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Development of New Therapies for Severe Asthma

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Persistent asthma has long been treated with inhaled corticosteroids (CSs), as the mainstay of therapy. However, their efficacy in patients with more severe disease is limited, which led to the incorporation of poor response to ICSs (and thereby use of high doses of ICS) into recent definitions of severe asthma. Several studies have suggested that severe asthma might consist of several different phenotypes, each with ongoing symptoms and health care utilization, despite the use of high doses of ICS, usually in combination with a second or third controller. Several new therapies have been approved for severe asthma. Long-acting muscarinic agents have recently been approved as an additional controller agent and appear to improve lung function, although their effect on symptoms and exacerbations is less. Although bronchial thermoplasty (BT) has emerged as a therapy for severe asthma, little is understood regarding the appropriate selection of these patients. Considerable data have emerged to support the presence of a group of patients with severe asthma who have ongoing Type 2 inflammation. These patients appear to respond to targeted biologic approaches which are at the current time mostly investigational. In contrast, few effective therapies for patients with less or no evidence for Type 2 inflammation have emerged. Many new and exciting therapies are at the forefront for severe asthma therapy and, in conjunction with precision medicine approaches to identify the group of patients likely to respond to these approaches, will change the way we think about treating severe asthma.

Key Words: Severe asthma; Type 2 inflammation; biologic medications; asthma phenotype

INTRODUCTION

Asthma has been broadly described as a chronic inflammatory disorder of the airways with bronchial hyper-responsiveness to a variety of stimuli and variable airflow obstruction that is often reversible either spontaneously or with treatment.[1] As an inflammatory disease, it has long been treated with corticosteroids (CSs), both inhaled and systemic, as the mainstay of therapy. In recent years, several major asthma research networks have suggested that severe asthma might be a different form of asthma representing more than just an inability to achieve asthma control. In 2014, the European Respiratory Society and American Thoracic Society (ERS/ATS) defined severe asthma as "asthma that requires treatment with highdose inhaled CSs plus a second controller and/or systemic CSs to prevent it from becoming "uncontrolled" or that remains "uncontrolled" despite this therapy."[2] This poor response to the standard treatment contributes to increased health care utilization, costs, and morbidity.[3] With this realization that not all asthmatics respond to the standard treatment and 5%-10% of asthmatics can be classified as severe,[2] many new approaches to treatment of severe asthma have been attempted. However, the introduction of the concept of molecular/inflammatory heterogeneity was critical to the emergence of therapies which appear to be effective in targeted populations/phenotypes of severe asthma.

In the past, therapeutic trials of new asthma medications have focused on a more mild population, without phenotypic differentiation. This may in part explain the lack of significant treatment response seen in many of these studies. However, in more recent years, the realization that different asthma phenotypes and endotypes exist which may respond differently to targeted therapies, combined with the unmet needs of the severe asthma population led to numerous clinical trials being performed in severe asthma. While it was reported in 1958 that asthmatics with eosinophils in the sputum responded to systemic steroids[4] and later shown that asthmatics with a lack of eosinophils did not respond to CSs,[5] many initial therapeutic studies did not divide patients based on the presence or absence of eosinophils.

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However, eventually treatments targeting IL-5 were shown to be effective, primarily in patients with ongoing evidence for eosinophilia.[6,7] As IL-5 is a Type 2 cytokine, with strong pro-eosinophilic qualities, this was the first evidence that Type 2 specific immune processes may be important in specific inflammatory phenotypes only.[6,7] These studies led to the identification of eosinophils, particularly blood eosinophils, as a Type 2 biomarker identifying treatment responses.

Additional evidence for the importance of Type 2 inflammatory phenotypes in asthma evaluated Type 2 biomarkers using *in vitro* and *ex vivo* approaches in human airway epithelial cells (HAEC) from mild asthmatics.[8,9] IL-13 stimulation upregulated 3 genes (chloride channel, calcium-activated family member-1 [CLCA1]; periostin; and SERPINB2) *in vitro* in cultured primary human airway epithelial cells.[8,9] The investigators then looked for these 3 genes in freshly brushed HAECs from asthmatics, finding that about 50% of the asthmatics had elevated expression of these genes, suggesting the presence of a Type 2, perhaps IL-13-associated, inflammatory process. Asthmatics with the presence of these Type 2 signature genes had higher tissue eosinophil counts, blood eosinophils, more airway hyperreactivity and atopy, and greater improvement with inhaled CSs than those without this signature.[8,9] This observation that periostin was associated with Type 2 inflammation led to additional studies looking at serum, which suggested a relationship between periostin and lung eosinophils,[10] and importantly to response to IL-13-directed therapy.[11] The fraction of exhaled nitric oxide (FeNO) is generated primarily by inducible nitric oxide synthase in airway epithelial cells and is also strongly induced by IL-13.[12,13] FeNO declines when IL-4 and IL-13 are blocked.[11,14,15]

At the current time, Type 2 biomarkers include periostin, FeNO, and sputum/blood eosinophils. In particular, recent trials of Type 2 targeted therapy have shown promise in asthmatic patients phenotyped by Type 2 inflammation. This review will focus on asthma therapies specifically targeted to a severe asthma population, including those developed for unphenotyped patients with asthma, and later, those in development for Type 2 phenotyped patients. We will begin with those treatments approved for severe asthma treatment and follow with those that are investigational.

