

New Targeted Therapies for Uncontrolled Asthma



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Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

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List of Design Committee Members: Jonathan Corren, MD (author); Michael Schatz, MD, MS (editor)

Learning objectives:

1. To review and understand important pathways responsible for phenotypes of asthma.
2. To examine new and evolving target-specific therapies for patients with poorly controlled asthma.

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Mechanistic studies have improved our understanding of molecular and cellular components involved in asthma and our ability to treat severe patients. An mAb directed against IgE (omalizumab) has become an established add-on therapy for patients with uncontrolled allergic asthma and mAbs specific for IL-5 (reslizumab, mepolizumab), IL-5R (benralizumab), and IL-4R (dupilumab) have been approved as add-on treatments for uncontrolled eosinophilic (type 2) asthma. While these medications have proven highly effective, some patients with severe allergic and/or eosinophilic asthma, as well as most patients with severe non-type-2 disease, have poorly controlled disease. Agents that have recently been evaluated in clinical trials include an antibody directed against thymic stromal

lymphopoietin, small molecule antagonists to the chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) and the receptor for stem cell factor on mast cells (KIT), and a DNA enzyme directed at GATA3. Antibodies to IL-33 and its receptor, ST2, are being evaluated in ongoing clinical studies. In addition, a number of antagonists directed against other potential targets are under consideration for future trials, including IL-25, IL-6, TNF-like ligand 1A, CD6, and activated cell adhesion molecule (ALCAM). Clinical data from ongoing and future trials will be important in determining whether these new medications will offer benefits in place of or in addition to existing therapies for asthma. © 2019 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (*J Allergy Clin Immunol Pract* 2019;7:1394-403)

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INTRODUCTION

Severe uncontrolled asthma has been estimated to afflict 2% to 3% of the total asthma population in the United States and has been shown to have considerable effects on both patient quality of life and health care expenditures.¹ Clinically, patients with poor asthma control frequently have difficulties with sleep, exercise, and activities of daily living, which are often not fully

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Abbreviations used

AHR- Airway hyperresponsiveness
ALCAM- Activated cell adhesion molecule
BAL- Bronchoalveolar lavage
CRTH2- Chemoattractant receptor-homologous molecule
expressed on T_H2 cells
FENO- Fractional exhaled nitric oxide
ICS- Inhaled corticosteroid
ILC2- Innate lymphoid cell type 2
NK- Natural killer
OCS- Oral corticosteroid
TL1A- TNF-like ligand 1A
TSLP- Thymic stromal lymphopoietin
T2- Type 2

appreciated by family or physician.² Recurrent exacerbations may occur in this group, occasionally on a frequent basis, which further compromise patient quality of life. Effects on health economics are significant, because these patients incur high direct costs due to medication use, physician utilization, and emergency room and hospital care.³ Indirect costs, largely accounted for by loss of work time for personal illness or care of a child, add further to the high social expense of asthma.⁴ Although non-adherence to or poor utilization of inhaled therapies remains an important cause of poorly controlled asthma, many patients continue to have severe chronic symptoms and exacerbations despite appropriate use of their controller therapies. A large prospective trial demonstrated that adherence to a regimen of high-dose inhaled corticosteroid (ICS) plus long-acting beta-adrenergic agonist resulted in well-controlled asthma in approximately 60% of patients with severe uncontrolled asthma.⁵ Although addition of a long-acting muscarinic antagonist may lead to improved asthma control in some patients, a small number will require treatment beyond the current regimens of inhaled, standard-of-care therapies.

PATHOGENESIS AND INFLAMMATORY PHENOTYPES

Type 2 inflammation

The most common molecular mechanism responsible for the pathogenesis of asthma is activation of the type 2 (T2) inflammatory pathway⁶ (Figure 1). T2 pathology is defined by the presence of key cytokines (IL-4, IL-5, and IL-13), immune cells (allergen-specific T_H2 and IgE-producing B cells, innate lymphoid cells type 2 [ILC2s], eosinophils, and mast cells), proteins (IgE), and small molecules (nitric oxide), many of which can be directly detected in sputum, blood, or exhaled gas.⁶ Although IL-4, IL-5, and IL-13 appear to be central to this molecular phenotype of asthma, emerging evidence indicates that additional cytokines may be involved, including IL-6, IL-17, IL-22, and IL-25.⁷

The most well-studied form of T2 asthma involves sensitization to airborne allergens. Following exposure of airway epithelium to an allergenic protein, dendritic cells present antigen to naive CD4 T cells in conjunction with the release of epithelial cytokines thymic stromal lymphopoietin (TSLP) and IL-33.⁸ The presence of these important costimulatory cytokines, referred to as alarmins, results in the development of

