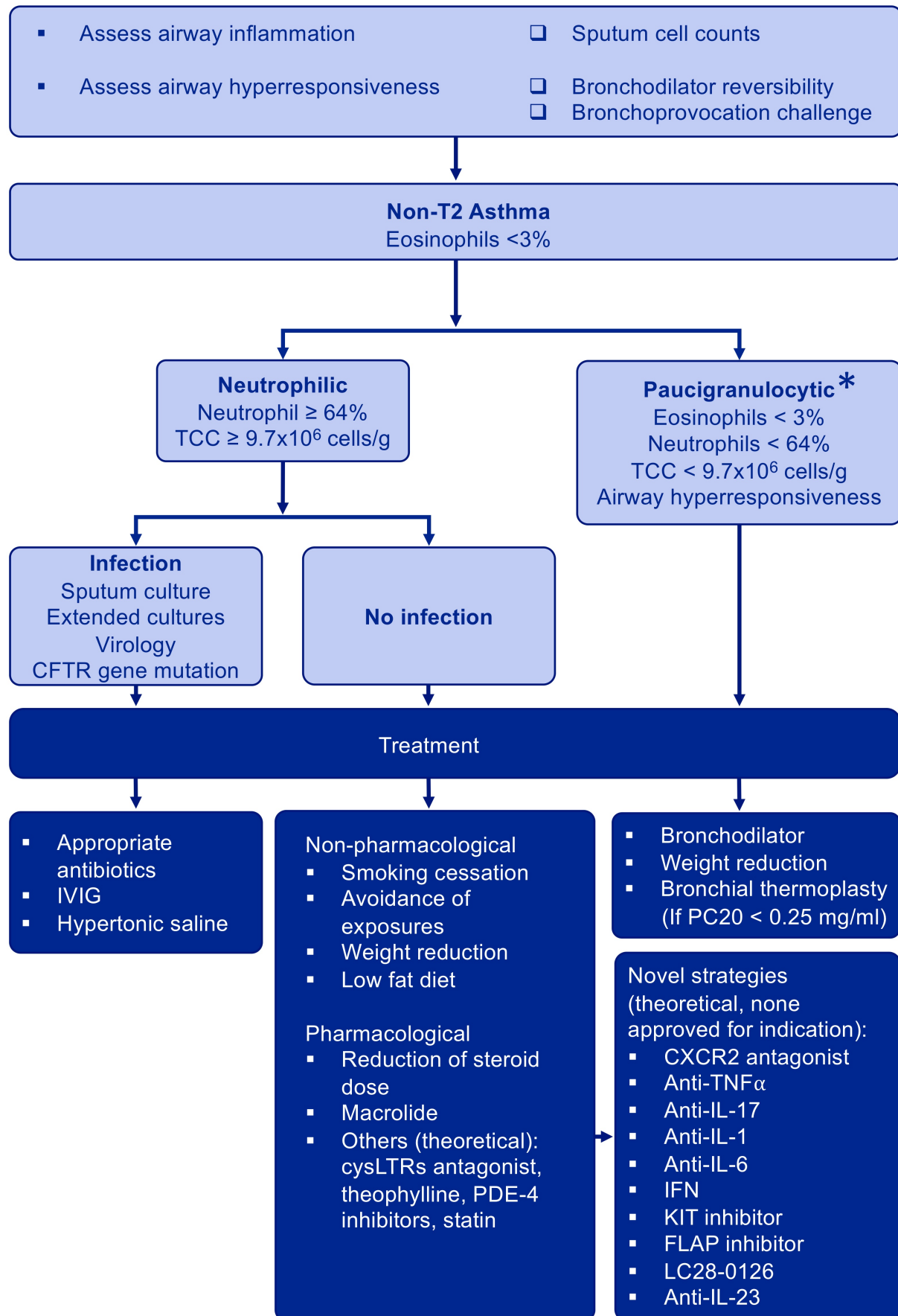


Fig.2_Parameswaran_ALL-2019-00368.R1