Approved therapies for severe asthma (Table and Figure)

Long-acting muscarinic agents LAMAs in unphenotyped severe asthma

Beta adrenergic receptor agonists have been the major bron-

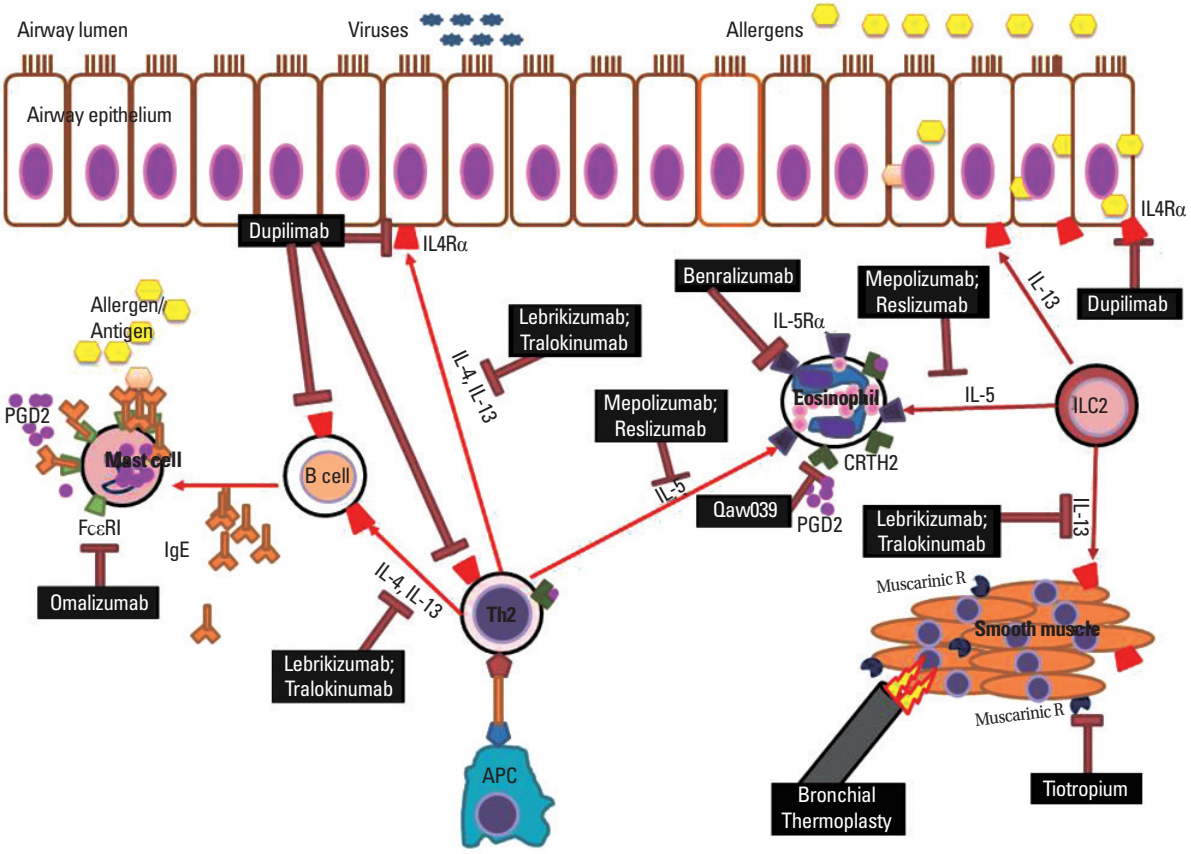

Figure. Selected therapeutic targets in severe ashtma.

Table. Summary of therapies used in severe asthma

A. Approved therapies

General target	Specific target	Therapies used	Baseline medications	Major outcome	Investigators
Smooth muscle tone	nerve	Tiotropium (inhaled)	ICS +LABA	↑ lung function, ↑time to severe asthma exacerbation, small ↓ exacerbation risk	[Kerstjens, 2011, 2012]
Smooth muscle mass	none	Bronchial thermoplasty	ICS +LABA	↓ severe exacerbations, modest ↑ asthma QOL and ↓ Health care utilization at 12 months	[Castro, 2010]
Mast cells/ Basophils	IgE (prevent binding to high affinity IgE receptor)	Monoclonal anti-IgE antibody: (SQ omalizumab)	ICS (no additional controllers) ICS+LABA (17% on OCS) *subanalyzed by **Type-2 High Phenotype** (↑ FeNO, blood eosinophils or serum periostin)	↓ asthma exacerbations, ↓ serum free IgE, ↓ ICS dose ↓ asthma exacerbations and ↑ asthma QOL; *Greater effect	[Busse, 2001] [Soler, 2001] [Holgate, 2004] [Hanania, 2011, 2013]
Eosinophils	IL-5	Anti-IL5 (IV or SQ mepolizumab) Anti-IL5 (SQ mepolizumab)	ICS + LABA; +/- OCS with **Type-2 High Phenotype** (sputum eosinophils >3% or ↑ blood eosinophils or ↑ FeNO) ICS + additional controller; 100% on systemic CS with **Type-2 High Eosinophilic phenotype** (blood eosinophils ≥300/μL prior year or ≥ 150/μL during optimization)	↓ asthma exacerbations, ↓ eosinophils in blood/sputum, ↑ AQLQ, ↑ symptom scores, ↑ lung function, ↓ systemic steroid requirements, ↓ exacerbations, ↑ AQLQ, ↑ asthma control	[Haldar, 2009] [Nair, 2009] [Pavord, 2012] [Ortega, 2014] [Bel, 2014]