allergen-specific T_H2 cells that then produce the cytokines IL-4, IL-5, and IL-13.⁶ This cluster of T_H2-derived cytokines plays key roles in the pathologic events that characterize allergic asthma. IL-5 is the central blood-borne factor leading to the maturation, activation, and survival of eosinophils in the blood and airway tissue.⁹ IL-4 and IL-13 have pleiotropic effects on airway cells and structures; 2 critical activities include up-regulation of vascular cell adhesion molecule 1 expression on blood vessels and the release of the chemokine eotaxin, both of which facilitate eosinophil entry into the airways.¹⁰ IL-4 and IL-13 also stimulate goblet cell development and secretion of mucus into the airway lumen, impair smooth airway responsiveness to beta-adrenergic agonists, increase fibroblast development and collagen deposition in the submucosa, and activate the epithelium to increase nitric oxide synthase activity and nitric oxide production.¹⁰ IL-4, but not IL-13, has been demonstrated to have important regulatory effects on the differentiation of T cells into T_H2 cells.¹⁰ Along with eotaxin, another potent inducer of eosinophil chemotaxis is prostaglandin D₂, which binds to eosinophils via the chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2, or DP2 receptor).¹¹ Once activated eosinophils have reached the airway, they release a number of cationic proteins (eg, eosinophil cationic protein), other oxidizing substances (eg, reactive oxygen species), and mediators (eg, cysteinyl leukotrienes), which may severely damage the epithelial lining of the airway and lead to bronchospasm and/or bronchial hyper-responsiveness. IL-4 and IL-13 also promote B cells to class-switch from IgM to IgE.¹⁰ IgE antibodies bind with high affinity to mast cells and basophils; cross-linkage by allergen triggers the release of mediators and cytokines. Mast cells largely account for immediate symptoms following allergen exposure and also contribute significantly to chronic inflammation. IgE also binds to dendritic cells,¹² which take part in facilitated antigen presentation and likely play an important role in the persistence of allergic sensitization.

Nonallergenic substances, including viruses and pollutants, may also elicit the epithelial release of TSLP and IL-33.⁸ Release of a third alarmin, IL-25, may be particularly prominent in response to viral infections.¹³ These cytokines have potent effects on the activation of ILC2s and mast cells, which release IL-5 and IL-13 and lead to many of the same inflammatory changes as noted above, particularly eosinophilia. Clinical asthma with airway eosinophilic inflammation in the absence of allergen-specific T_H2 cells and IgE is best described as T2 nonallergic asthma.

Non-T2 inflammation

Patients with asthma who have no evidence of T2 inflammation, referred to as non-T2 or low-T2 asthma, appear to represent a heterogeneous group.¹⁴ Patients with a non-T2 phenotype frequently develop airway disease at a later age, do not have the typical comorbid conditions of T2 asthma (ie, food allergy, atopic dermatitis, allergic rhinitis, nasal polyposis), have no or limited amounts of specific IgE, and often do not respond well to ICSs.¹⁵ In many patients, persistent bronchial infection and gastroesophageal reflux are identified and may contribute to non-T2 asthma pathogenesis.¹⁶ In many cases, patients with non-T2 disease have been observed to have sputum neutrophilia (>60% of cells)¹⁷ or minimal pathologic signs of active inflammation (paucigranulocytic).¹⁸ There is limited evidence to support a primary pathway of inflammation

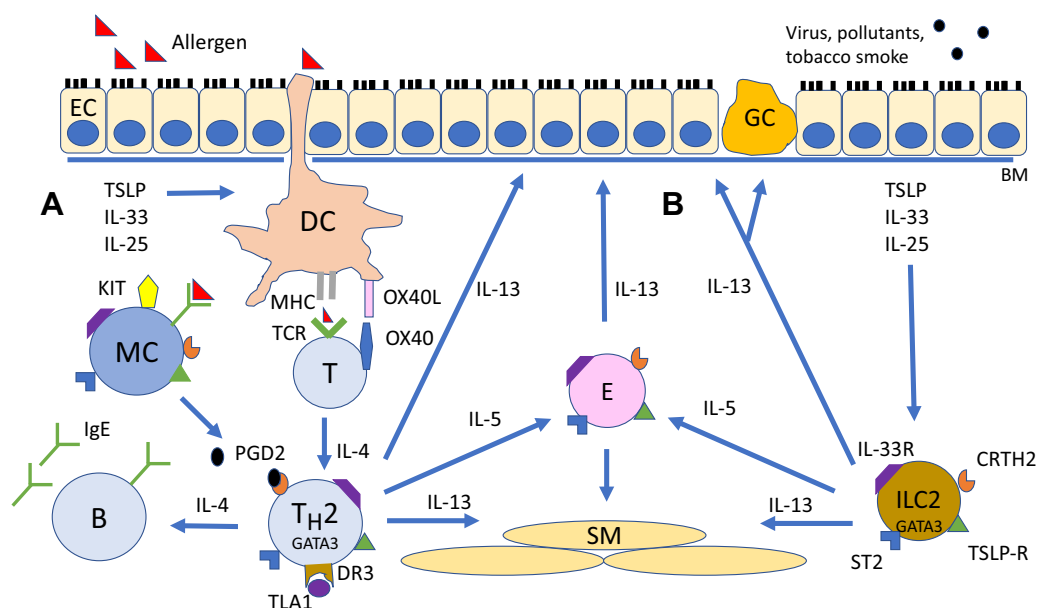


FIGURE 1. Mechanisms of T2-high asthma. **A**, Allergic asthma. **B**, Nonallergic T2 asthma. *B*, B cell; *BM*, basement membrane; *DC*, dendritic cell; *DR3*, death receptor 3; *E*, eosinophil; *EC*, epithelial cell; *GATA3*, GATA3 transcription factor; *KIT*, stem cell receptor; *GC*, goblet cell; *MC*, mast cell; *OX40L*, OX40 ligand; *PGD2*, prostaglandin D2; *SM*, smooth muscle cell; *ST2*, IL-33 receptor; *T*, naive T helper cell.