B. Investigational therapies

General target	Specific target	Therapies used	Baseline medications	Major outcome	Investigators
Eosinophils	IL-5	Anti-IL5 (IV reslizumab)	High dose ICS + additional controller, with ≥1 exacerbation in prior yr with **Type-2 High Phenotype** (sputum eosinophils ≥3% or ↑blood eosinophils)	↑ ACQ , ↑ FEV1, ↓ eosinophils in blood/sputum, ↓ exacerbations	[Castro, 2011] [Castro, 2015]
Eosinophils and basophils	IL-5Rα	Anti-IL-5Rα (SQ benralizumab)	High dose ICS +LABA with **Type-2 High Phenotype** (eosinophilic index, ↑ FeNO or ↑ blood eosinophils)	↓ exacerbations *↑ blood eosinophils more predictive of tx response	[Castro, 2014]
Type2 inflammation	IL-4Rα	Anti-IL4R alpha (SQ AMG 317) Anti-IL4R alpha (SQ dupilumab)	ICS ICS+LABA with **Type 2-high phenotype** (blood eosinophils ≥300/μL or sputum eosinophils ≥3%)	↓ Serum IgE, no clinical efficacy ↓ Asthma exacerbations, ↓ FeNO, ↓ B-agonist use, ↑ FEV1	[Corren, 2010] [Wenzel, 2013]
	IL-13	Anti-IL13 mAb (SQ lebrikizumab) Anti-IL13 mAb (SQ tralokinumab)	ICS +LABA ICS+2nd controller Divided by **Type 2-high phenotype** (↑ serum periostin) ICS+LABA ICS+LABA + exacerbation hx	Small ↑ FEV1 in all-comers; *Greater ↑ FEV1 in sub-analysis of **Type 2 high phenotype** (↑ serum periostin, FeNO) ↓ Asthma exacerbations, ↑ FEV1; no dose response ↓ B-agonist use, ↑ FEV1 in all-comers *Greatest benefit in subanalysis of **Type 2-high phenotype** (↑ sputum IL-13) No effect on exacerbations *Modest effect on exacerbation and FEV1 when subanalyzed by **Type 2-high phenotype** (↑ serum periostin)	[Corren, 2011] [Hanania, 2015] [Piper, 2013] [Brightling, 2015]

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Table. Continued

General target	Specific target	Therapies used	Baseline medications	Major outcome	Investigators
		Anti-IL-13 mAb (IV GSK679586)	ICS+LABA, 16% OCS	No effect on prespecified clinical asthma Outcomes even when sub-analyzed by IgE and blood eosinophils	[DeBoever, 2014]
PGD2	CRTH2 (PGD2 receptor)	Anti-CRTH2 (Qaw039 oral)	ICS+LABA, 25% on OCS	↑ FEV1 and asthma quality of life, ↓ eosinophils (sputum, bronchial submucosa)	[Berair, 2015]
Th17 cells/ Neutrophils	IL-17R (blocks receptor binding to IL-17A, IL-17F, and IL-17E/IL-25)	Anti-IL-17 receptor Ab (brodalumab SQ)	ICS+additional controller	No treatment differences vs placebo; Minimal improvements in ACQ seen only in a high-reversibility subgroup, no effect by blood neutrophils or eosinophil subgrouping	[Busse, 2013]
Neutrophils	CXCR2 (IL-8 receptor)	Selective CXCR2 receptor antagonist (SCH527123 oral)	ICS+additional controller, Sputum neutrophils >40%	↓ neutrophils (blood and sputum), slight ↓ mild exacerbations, no other clinical benefits observed	[Nair, 2014]
Bacteria or Neutrophils	50S subunit of bacterial ribosomes	Oral macrolide antibiotics (Azithromycin oral)	ICS+LABA with **low evidence for Type-2 inflammation** (low FeNO)	No difference in severe exacerbations and lower respiratory tract infections *Post-hoc sub-analysis of subgroup with the **lowest Type-2 inflammation** (no/low eosinophils and low FeNO): lower rate of exacerbations and infections vs placebo	[Brusselle, 2013]

chodilator used for severe asthma-treatment. While muscarinic antagonists have been successfully used to treat chronic obstructive pulmonary disease, use in asthma has been limited to short-acting muscarinic antagonists in acute asthma exacerbations and those with side effects from short-acting beta- agonists. Long-acting muscarinic antagonists (LAMAs), such as tiotropium, competitively inhibit the action of acetylcholine at type 3 muscarinic (M_3) receptors in bronchial smooth muscle, reducing basal airway tone, making them a good target for some patients with severe asthma. Tiotropium, delivered by inhalation, has been shown to improve FEV1 in several studies of severe asthma. In a randomized double-blind, placebo-controlled (RDBPC) study of 107 severe symptomatic asthmatics, the addition of once daily inhaled tiotropium to high-dose inhaled CS (ICS) plus long-acting β-agonist (LABA) improved trough and peak FEV1 and peak expiratory flow rate compared to placebo.[16] In 2 subsequent large RDBPC parallel group trials of 912 patients with poorly controlled asthma despite high-dose ICS and LABA, treatment with tiotropium once daily again improved lung function, increased the time to a severe asthma exacerbation and had a small effect on exacerbation rate compared to placebo (18% reduction).[17] Since 2014, tiotropium bromide administered through a soft-mist inhaler has been approved for asthma treatment in the European Union and Japan and has recently been approved by the US FDA for the long-term, once-daily maintenance treatment of asthma in patients at 12 years of age and older. For asthma, the FDA approved a once-daily dose of 2.5 μg (delivered in 2 puffs of 1.25 μg each).[18] Tiotropium was recently added to the 2015 Global Initiative for Asthma (GINA) treatment guidelines as add-on therapy for severe asthmatic adult patients with a history of exacerbations at Steps 4 or 5.[19] A Cochrane review of the sole study comparing LAMA plus ICS to increased doses of ICS for adult asthmatics found that the differences between the treatments were too small or imprecise to understand if adding a LAMA to ICS is more effective than increasing the dose of ICS but concluded that LAMA add-on may lead to greater improvement in lung function (FEV1) compared to an increased ICS dose in adult asthmatics.[20] A Cochrane review of the 5 studies in uncontrolled adult asthmatics assessing the efficacy and safety of a LAMA added to ICS vs the same dose of ICS alone, concluded that in adults taking ICS without a LABA, LAMA add-on therapy reduces the likelihood of exacerbations requiring OCS treatment and improves lung function. However, the benefits of LAMA combined with ICS for hospital admissions, quality of life, and asthma control are unknown.[21] These studies suggest that once-daily inhaled tiotropium may be used as add-on therapy in patients with uncontrolled severe persistent asthma to improve lung function and possibly decrease exacerbations, but additional studies may be helpful.

Bronchial thermoplasty

Airway smooth muscle hypertrophy/hyperplasia has been considered a hallmark of severe asthma. Thus, a thermal ap-

proach to potentially decrease smooth muscle mass through applying excess heat in the airways was developed. During a series of 3 bronchsoscopies, thermal energy is delivered to the airway wall to reduce airway smooth muscle in a procedure known as BT. A single multi-center RDB sham-controlled trial (Asthma Intervention Research2 [AIR2] Trial) of 288 adult severe asthmatic subjects, symptomatic despite high-dose ICS/LABA, found that BT had a modest effect to improve asthma quality of life and reduced health care utilization and severe exacerbations over a 12-month follow-up period.[22] However, there were also significant increases in asthma exacerbations during the BT treatment phase in the BT group.[22] Other measures, such as lung function, symptom scores and rescue medication use, were not significantly different between groups.[22] Importantly, subjects excluded from the AIR2 trial included those with pre-bronchodilator FEV1 <60%, life-threatening asthma, chronic sinus disease, use of immunosuppressants, and history of 3 or more hospitalizations or 4 or more bursts of OCS for asthma, or 3 or more lower respiratory tract infections in the previous year, suggesting efficacy of the procedure in some of the most severe asthmatics is unknown.[22] Long- term safety and efficacy are not yet clear as no placebo group has been included in the follow-ups. However, the proportion of subjects experiencing an exacerbation remained significantly decreased in the BT group at years 2 and 5 compared to the 1st year post thermoplasty.[23,24] These studies suggest that thermoplasty may provide long-lasting benefit in some severe asthmatics. In a small observational study of bronchial biopsies from 10 severe, uncontrolled asthmatics (all on ICS/LABA and 7 on oral CS) who underwent BT and had a baseline airway smooth muscle (ASM) area of ≥15%, ASM mass was reduced at 3 months post-BT compared to baseline biopsies (absolute decrease in ASM area=12.9%) but was not different between lung areas nor comparing the right vs left sides of the lung, or even when comparing airways that had been treated vs those that had not.[25] Due to abnormalities seen on CXR following the third BT procedure in 1 patient, CT scans were performed the day after each procedure in the next 7 patients and showed alveolar and ground glass opacities in all patients in the treated lobe and in the middle (untreated) lobe in 5 of 7 patients, suggesting possible pathologic changes extending beyond the smooth muscle.[25] In another small prospective study of 17 severe asthmatics who completed BT, collagen deposition and ASM mass decreased in the treated airway segments (absolute decrease of 8.3%).[26] Sub-analysis of 5 subjects with a baseline ASM area of ≥15% showed a mean absolute ASM reduction of 16.2%, suggesting that those with more baseline ASM may be more responsive to BT, although the post-bronchodilator FEV1 values did not change at 1-year follow-up, suggesting that the mechanisms are not completely understood.[26] In another recent prospective biopsy study of 11 severe asthmatics (8 with a baseline FEV1 <60% predicted and on chronic OCS), endobronchial biopsy post-BT showed decreased α-smooth muscle actin expression in 7 of 11 subjects and several inflammatory mediators (including transforming growth factor-β and CCL5) were decreased in all subjects in the bronchoalveolar lavage 6 weeks post-BT.[27] Despite these findings, FEV1 at 3 and 6 weeks post-BT did not improve in 6 of these patients (3 with an initial FEV1 >60% and 3 with FEV1 <60% predicted), again suggesting that it may be challenging to identify those who will benefit from BT.[27] A Cochrane review of 3 randomized controlled trials (totaling 429 patients) that compared BT vs any active control in adults with moderate or severe persistent asthma concluded that there were lower rates of asthma exacerbations, but no difference in asthma control scores and an increased risk of adverse event during treatment.[28] While there was a modest clinical benefit in quality of life, 2 of the studies did not include a sham arm.[28] Therefore, although the ERS/ATS guidelines on severe asthma do not advise against this FDA-approved procedure, they cite very low confidence in the current data on this procedure, as the potential benefits and harms may be large, the long-term consequences of this invasive approach are unknown, and there is currently a lack of understanding which patients may benefit.[2] They strongly recommended that any patient undergoing this procedure be entered into an IRB-approved registry or clinical trial. Additional studies are needed to determine if patients with systemic CS-dependent, type 2 inflammation and severe obstruction would benefit from BT.