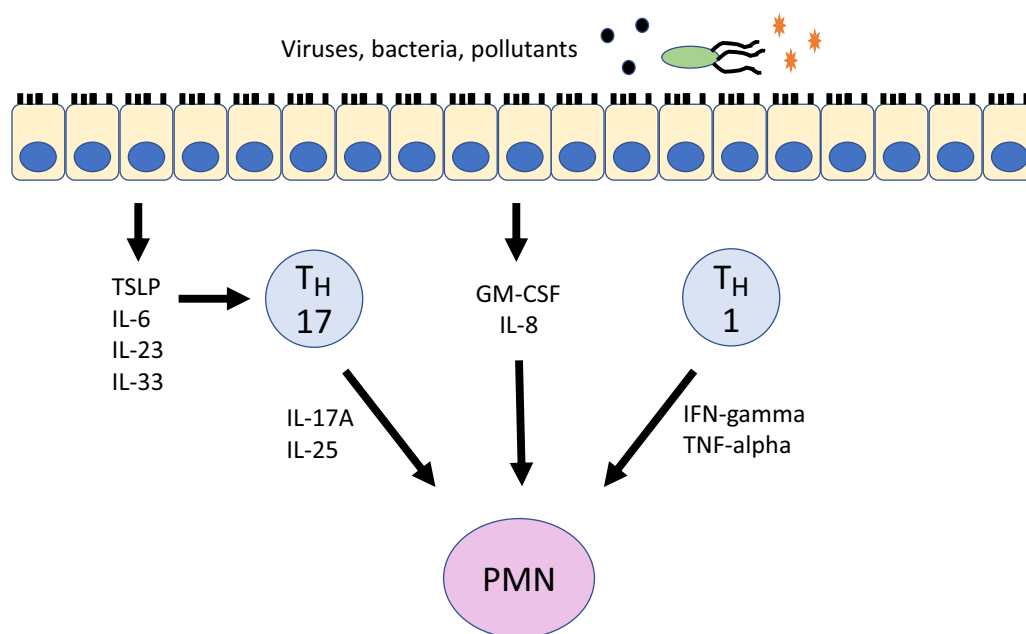


FIGURE 2. Possible mechanisms of T2-low asthma. *BM*, Basement membrane; *EC*, epithelial cell; *PMN*, polymorphonuclear leukocyte.

in patients with non-T2 asthma, although activation of T1/T17 pathways has been implicated in some patients with T2-low disease^{19,20} (Figure 2). The importance of T1- and T17-derived mediators is unclear at present, and in some cases upregulation of these pathways may be the result of pharmacologic suppression of T2 biology.²¹

Mixed eosinophilic and neutrophilic inflammation

A subset of patients with particularly severe asthma has been demonstrated to have a mixed infiltrate of eosinophils and neutrophils in their sputum. This group has been characterized as having the greatest clinical burden of disease and most severe airflow limitation.²² It is currently unclear what mechanisms

TABLE I. Cytokine-specific medications approved as add-on therapy in uncontrolled asthma

Target	Medication	Dose/route/frequency	Indication/age (y)
IgE	Omalizumab	75-375 mg SC, Q 2-4 wk (based on IgE and wt)	Moderate-severe allergic, >6
IL-5	Mepolizumab	100 mg, SC, 4 wk	Severe eosinophilic, >12
IL-5	Reslizumab	3 mg/kg, IV, 4 wk	Severe eosinophilic, >18
IL-5R	Benralizumab	30 mg, SC, 4 wk × 3, then 8 wk	Severe eosinophilic, >12
IL-4R	Dupilumab	200-300 mg, SC, 2 wk	Moderate-severe eosinophilic, >12
		300 mg, SC, 2 wk	Oral-steroid dependent, >12

IV, Intravenously; Q, every; SC, subcutaneously; wt, weight.

underlie this unique and potentially important inflammatory phenotype.

Biomarkers

The above-noted cytokine-cell-enzyme interactions allow us to infer logical biomarkers for identification of patients with T2 asthma, including blood and sputum eosinophil counts, fractional exhaled nitric oxide (FENO) levels, and specific IgE.²³ Although sputum neutrophilia (in the absence of sputum eosinophilia) may be present in some patients with T2-low asthma, no reliable biomarkers have been developed for this subtype of asthma.¹⁷ Therefore, the diagnosis of T2-low disease is usually inferred from the absence of elevation of T2 biomarkers.

TARGETED THERAPIES APPROVED FOR SEVERE ASTHMA

Beginning 15 years ago, targeted biologic treatments became available for patients with severe asthma (Table I). Omalizumab, an mAb directed against IgE, was the first drug approved for patients with severe asthma and an allergic phenotype and has been shown to reduce exacerbations and improve symptoms and quality of life.²⁴ Three mAbs that inhibit activities of IL-5 have been approved for patients with severe asthma and an eosinophilic phenotype. Mepolizumab and reslizumab both bind directly to IL-5, whereas benralizumab binds to the IL-5 receptor and causes eosinophil apoptosis; all 3 of these antibody therapies cause robust reductions in blood eosinophil counts and significant improvements in asthma exacerbation rates, pulmonary function, and patient-reported outcomes.²⁵⁻²⁷ Mepolizumab and benralizumab have both been shown to reduce the need for oral corticosteroids (OCSs) in patients chronically steroid-dependent while simultaneously reducing the rate of asthma exacerbations and improving pulmonary function.^{28,29} Most recently, dupilumab, an mAb that binds to the IL-4 receptor alpha chain and prevents binding of both IL-4 and IL-13, has been approved for patients with moderate-to-severe eosinophilic asthma³⁰ and patients requiring chronic OCSs.³¹ In the trial of OCS-requiring patients, OCS doses were reduced while the rate of asthma exacerbations and pulmonary function improved significantly.³¹ The above biologic therapies have all been proven to be extremely safe, and significant long-term adverse events have not surfaced to date. Of note, all of these therapeutic antibodies appear to be more efficacious in patients with increasing levels of T2 biomarkers relative to those with lower levels, likely related to the magnitude of the inflammatory signal.^{25,30} However, there remains a significant group of patients who are classified as having nonallergic, non-T2 asthma who do not respond well to current standard-of-care treatments and likely do not respond to

any of the above biologic treatments. Thus, there continues to be an important and unmet therapeutic need in patients with severe uncontrolled asthma.