Targeting IgE in severe asthma

While no longer a new therapy for severe asthma, omalizumab was the first monoclonal antibody used in asthma treatment. Omalizumab binds to free IgE and prevents IgE from binding to its high-affinity receptors (FcεRI). It was initially approved for treatment of moderate to severe allergic asthma, defined by atopy (presence of perennial specific IgE on skin or serum testing) and a total serum IgE level between 30 and 700 IU/mL. In an early phase III RDBPC study of 525 allergic persistent asthmatics on ICS defined by earlier NHLBI EPR-2 criteria, subcutaneous omalizumab every 2 or 4 weeks reduced asthma exacerbations and rescue medication use.[29] In another large RDBPC trial of 546 adult persistent allergic asthmatics, symptomatic despite ICS, subcutaneous omalizumab every 2 or 4 weeks also reduced asthma exacerbations during a 16-week stable steroid phase and a 12-week subsequent steroid dose-reduction phase.[30] In a RDBPC study of 246 patients with more severe allergic asthma on high dose ICS, subcutaneous omalizumab every 4 weeks for 32 weeks allowed reduction in ICS dose without worsening of asthma control.[31] However, these patients were not on any additional controllers. These attempts to "phenotype" asthma based on the presence of atopy and IgE level, were not very predictive of omalizumab response.

A more recent RDBPC study of 850 severe asthmatics, defined as inadequately controlled despite treatment with high-dose

ICS plus LABA with 17% on systemic CS, showed that omalizumab therapy for 48 weeks decreased exacerbations by 25% and improved asthma quality of life scores, but without effect on symptoms or lung function.[32] Given the modest observed efficacy and evolving biomarker development beyond IgE and atopy, a *post hoc* analysis of this study was performed, dividing patients by their baseline levels of Type 2 inflammatory biomarkers, particularly blood eosinophils, FeNO, and serum periostin. Those patients with levels higher than the median values for these biomarkers had greater reduction in exacerbations than those severe asthmatics without elevations in these biomarkers.[33] However, differences in other outcomes (symptoms or lung function) were not affected by this approach.

While these studies suggest that omalizumab is effective in a subset of severe, allergic asthma, perhaps particularly in those with ongoing Type 2 inflammation, concerns about anaphylaxis and cardiovascular risk, cost and lack of efficacy in some patients suggest that other treatments are needed. The ERS/ATS guidelines on severe asthma suggest that anti-IgE could be considered in patients with severe allergic asthma, although the degree of improvement and the evidence to support it in guidelines-defined severe asthma was modest.[2] A Cochrane review analyzed the subset of patients receiving OCS in 25 omalizumab asthma trials. Although the asthmatics receiving OCS may have had better asthma control while receiving omalizumab, the maintenance OCS dose is not significantly impacted. Thus, its utility in systemic CS-dependent patients is not clear.[34,35]

Emerging biologic therapies for severe asthma: IL–5 targeted therapies

Studies suggest that eosinophilia is present in approximately 50% of asthma cases, across the spectrum of severity. However, it is most strongly associated with a severe, generally adult-onset asthma phenotype with persistence of eosinophils despite high doses of CS and less evidence for traditional allergic markers.[36-39] As a potent pro-eosinophilic cytokine, IL-5, and its receptor, IL-5R, have been targeted in severe asthma. Although earlier studies of monoclonal antibodies against IL-5 in non-phenotyped, mild-moderate asthma failed to show efficacy, subsequent studies targeting an eosinophilic phenotype of severe asthma (sputum eosinophilia of >3% in the past year), were successful.[6,7] A RDBPC trial of 61 moderate-severe asthmatics showed that monthly intravenous (IV) mepolizumab, a monoclonal antibody against IL-5, reduced asthma exacerbations, improved asthma quality of life questionnaire (AQLQ), and decreased airway wall thickening on computed tomography of the chest.[6] In 22 systemic CS-dependent asthmatics, monthly IV administration of mepolizumab decreased steroid requirements and led to some symptom improvement.[7] These small studies were followed by a large multicenter RDBPC trial of anti-IL5 in 621 severe asthmatics who met the ATS criteria for refractory asthma,[40] requiring at least 880 ug of fluticasone equivalent per day with or without maintenance oral CS, additional controller drugs and a history of at least 2 exacerbations requiring systemic CS in the prior year. The patients were defined as having Type 2/eosinophilic inflammation on the basis of sputum eosinophils ≥3%, blood eosinophil counts ≥300/μL, or FeNO ≥50 ppb) in the Dose Ranging Efficacy And safety with Mepolizumab (DREAM) study.[41] Despite continued use of high dose ICS/LABA, IV mepolizumab decreased asthma exacerbations by 52%.[41] While the earlier studies used sputum eosinophils to define a population of severe asthmatics responsive to anti-IL5, this and subsequent studies showed that blood eosinophils could be successfully used to define a responsive severe asthma phenotype. In a subsequent study of 576 severe, eosinophilic asthmatics, with a history of 2 more exacerbations in the previous year, despite high-dose ICS, subcutaneous (SQ) or IV mepolizumab, decreased exacerbations by 53% (SQ) or 47%, (IV) respectively.[42] Compared to placebo, mepolizumab also had a small effect to improve lung function (FEV1% predicted) and symptom scores.[42] Mepolizumab has also been studied for its systemic CS-sparing effects. In a sub-analysis of the 188 severe asthmatics in the DREAM study on high-dose ICS/LABA and additionally on daily oral CS, mepolizumab was equally effective in reducing exacerbation risk and peripheral eosinophils compared to the group not on OCS.[43] This led to a prospective OCS-sparing study in 135 severe, systemic-CS-dependent patients (5-35 mg of prednisone per day). Monthly SQ mepolizumab for 20 weeks increased the likelihood of a 75% or greater reduction in CS dose by 2.39 times vs placebo and reduced the daily CS dose by a median of 50%.[44] Despite a baseline FEV1% predicted <60% and a mean of 3 severe exacerbations in the prior year, SQ mepolizumab significantly improved quality of life and asthma control, and reduced exacerbations with a safety profile similar to placebo in this population of severe asthmatics.[44]