Targeted therapies (with published results)

Anti-IL-17. T_H17 cells appear to contribute to asthmatic inflammation primarily through the production of the IL-17 family of cytokines, of which IL-17A and IL-17E (IL-25) bind to the IL-17RA receptor.³² In patients with active asthma, airway concentrations of IL-17A and IL-17F are increased and are positively correlated to neutrophilic inflammation and disease severity.^{33,34} One possible explanation for this observed increase in disease severity is IL-17RA-mediated airway hyper-responsiveness (AHR) via enhanced smooth muscle cell contraction.³⁵

Brodalumab (AMG 827) is a human, anti-IL-17RA IgG₂ mAb that binds with high affinity to human IL-17RA, blocking the biologic activity of IL-17A, IL-17F, IL-17A/F heterodimer, and IL-17E (IL-25). In a phase 2b study of 302 adult patients with moderate-to-severe asthma, 3 dose levels of brodalumab (140, 210, 280 mg) or placebo were administered subcutaneously at day 0, 7, 14, 28, 42, 56, and 70 during a 12-week trial.³⁶ For the overall study population, no treatment differences were observed. However, in a subgroup of patients with high FEV₁ reversibility to inhaled bronchodilator (≥20% improvement; n = 112), the Asthma Control Questionnaire (ACQ) demonstrated a nominal improvement (estimated treatment difference, 0.53). It is unknown whether brodalumab would have been more efficacious if patients had been selected for study using a phenotype-specific approach, that is, the presence of significant sputum neutrophilia or absence of T2 biomarkers.

Currently, CJM112, an IL-17A antibody, is being evaluated in a phase 2a study of adults with moderate-to-severe, poorly controlled asthma who have a blood eosinophil level of less than 300 cells/μL and total serum IgE level less than 150 IU/mL (NCT03299686); the results of that study are expected toward the end of 2019.

Anti-TSLP. TSLP is a member of the IL-2 cytokine family, and, as noted above, is released by the bronchial epithelium in response to tissue stimulation or damage caused by mechanical injury, proinflammatory cytokines (TNF-α, IL-1-α, IL-4, IL-5), viruses (particularly rhinovirus and respiratory syncytial virus), proteases, allergens, and other proinflammatory substances, such as tobacco smoke.³⁷ Following release, TSLP leads to activation, as well as potential suppression, of a number of immune cells. A critical effect of TSLP appears to be regulation of dendritic cell function, which then promotes the maturation of naive CD4 T cells into allergen-specific CD4 T_H2 cells.³⁸ TSLP has also been

shown to enhance cytokine production from multiple types of innate immune cells, including ILC2s, mast cells, natural killer (NK) T cells, and eosinophils, and to promote the development and function of a subset of basophils.³⁹⁻⁴¹ Finally, TSLP may have effects on both T_H1 and T_H17 cells, although likely to a much lesser extent than observed effects on T_H2 cells.

Genetic studies have shown that certain alleles at the human TSLP locus are related to the development of asthma and bronchial hyperresponsiveness, suggesting that TSLP may affect disease expression.⁴² TSLP expression has been shown to correlate inversely with lung function and asthma,⁴³ positively with AHR in patients with asthma.⁴⁴

Tezepelumab is a fully human and highly specific antibody to TSLP that prevents TSLP from binding to its receptor.⁴⁵ In a proof-of-concept allergen challenge study, tezepelumab was administered at an intravenous dose of 700 mg every 4 weeks for 12 weeks to patients with mild allergic asthma.⁴⁶ Patients underwent inhaled allergen challenge at baseline and after 6 and 12 weeks of treatment; serial spirometry, blood and sputum eosinophil counts, and FENO were all measured during or after each allergen challenge. The results demonstrated that both the early and late asthmatic responses were significantly attenuated, and the late response was reduced by 34% and 46% compared with placebo at the 6-week and 12-week time points, respectively. The mean blood eosinophil count at week 4 was significantly decreased compared with placebo (59% vs 21%), and post-allergen rises in blood eosinophils were blunted significantly with active treatment. The mean sputum eosinophil count before allergen challenge was reduced as well, from 4.1% at baseline to 0.4% after 6 weeks and the postallergen rise in sputum eosinophils was near-completely blocked. Similarly, FENO levels dropped throughout the study, and the postallergen increases were significantly reduced. These results indicate strong suppressive effects on immediate reactivity to allergen, suggesting the possible inhibition of mast cell activation. Reduction of the late asthmatic FEV₁ response, along with decreases in blood and sputum eosinophils and FENO, may implicate inhibitory effects upon other possible cell types, including T_H2 and ILC2s. Overall, these changes in blood eosinophil counts and FENO levels suggest that tezepelumab has a broad anti-T2 effect with reductions in both IL-5 and IL-4/IL-13.