Similar to mepolizumab, the humanized anti-IL-5 antibody reslizumab has also been studied in a severe asthma population as defined by high-dose ICS use with at least 1 other controller medication and poor control with an asthma control questionnaire (ACQ) score ≥1.5, but with blood eosinophils >400/μL. In an earlier study of eosinophilic asthmatics (sputum eosinophils ≥3%) inadequately controlled despite high-dose ICS and additional controller medications, monthly IV reslizumab modestly improved ACQ scores and FEV1, while decreasing eosinophil counts in the sputum and blood.[45] In 2 recent duplicate multicenter RDBPC phase 3 trials, 953 inadequately controlled asthmatics on medium-high doses of ICS (with 15% using daily OCS) with blood eosinophils ≥400 cells/μL and ≥1 exacerbation in the prior year, were given IV reslizumab or placebo every 4 weeks for 1 year.[46] Quite similar to mepolizumab, reslizumab significantly reduced the asthma exacerbation frequency (rate ratio of 0.5 and 0.41 for Studies 1 and 2, respectively) compared to placebo.[46] Finally, in addition

to IL-5, the receptor for IL-5 (IL-5Rα) has been targeted using the humanized monoclonal antibody benralizumab. Unlike mepolizumab or reslizumab, benralizumab binds to the IL-5Rα and then destroys eosinophils and basophils, expressing this receptor through an opsonization process.[47] In a recent trial in uncontrolled eosinophilic asthma despite ICS/LABA, SQ benralizumab (100 mg dose) for 1 year reduced exacerbations by 41% compared to placebo.[48] However, there were differences in efficacy when evaluating response in patients defined as eosinophilic by the formulaic "eosinophilic index" (primary endpoint) or simple blood eosinophil counts. Interestingly, simple blood eosinophil counts were more predictive of response to treatment than the more complex eosinophil index. Of note, most of the outcomes in this trial achieved a pre-specified significance level of $P=0.20$ rather than the traditional $P<0.05$.[48] In a RDBPC trial of 110 asthmatics who presented to the ED with an acute asthma exacerbation, a single dose of IV benralizumab did not achieve the primary outcome (proportion of subjects with ≥1 exacerbation at 12 weeks), but did reduce asthma exacerbation rates and exacerbations resulting in hospitalization compared to placebo when added to the usual standard of care.[49] While these drugs all target the IL-5 pathway, additional studies will be needed to determine if these 3 drugs all have the same efficacy. Furthermore, the definition of eosinophilia (source and absolute numbers) will be important in moving forward as these studies used slightly different thresholds. The DREAM study showed that blood eosinophils, but not sputum eosinophils, were correlated with response to mepolizumab and *post hoc* analysis of this data showed that a single screening peripheral blood eosinophil count ≥150 cells predicted response to mepolizumab.[41] Asthmatics with screening eosinophil count <150 cells had less reduction in asthma exacerbation rates.[41] In a *post hoc* analysis of placebo subjects enrolled in the DREAM trial, a single measurement of blood eosinophils ≥ 150 cells at screening predicted the average of subsequent measurements being ≥150 in 85% of this population treated with placebo.[50] Interestingly, baseline sputum eosinophils (≥3%) did not predict treatment response with mepolizumab (asthma exacerbation reduction of 69% vs 66%, respectively).[50] In the benralizumab study, subgroup analysis by a baseline blood eosinophil count ≥300 cells showed reduced exacerbations vs placebo, but this effect was not seen in the subgroup with blood eosinophils <300 cells.[48] Importantly, all of these targeted approaches to IL-5 or its receptor, reduced blood eosinophil counts to nearly undetectable. These studies suggest that blood eosinophil counts may be a good predictive biomarker for responses to anti-IL5 and IL-5Rα and are easily obtainable in the clinic.

The US FDA recently approved mepolizumab as an add-on maintenance treatment of patients with severe asthma aged 12 years and older with an eosinophilic phenotype given as a 100 mg SQ dose every 4 weeks.[51] In severe, uncontrolled asthma with evidence for persistent eosinophilia (likely blood eosinophils of 150/μL or more) despite inhaled and systemic CSs, targeting IL-5 or its receptor appears to decrease asthma exacerbations by ~50%, considerably greater than any other currently marketed drug or device for severe asthma.

Investigational drugs: Therapy directed against the Type 2 cytokines IL–4 and IL–13 in severe asthma

As canonical Type 2 cytokines, IL-4 and IL-13 are important drivers of Th2 and Type 2 inflammation. In a RDBPC study of a humanized monoclonal antibody to IL-4 receptor α (AMG 317), weekly SQ injections in moderate-to-severe otherwise non-Type 2 phenotyped asthmatics failed to show clinical efficacy (in terms of ACQ scores) but did reduce IgE levels in the serum, suggesting some evidence for biologic efficacy.[52] A significant advance was made in this area when a monoclonal antibody against IL-13 (lebrikizumab) in 219 moderate-severe uncontrolled asthmatics, despite medium-high dose of ICS/LABA, showed a small improvement in FEV1.[11] Importantly, a pre-specified sub-analysis showed that asthmatics with elevations in Type 2 biomarkers (serum periostin and FeNO) had greater increases in FEV1 with blockade of IL-13 than those without.[11] However, no other outcomes beyond FEV1 were improved. Similarly, another monoclonal antibody against IL-13, tralokinumab given SQ every 2 weeks in 194 moderate-severe asthmatics, modestly decreased rescue β-agonist use and improved FEV1.[53] Like the lebrikizumab study, those with measurable IL-13 (as a Type 2 biomarker) in sputum, had larger improvement in ACQ scores and FEV1 on tralokinumab than those without.[53] A Phase 2b RDBPC trial of tralokinumab in 452 severe asthmatics on high-dose ICS/LABA with a history of 2-6 exacerbations in the previous year failed to reduce asthma exacerbation rates vs placebo.[54] In a *post hoc* subanalysis of patients with elevated serum periostin, tralokinumab therapy modestly improved asthma exacerbation rates and prebronchodilator FEV1.[54] Interestingly, a shortened study of lebrikizumab in a Phase 2b study of moderate-severe uncontrolled asthma patients with elevated periostin showed more modest efficacy without consistent dose-dependent effects on lung function, symptoms or exacerbations. However, this study was shortened due to discovery of guinea pig IgG peptide contamination in the monoclonal.[55]

While most studies have shown favorable therapeutic outcomes with blockade of IL-13 in severe asthma, use of the monoclonal antibody to IL-13 (GSK679586) in severe asthmatics on high-dose ICS and 16% on OCS failed to show clinical efficacy (exacerbation rates or symptom scores) compared to placebo even when subgrouped by those with elevated serum IgE and/or blood eosinophils.[56] The reasons for these differences are not known.

The first study to prospectively target severe asthmatics with elevations in a Type 2 biomarker (blood or sputum eosinophils) with an IL-4/13 approach was performed with dupilumab, a

monoclonal antibody to IL-4Rα. In a Phase 2a proof of concept study of moderate-to-severe asthmatics uncontrolled despite moderate-to-high dose of ICS/LABA and eosinophilia in the blood (≥300 cells/μL) or sputum (≥3%), weekly SQ dupilumab treatment resulted in 87% fewer patients losing control of their asthma upon withdrawal of LABA and then ICS compared to placebo.[15] In contrast to studies with antibodies to IL-13, there were improvements in lung function and symptom scores both on top of background ICS/LABA therapy and when background therapy was withdrawn.[15] Decreases in type 2 biomarkers, including FeNO, IgE, and eotaxin-3, were also observed with dupilumab treatment. The improvement in FEV1 inversely correlated with the change in FeNO, emphasizing the biologic mechanism for the clinical impact of this strategy.[15] A phase 2b study of dupilumab has been completed with similar results, but suggesting that patients with lower levels of blood eosinophils may also respond.[57]

These studies confirm that IL-13 (and perhaps IL-4 as well) is a central cytokine in airway inflammation and hyperresponsiveness in asthma and in moderate-to-severe asthmatics with evidence for Type 2 inflammation, perhaps even at a lower level that initially expected. It is anticipated that further studies using IL-13 and IL-4Rα approaches will need to be performed to determine whether one approach is superior to the other and whether different patients will respond to treatment with these drugs as compared to IL-5-directed approaches.

Targeting CRTH2 in severe asthma

Prostaglandin D2 (PGD2) can be generated by activated mast cells during allergic reactions and has been shown to be increased in the bronchoalveolar lavage fluid of severe asthmatics, particularly in association with recent asthma exacerbations and Type 2 biomarkers (FeNO and blood eosinophils).[58,59] PGD2 acts through its G-protein coupled receptor, chemoattractant receptor–homologous molecule expressed on TH2 lymphocytes (CRTH2/DP2), which is present on Th2 lymphocytes, eosinophils, and group 2 innate lymphoid cells (ILC2).[60] CRTH2 activation by PGD2 can also stimulate Type 2 cytokine production by ILC2s.[61] In a RDBPC trial of the CRTH2 antagonist (OC000459) in moderate persistent asthma (not on ICS), this drug was associated with improvement in asthma quality of life, night-time awakenings and FEV1 compared to placebo.[62] In a RDBPC trial of 519 mild-moderate asthmatics not on ICS, oral OC000459 for 12 weeks increased FEV1 vs placebo in those with blood eosinophil counts ≥250/μL while no improvements were seen in those with blood eosinophil counts <250/μL.[63] In 2 trials of poorly controlled asthmatics, the CRTH2 antagonist (BI671800) was also associated with small improvements in FEV1 in symptomatic patients not on controller medications (Trial 1) and those on ICS (Trial 2).[64] Importantly, in a recent 12-week Phase 2a RDBPC trial of the oral CRTH2 antagonist (Qaw039) in 61 severe, uncontrolled asthmatics, CRTH2 blockade reduced eosinophils in the sputum and bronchial submucosa, and improved asthma quality of life and FEV1 vs placebo.[65] While additional studies are needed, CRTH2 antagonism could be a useful adjunctive approach in severe poorly controlled asthma with evidence for eosinophilia, perhaps prior to addition of more expensive biologic approaches.