Based on these initial results, a large dose-ranging study of tezepelumab (70, 210, and 280 mg) or placebo subcutaneously every 4 weeks × 52 weeks was conducted in patients with moderate-to-severe uncontrolled asthma with at least 2 prednisone-requiring exacerbations in the previous year, and included patients with both T2 (eosinophils > 250/μL) and non-T2 asthma.⁴⁷ All groups receiving active treatment had significantly fewer exacerbations per patient per year (tezepelumab 0.26, 0.19, 0.22 vs placebo 0.67) and these changes occurred irrespective of T2 biomarker status. Pulmonary function and multiple measures of patient-reported outcomes improved within the first month of tezepelumab treatment relative to placebo and persisted throughout the 52-week trial. Blood eosinophils, FENO, and total serum IgE all declined during active therapy compared with placebo, and these changes either reached a stable plateau or continued to decrease during the trial period. Adverse events were similar in placebo- and tezepelumab-treated groups. Data from these 2 trials of tezepelumab confirm that TSLP plays an important role in asthma and inhibition of this cytokine results in significant improvements in exacerbation

rates, asthma physiology, subjective measures of asthma, and T2 inflammatory and immune processes. As was seen in the proof-of-concept study, tezepelumab reduced both blood and sputum eosinophils as well as FENO, confirming the important role of TSLP in regulating expression of the T2 phenotype with expression of both IL-5 and IL-4/IL-13 activities. A phase 3 study of tezepelumab in adults with moderate-to-severe uncontrolled asthma, including patients with both T2-high and T2-low asthma, is ongoing, with results expected early in 2021 (NCT03347279).

In addition to tezepelumab, an antibody fragment that binds to TSLP and is delivered by inhalation (CSJ117, [ClinicalTrials.gov](#)) is currently in development in a phase 1 trial. To date, there are no published data regarding this medication.

Inhibitor of CRTH2. CRTH2 (or DP2 receptor) serves as the receptor for prostaglandin D2 and is expressed by T_H2 cells, eosinophils, basophils, epithelial cells, and ILC2s.¹¹ Prostaglandin D2-CRTH2 signaling has been shown to be involved in the recruitment and activation of all the above cells, as well as induction of mucin production and metaplasia of the epithelium.¹¹ Evidence for the importance of this receptor-ligand interaction in asthma includes data showing elevated concentrations of both prostaglandin D2 and CRTH2 in patients with severe asthma compared with those with mild-to-moderate disease and normal controls⁴⁸ and a correlation between the number of CRTH2-positive cells and asthma severity.⁴⁹

A number of CRTH2 antagonists have been developed and studied in patients with mild asthma, in many cases showing limited or no efficacy.⁵⁰ Most recently, fevipiprant (225 mg twice a day) or placebo was administered to 61 patients with moderate-to-severe, poorly controlled asthma with sputum eosinophilia in a controlled trial lasting 12 weeks.⁵¹ At the end of treatment, sputum eosinophil percentage decreased significantly from a geometric mean of 5.4% to 1.1% in the fevipiprant group and from 4.6% to 3.9% in the placebo group. Bronchoscopic biopsies demonstrated that fevipiprant-treated patients had improved measures of epithelial integrity and a reduction in airway smooth muscle mass. Clinically, patients receiving fevipiprant also had improved patient-reported outcomes and pulmonary function compared with placebo, despite the relatively small sample size. These positive signals for both pathogenetic and clinical improvement led to a much larger dose-ranging study of fevipiprant (vs placebo and montelukast) of 12-week duration in patients with uncontrolled asthma despite the use of low-dose ICS.⁵² At study end, fevipiprant was associated with a maximum model-averaged difference of 0.112 L in FEV₁ compared with placebo, with greatest differences observed at the 150-mg daily and 75-mg twice daily doses, which was noted after 4 weeks of treatment and remained stable over the entire trial. Although there were trends toward improvements in exacerbations, the sample size was not powered to detect changes in that end point. Measures of patient-reported outcomes were not significantly improved in either the fevipiprant group or the montelukast group. Analysis of adverse events showed an imbalance in mild cardiac rhythm events in patients receiving fevipiprant; subsequent adjudication suggested that these were not causally related to the active study drug. The above data showing improvements in sputum eosinophilia, lung function, and airway pathology, particularly smooth muscle mass, suggest that fevipiprant may be useful in patients with uncontrolled

asthma. Fevipiprant is currently being assessed in a phase 3 clinical trial program in patients with asthma that is inadequately controlled while taking standard-of-care therapy (NCT03052517).

Inhibitor of KIT proto-oncogene receptor tyrosine kinase. Mast cells are long-lived, tissue-dwelling cells that have been shown to contribute to the pathogenesis of asthma.⁵³ Mast cell numbers are correlated with AHR and asthma disease severity, and the mast cell mediator, tryptase, is elevated in the bronchoalveolar lavage (BAL) fluid of patients with asthma compared with control patients without asthma.^{54,55} Stem cell factor is a multipotent growth factor that binds to its receptor, KIT proto-oncogene receptor tyrosine kinase (referred to as KIT), and induces proliferation and differentiation of immature mast cells from CD34⁺ progenitors, chemotaxis of mast cells, enhanced survival of mast cells, and allergen-induced degranulation of human lung-derived mast cells.⁵⁶ Increased concentrations of stem cell factor have been identified in the airways of patients with asthma compared with controls without asthma.⁵⁷