Investigational approaches in Type 2, low asthma

While previous studies suggest that monoclonal antibodies targeting IgE, IL-5, IL-4, and IL-13 may be effective for some severe asthmatic patients with evidence for Type 2 inflammation, approximately half of asthma patients do not have Type 2 inflammation.[9,13] Thus, other pathways have been targets for therapy, particularly in a severe, uncontrolled population without evidence for Type 2 inflammation ("Type 2, low asthma"). However, very few targets have been identified. Although anti-TNF is highly effective in rheumatoid arthritis, studies in asthma have not consistently shown efficacy, and the efficacy to safety ratio is unacceptable, which has limited further development of drugs targeting TNF-α in severe asthma.[66-68]

T helper 17 (Th17) cells produce 2 cytokines (IL-17A and IL-17F) which indirectly recruit neutrophils.[69] One study has targeted the IL-17 receptor in severe asthma. Treatment with brodalumab, an anti-IL-17 receptor antibody (blocking receptor binding to IL-17A and IL-17 F and also IL-17E/IL-25) in 322 severe asthmatics in a RDBPC trial did not show any treatment differences compared to placebo.[70] Minimal improvements in ACQ responses were seen only in a high-reversibility subgroup (post-bronchodilator FEV1 improvement >20%), but subgrouping by blood neutrophils or eosinophils did not affect outcomes.[69] A follow-up Phase 2b study targeting the high-reversibility subgroup failed to show any efficacy, and the study was stopped.[71]

The presence of neutrophils in the airway may be important in the pathogenesis of severe asthma.[72-75] CXCR2, a receptor for IL-8, a potent chemoattractant for neutrophils, has been a therapeutic target in severe asthma. In a RDBPC study, 34 severe asthmatics with sputum neutrophils >40% received SCH527123, a selective CXCR2 receptor antagonist, or placebo daily for 4 weeks.[76] Despite decreases in blood and sputum neutrophils and fewer mild exacerbations, no other clinical benefits were observed.[76]

While macrolide antibiotics are FDA-approved for treating bacterial infections, use in treatment for asthma/severe asthma is investigational. In several chronic neutrophilic airway diseases, such as exacerbation-prone COPD, non-cystic fibrosis bronchiectasis, and bronchiolitis, treatment with low-dose macrolides, such as azithromycin and erythromycin, decreases exacerbations.[77-79] Macrolide treatment has also been studied in severe asthmatics with frequent exacerbations. In a RDBPC trial of 109 exacerbation-prone severe asthmatics but with low evidence for Type 2 inflammation on the basis of low FeNO, daily

azithromycin was added to combination ICS/LABA for 6 months.[80] The rate of severe exacerbations and lower respiratory tract infections requiring antibiotic treatment was not different between the azithromycin and placebo groups. However, when subanalyzed in a *post hoc* analysis, the severe asthma subgroup with the least evidence for any Type 2 inflammation (no/low eosinophils and low FeNO) had a lower rate of exacerbations and infections compared to placebo.[80] However, the small numbers and *post hoc* analysis make these results less robust. Until more data are available, the ERS/ATS guidelines suggest that clinicians do not use macrolide antibiotics for the treatment of severe asthma due to concerns about the development of macrolide antibiotic resistance and uncertain clinical benefits.[2]

Alternative immunosuppressives in severe asthma

Immunosuppressive agents, such as methotrexate, have been studied for their steroid- sparing ability in systemic CS-dependent asthma for many years. However, all the studies of these agents were performed before ICSs (and LABAs) became a mainstream treatment for asthma, and before any concept of phenotyping. Therefore, their applicability today remains unclear. In 31 asthmatics who required daily prednisone (mean dose=26.8 mg/day, for 4.7 years), long-term methotrexate therapy for 18-28 months enabled a prednisone dose reduction to 6.3 mg/day, and improved FEV1 and asthma symptom scores.[81] In contrast, in a RDBPC 13 weeks trial of methotrexate intramuscularly weekly in 19 patients with severe CS-dependent asthma, no differences between methotrexate and placebo were observed.[82] In a Cochrane Database review of 10 randomized trials of 185 adult CS-dependent asthmatics where methotrexate was added to usual therapy for at least 12 weeks there was a small reduction in oral CS dose favoring methotrexate but no difference in FEV1 and hepatotoxicity was seen more frequently with methotrexate.[83] Based on these minimal data, the lack of more recent data in patients treated with ICS/LABA and lack of any phenotyped studies, the ERS/ATS guidelines on severe asthma do not recommend the use of methotrexate in adults with severe asthma.[2] If methotrexate is used, the guidelines recommend use in specialized centers in patients who require daily OCS and that labs (including CBC with differential), liver function, creatinine), chest X-ray, and DLCO are obtained before starting therapy and upon completion.[2]

In an observational study of 19 patients referred for evaluation of severe CS-refractory asthma, 10 patients had inconsistent abnormalities on chest CT scan and underwent video-assisted thoracoscopic biopsies.[84] Pathology of these 10 cases showed findings consistent with asthma (eosinophilia, goblet cell hyperplasia), but also showed interstitial nonnecrotizing granulomas with a high frequency (70%) of personal or family history of autoimmune-like disease, termed "asthmatic granulomatosis." Of these asthmatic granulomatosis patients treated with alternative agents, such as azathioprine, mycophenolic acid, methotrexate, or infliximab, 90% showed decreased CS requirements and improved or maintained FEV1 despite lower CS doses.[84] While there are no RDBPC trials in asthmatic granulomatosis, this study was the first to suggest that some severe asthmatics may have an autoimmune form of asthma and benefit from immunosuppressive therapies not previously used in asthma treatment. Further studies are clearly needed.

CONCLUSION

Asthma is an important national health problem, with 10% of all asthmatics having severe disease and responding poorly to standard asthma treatment, such as inhaled and systemic corticosteroids. With recent studies suggesting that asthma is a heterogeneous disease of different phenotypes and responses to selected therapies, studies which incorporate the interaction between various biomarkers and responses to specific treatments in severe asthmatics should increases. By identifying the distinct immunologic mechanisms involved in severe, poorly controlled asthma, new targeted therapies could improve patient quality of life and our understanding of human asthma. While several monoclonal antibodies show promise in asthma with evidence for Type 2 inflammation, further work is needed for severe asthmatics who do not show evidence of Type 2 inflammation.

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