Imatinib is a small molecule inhibitor of KIT and has been shown to markedly reduce bone marrow mast-cell numbers and serum tryptase concentrations in patients with chronic myeloid leukemia.⁵⁸ Imatinib, started at 200 mg daily and increasing to 400 mg daily, or placebo was administered to a group of 62 patients with severe uncontrolled asthma for 24 weeks in a proof-of-concept study design.⁵⁹ The primary end point, methacholine PC₂₀, was improved significantly in the imatinib group at month 3 by 1.20 doubling doses and at month 6 by 1.73 doubling doses, as compared with increases of 0.03 and 1.07 doubling doses, respectively, in the placebo group. Serum tryptase was reduced and FEV₁ improved significantly (increase of 46 mL compared with placebo) in patients receiving imatinib and the change in peripheral blood eosinophil count was significantly correlated with the reduction in bronchial hyperresponsiveness. A small number of adverse events primarily related to muscle cramping were reported in the trial. At present it is uncertain whether imatinib will be studied further in patients with severe refractory asthma.

GATA3-specific DNA enzyme. GATA3 belongs to the GATA family of transcription factors, which has an essential role in the earliest steps of T-cell development.⁶⁰ GATA3 was initially identified as a key T_H2 differentiation factor, with upregulation of GATA3 expression in cells differentiating along the T_H2 lineage, as well as an important regulator of T_H2 cytokine expression.⁶¹ More recently, GATA3 has been identified to also have essential functions in ILC2s, mast cells, and eosinophils, where it controls T2 cytokine expression.⁶²

In airways of patients with asthma, an increase in GATA3 expression was demonstrated compared with that in control subjects and correlated significantly with IL-5 expression and AHR.⁶³ In a mouse model of asthma, expression of a dominant negative mutant of GATA3 resulted in significant blunting of T_H2 effects, including tissue eosinophilia, mucus production, and IgE production.⁶⁴ Importantly, GATA3 is expressed only in intracellular processes and not on cell surfaces, making it difficult to reach with conventional approaches such as mAb or small-molecule therapies.

Given the importance of GATA3 in regulating T_H2 development and T_H2 cytokine expression, researchers have

developed a GATA3-specific DNA enzyme (GS-DNAzyme) that is capable of penetrating into the cell interior (SB010). DNAzymes are catalytically active, single-stranded synthetic DNA antisense molecules that bind to and then cleave specific sequences of mRNA.⁶⁵ In an animal model of airway inflammation, the GS-DNAzyme has been shown to significantly reduce levels of both GATA3 mRNA and protein and the production of T_H2 cytokines in human T cells and tissue explants.⁶⁶ Thirty-nine patients with mild allergic asthma underwent an inhaled allergen challenge at baseline and after 28 days of treatment with the inhaled GS-DNAzyme SB010, 10 mg, or placebo, once daily, with each allergen challenge followed by bronchial methacholine challenge, sputum cell analysis, and measurement of FENO.⁶⁷ The late asthmatic response was reduced after treatment with SB010, with a significant improvement in the mean area under the curve from the baseline value (66.4) to the posttreatment value (44.0), reflecting a reduction of 33.7%. The early asthmatic response was also inhibited, with a significant improvement in the mean area under the curve from 37.9 to 33.6, or 11.3% (as compared with a worsening of 10.5% in the placebo group). Patients receiving SB010 had significantly lower sputum tryptase levels compared with placebo (median, 6.39 ng/mL vs 13.10 ng/mL) and a trend toward lower sputum eosinophil counts relative to placebo. The postallergen challenge increase in IL-5 levels observed in the placebo group was significantly attenuated in the group receiving SB010. There was no protective effect of SB0101 on allergen-induced levels of FENO or AHR to methacholine. During the 4-week study period, there were no noteworthy differences in adverse events observed between patients receiving SB010 and those receiving placebo. This proof-of-concept study demonstrates that inhibition of GATA3 using DNAzyme technology reduces the early asthmatic response by inhibiting mast-cell mediator release and attenuates the late response by down-regulating cytokine release from T_H2 and/or ILC2 cells and eosinophils. Future long-term studies, focusing on clinical parameters such as exacerbations, lung function, patient-reported outcomes, and long-term safety, are needed to determine whether GATA3 inhibition will be a useful treatment for severe uncontrolled asthma.

New targeted therapies in clinical trials (without published results)

Anti-IL-33 and anti-ST2. IL-33 is a tissue-derived nuclear cytokine from the IL-1 family that is expressed in epithelial, endothelial, and fibroblast-like cells.⁶⁸ The biologic activity of IL-33 is increased by a number of proteases, including neutrophil elastase, and reduced by caspases.⁶⁹ Similar to TSLP, injury of the epithelium by various factors, including allergen exposure, viral infections, and trauma, leads to the release of IL-33, which then binds to its receptor ST2 (IL-1RL1) on a wide variety of cells, including mast cells, ILC2s, T_H2 cells, and eosinophils, basophils, dendritic cells, T_H1 cells, CD8⁺ T cells, NK cells, invariant NK T cells, B cells, neutrophils, macrophages, and fibroblasts.⁶⁸ IL-33 has been shown to be a key regulator of CD4 cell differentiation into T_H2 cells⁷⁰ and is a chemoattractant for T_H2 cells, recruiting T_H2 cells to lymph nodes and tissue.⁷¹ IL-33 has been shown to have potent effects on mast cells, as binding of IL-33 and subsequent signaling leads to expression of a large variety of proinflammatory cytokines, chemokines, and lipid mediators, including CXCL8 (IL-8), IL-5, IL-13, IL-6,

TNF- α , and prostaglandin D₂.⁷² Eosinophil survival and activation are increased by IL-33.⁷³ Another potentially important effect of IL-33 may be the activation of ILC2s, which are a rich source of IL-5 and IL-13 and may contribute significantly to persistent airway inflammation.^{74,75}

Studies of population genetics have shown that the loci for both IL33 and ST-2 are strongly associated with the development of asthma⁷⁶ and the expression of IL-33 has been shown to be increased in patients with severe asthma compared with normal controls.⁷⁷ mAbs directed against IL-33 have been used in mouse models of asthma and been shown to inhibit allergen-induced T_H2 cytokine production, eosinophilic airway inflammation, and mucus secretion.⁷⁸

There are a number of mAbs directed against IL-33 and ST2 currently in early phase clinical development in patients with severe asthma (Table II). The results from these trials will be instrumental in determining whether there will be a therapeutic role for IL-33 antagonism in patients with uncontrolled asthma.

Other potential therapeutic targets in severe asthma

IL-25. IL-25, also referred to as IL-17E, is an epithelial-derived cytokine and member of the IL-17 cytokine family, and is the only member of this cytokine group that is capable of eliciting T2 inflammatory effects.⁷⁹ In a manner similar to TSLP and IL-33, IL-25 is secreted in response to epithelial stimulation and functions as an alarmin with significant downstream effects. In addition to epithelium, it has been identified in eosinophils, mast cells, and endothelial cells, and to a lesser extent in macrophages, neutrophils, T cells, NK T cells, and ILC2s.⁸⁰ IL-25 binds to a heterodimeric complex composed of IL-17-receptor A and IL-17-receptor B, which is found on antigen-presenting cells, airway smooth muscle cells, airway epithelial cells, fibroblasts, eosinophils, and NK T cells.⁸¹ IL-25 secretion leads to the recruitment of T_H2 cells and stimulates the production of IL-4, IL-5, and IL-13 in the lungs.⁷⁹ IL-25 also contributes to the development and activation of ILC2s, although less effectively than IL-33, and thereby provides another important source of T2 cytokines to areas of inflammation.⁸⁰ In addition, IL-25 has been shown to have significant effects on the expansion and activation of eosinophils during allergic responses.⁸¹ Perhaps most importantly, IL-25 appears to play an important role in T2 inflammatory events that occur during human rhinovirus-induced exacerbations of asthma.¹³

Genetically, a rare allele of the *IL-17RB* gene has been associated with a reduced incidence of asthma,⁸² whereas a number of minor alleles of the *IL-17RA* gene are associated with a reduced risk of developing aspirin-exacerbated respiratory disease.⁸³ In an important mechanistic study examining the role of IL-25 in newly diagnosed, corticosteroid-naïve patients with asthma, patients were identified as having high or low levels of bronchial mRNA transcripts for IL-25.⁸⁴ The IL-25-high subgroup was more highly allergic and demonstrated evidence of eosinophil activation, increased expression of biomarkers for IL-13, and greater AHR to methacholine when compared with the IL-25-low subgroup. Blocking IL-25 in a mouse model of allergic asthma caused a significant reduction in BAL fluid levels of inflammatory cells and cytokines.⁸⁵ These data indicate that IL-25 plays a potentially important role in T2 asthma. However, given the activities that it shares with IL-33 and TSLP, it is difficult to determine whether IL-25 contributes uniquely to T2 asthma. It is possible that IL-25 may be more central to

TABLE II. Emerging targeted therapies for moderate-to-severe uncontrolled asthma

Target	Medication	Type of drug	Published results	Phase
TSLP	Tezepelumab	mAb	Phase 2a, 2b	3
TSLP	GS117	mAb fragment	—	1
IL-17A	CJM112	mAb	—	2a
CRTH2	Fevipirant	Small molecule	Phase 2b	3
KIT	Imatinib	Small molecule	Proof-of-concept	—
GATA-3	DNA-zyme	DNA molecule	Proof-of-concept	—
IL-33	RGN3500	mAb	—	2a
ST2	MSTT1041A	mAb	—	2a
ST2	CNTO 7160	mAb	1	—
IL-25	ABM125	mAb	—	—
TL1A	CO3V	mAb	—	—
CD6	Itolizumab	mAb	—	—

GATA3, GATA3 transcription factor; KIT, stem cell receptor; ST2, IL-33 receptor; TL1A, TNF-like ligand 1A.

viral-induced exacerbations of asthma. Adding anti-IL-25 on a regular basis or possibly using it in a preventative fashion at the beginning of the fall virus season could serve a unique purpose in patients with severe, viral-related attacks of asthma.

IL-6. IL-6 has both proinflammatory and anti-inflammatory effects and is produced by innate immune cells, including dendritic cells, macrophages, mast cells, neutrophils, endothelial cells, and epithelial cells.⁸⁶ IL-6 binds to the IL-6 receptor found on monocytes, B cells, hepatocytes, and neutrophils. Factors that induce cell stress or damage, including ultraviolet irradiation, microbial products, viruses, or proinflammatory cytokines, are capable of stimulating the release of IL-6, IL-1- α , and TNF- α .⁸⁶ These cytokines contribute to the “acute phase response,” during which liver cells release plasma proteins (C-reactive protein, fibrinogen, alpha-1-antitrypsin, and others), complement proteins are activated, and a number of metabolic changes begin.

In genetics association studies, certain alleles at the IL-6-receptor gene are associated with increased asthma susceptibility.⁸⁷ Increased levels of IL-6 have been identified in the serum of patients with asthma with clinically active disease⁸⁸ and BAL of patients with status asthmaticus.⁸⁹ Bronchoalveolar IL-6 has also been demonstrated to be higher in patients with nonallergic as compared with allergic asthma,⁹⁰ and serum IL-6 is elevated and has a negative relationship with pulmonary function in obese patients with asthma.⁹¹

The above findings suggest that IL-6 may have importance in patients with asthma, particularly in those who are obese and have nonallergic disease. As such, drugs that block IL-6 effects, such as the anti-IL-6 mAb tocilizumab, may be useful in some patients with asthma. Using a mouse model of allergic asthma, tocilizumab significantly reduced cockroach allergen challenge-induced airway responses and inflammatory cell infiltration, which was primarily mediated by inhibition of IL-17A expression by $\gamma\delta$ -T cells.⁹² These animal model data suggest that there is a biologic rationale for the use of IL-6 inhibitory therapy in asthma. Future trials in patients with elevated IL-6 will be critical in determining the utility of anti-IL-6 in asthma.

TNF-like ligand 1A. TNF-like ligand 1A (TL1A), also referred to as vascular endothelial growth inhibitor-251, is a

member of the TNF superfamily of ligands that binds to its primary receptor, death receptor 3.⁹³ TL1A is expressed constitutively at low levels by endothelial cells and at higher levels by tissue macrophages, submucosal lymphocytes, and dendritic cells under inflammatory conditions.⁹⁴ Death receptor 3 signaling is not a primary driver of immunity, but rather amplifies activation under certain conditions.⁹⁵ TL1A has been shown to have important effects as a costimulator of maturation and function of various cell types, including T_H1, T_H17, T_H2, NK, NK T, regulatory T, and B cells. Depending on the culture conditions used, these effects include increased secretion of T_H1 cytokines (including IFN- γ , GM-CSF, and TNF- α), increased differentiation of CD4⁺ cells into T_H17 cells, augmented release of IL-4, IL-5, and IL-13 from T_H2 cells, expansion of ILC2 cells with increased IL-5 and IL-13 production, and enhanced T_H9 pathogenicity.⁹⁶⁻⁹⁸ Taken together, this broad mechanism of action suggests that modulation of TL1A may benefit patients with both T2 and non-T2 asthma via effects on key effector cell populations.

In patients with mild asthma, inhaled allergen challenge led to a significant increase in death receptor 3⁺ ILC2s and supernatant levels of TL1A 24 hours after allergen challenge compared with preallergen and 24 hours after diluent challenge.⁹⁸ These data indicate that TL1A may be involved in allergic asthma via effects on ILC2s. Additional mechanistic studies will help define whether other cell types, particularly T_H2 cells, are similarly involved in allergic asthma.

To date, there have been no trials of TL1A antagonism in human asthma. However, in a mouse model of allergic asthma, administration of an anti-TL1A antibody reduced the expression of T_H2 cytokines and eosinophilic airway inflammation, even when administered following allergen challenge.⁹⁹ These data suggest that neutralization of TL1A does have a significant effect on T_H2 cytokine production and T2 inflammation, and that it is capable of disrupting established immune activation. Recent development of a potent and highly specific anti-TL1A antibody may hold promise in human trials of asthma.

CD6 and activated cell adhesion molecule (ALCAM).

The cell surface marker CD6 is found on most T cells as well as a subgroup of B cells.¹⁰⁰ CD6 binds to activated cell adhesion molecule (ALCAM, CD166), which is found on T cells, monocytes, epithelial cells, and mesenchymal cells; it also binds to CD318, which has been identified in synovial tissues.¹⁰¹ CD6 mainly functions as an adhesion and an intracellular signal-transducing receptor and participates in the formation and stabilization of T-cell contacts with antigen-presenting cells. CD6 is also a receptor for several pathogen- and damage-associated molecular patterns.¹⁰²

Multiple lines of evidence are now emerging to implicate CD6 and ALCAM in the pathogenesis of autoimmune diseases, such as multiple sclerosis and inflammatory bowel disease.^{103,104} In an allergic mouse model relevant to human airway disease, ALCAM was detected in BAL fluid and serum following allergen challenge, and the inflammatory response to allergen was markedly diminished using ALCAM-knockout mice.¹⁰⁵ Given the emerging evidence that CD6 may modulate inflammatory responses to inhaled allergen and affects diverse populations of cells that may be involved in airway disease, there may be a potential role for anti-CD-6 therapy in patients with asthma.

CONCLUSIONS

Clinical research performed over the last 2 decades has produced highly efficacious biologic treatments for asthma that is uncontrolled on standard-of-care inhaled therapies. MABs which specifically target IgE, IL-5 and its receptor, and the IL-4 receptor have demonstrated significant improvements in both severe asthma exacerbations and patient-reported measures of asthma. Importantly, all 5 of the biologic therapies approved for asthma address T2 inflammation only. Currently, the greatest unmet need is among patients with non-T2 inflammation, who are least likely to be responsive to ICS therapy. In this regard, the anti-TSLP mAb tezepelumab has shown potential efficacy in both T2 and non-T2 airways disease, and ongoing phase 3 trials will further define the populations that will benefit most from this medication. It is unclear whether other molecular approaches, such as anti-TL1A or anti-IL-6, will also have significant therapeutic effects in patients with non-T2 asthma.

Great progress has been recently made in the development of targeted, phenotype-specific asthma therapies. As basic and translational research into the pathogenesis of asthma yields new and important information, we are likely to witness the continued development of more effective therapies for this difficult disease.

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