

Asthma phenotypes and the use of biologic medications in asthma and allergic disease: The next steps toward personalized care

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Activity Objectives

1. To understand the molecular phenotypes of asthma and how these can be used to predict response to therapy.
2. To understand which interventional trials have most shaped the paradigm of phenotype-directed, targeted asthma therapies

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Traditionally, asthma and allergic diseases have been defined by broad definitions and treated with nonspecific medications, including corticosteroids and bronchodilators. There is an increasing appreciation of heterogeneity within asthma and allergic diseases based primarily on recent cluster analyses, molecular phenotyping, biomarkers, and differential responses to targeted and nontargeted therapies. These pioneering studies have led to successful therapeutic trials of molecularly targeted therapies in defined phenotypes. This review analyzed randomized double-blind, placebo-controlled trials of molecularly targeted therapies in defined allergic disease and asthma phenotypes. IgE was the first successful biological target

used in patients with allergic disease and asthma. This review shows that therapies targeting the canonical type 2 cytokines IL-4, IL-5, and IL-13 have shown consistent efficacy, especially in asthmatic patients with evidence of T_H2/type 2 inflammation (“type 2 high”). As of yet, there are no successful trials of targeted therapies in asthmatic patients without evidence for type 2 inflammation. We conclude that further refinement of type 2 therapies to specific type 2 phenotypes and novel approaches for patients without type 2 inflammation are needed for asthma and allergic disease treatment. (*J Allergy Clin Immunol* 2015;135:299-310.)

Key words: Asthma phenotypes, biologic therapies, eosinophils, IgE, IL-4, IL-5, IL-13, T_H2/type 2 inflammation

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Asthma and allergies are common yet heterogeneous chronic diseases. The definition of asthma, reversible airflow limitation or bronchial hyperresponsiveness with appropriate clinical symptoms, is relatively broad and nonspecific, such that multiple clinical phenotypes meet this simple definition.¹ Asthma

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Abbreviations used

ACQ:	Asthma Control Questionnaire
DBPC:	Double-blind, placebo-controlled
FDA:	US Food and Drug Administration
FENO:	Fraction of exhaled nitric oxide
ICS:	Inhaled corticosteroid
IL-4R α :	IL-4 receptor α
IL-5R α :	IL-5 receptor α
LABA:	Long-acting β -agonist
OCS:	Oral corticosteroid
TSLP:	Thymic stromal lymphopoietin

treatments are predominantly nonspecific anti-inflammatory drugs (corticosteroids) and bronchodilators (β_2 -agonists), which work in most patients. However, even responses to these treatments vary. Although multiple factors can contribute to poor responses, underlying pathobiological differences are increasingly recognized to play a role.

In contrast to asthma, allergy is defined as “the result of immune reactions to antigens known as allergens” in which the body is predisposed to produce specific IgE antibodies after exposure to these allergens.² Type I immediate hypersensitivity allergic reactions include allergic rhinitis, allergic asthma, and food allergy.² Although there is some heterogeneity in patients with allergic disease as well, less phenotypic characterization has been reported.

A phenotype involves the complex interaction of many genetic and environmental factors in conjunction with observable characteristics, such as lung function (for asthma) or specific IgE responsiveness to particular allergens.² Asthma phenotyping has involved biased and unbiased approaches to grouping clinical, physiologic, and hereditary characteristics.³⁻¹¹ Studies have supported the importance of age of onset, eosinophils, and lung function, but definitive clustering of these characteristics or their relation to pathobiology remains uncertain.^{4,5,11,12}

IDENTIFYING TYPE 2 CHARACTERISTICS FOR TARGETED THERAPY

Identification of T_H1 and T_H2 immunity in the early 1990s and widespread efficacy of inhaled corticosteroids (ICSs) led to the hypothesis that asthma/allergies were primarily driven by T_H2 immunity involving the cytokines IL-4, IL-5, and IL-13.¹³⁻¹⁶ Despite this, pathobiologic studies suggested differences in inflammatory/immune processes across asthmatic patients,¹⁷ and early studies of T_H2-targeted therapies were not efficacious.^{18,19}

Although it had been reported for years that corticosteroid responses were dependent on lung eosinophils, asthma phenotype was rarely considered when planning therapy.^{5,11,17} Phenotype-directed therapy began to evolve when an mAb to IL-5 (mepolizumab) was specifically developed for eosinophilic asthma. In contrast to previous studies in nonselected asthmatic patients, antieosinophilic therapy reduced exacerbations and systemic corticosteroid requirements in patients with eosinophilic asthma.^{20,21} By using a different approach, epithelial biomarkers for type 2/IL-13 inflammation were first identified *in vitro* and then identified *ex vivo* in a subset of patients.^{22,23} Three genes (chloride channel, calcium activated,

family member-1 [*CLCA1*]; periostin [*POSTN*]; and *SERPINB2*) upregulated in response to IL-13 *in vitro* were identified in fresh human airway epithelial cells from approximately 50% of corticosteroid-naïve patients with mild asthma. The remaining asthmatic patients and healthy (nonatopic) control subjects had low expression of these “T_H2/type 2” genes. Those in the type 2–high cluster were more atopic, had higher tissue eosinophil counts, and had more bronchial hyperresponsiveness. Importantly, the T_H2/type 2–high cluster improved with inhaled fluticasone, whereas those without the type 2 signature did not.²³ These studies were some of the first to demonstrate improved efficacy of targeted and untargeted therapies when directed to patients whose characteristics suggest they should be more responsive to those therapies. Identifying molecular pathways that contribute to clinically meaningful outcomes (through targeted therapy) could ultimately lead to identification of disease endotypes, none of which have yet been fully described in asthmatic patients.^{24,25}

TYPE 2-RELATED THERAPIES

The evolution of asthma treatment holds the promise of development of precision medicine, biologic therapies specifically targeted to the right patients to improve efficacy and decrease risk. Pathobiologic studies combined with therapeutic trials of type 2–targeted therapies have confirmed the existence of a type 2 asthma phenotype. These findings spurred further studies of type 2–targeted therapy in patients with biomarker evidence for type 2 inflammation.

Overview: Type 2 immune-related targets

For more information, see Fig 1, Table I,^{18-21,26-56} and Table E1 in this article’s [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Type 2–targeted biologic therapies either approved or in clinical trials include those targeted to IgE, IL-5, IL-13, IL-4 receptor α (IL-4R α), and thymic stromal lymphopoietin (TSLP). IL-4 and IL-13 are central to type 2 inflammation, with GATA3 being their master transcription factor. IL-4R α is the common receptor subchain for both IL-4 and IL-13, which is present in both the type 1 (dimerized with γc) and type 2 (dimerized with IL-13 receptor $\alpha 1$) receptors. IL-4 activates both type 1 and type 2 receptors, whereas IL-13 only activates type 2 receptors. Thus IL-13 cannot activate T cells, but both IL-4 and IL-13 can promote IgE isotype switching in B cells.^{15,16,57} IgE binds to the high-affinity IgE receptor, which activates mast cells/basophils associated with type I hypersensitivity when cross-linked by allergen. IL-5 is critical to eosinophil development and survival and, interestingly, is generated in high amounts (as is IL-13) by the newly identified type 2 innate lymphoid cells.⁵⁸ Although IL-4 is required for T_H2 priming and maturation, T_H2 differentiation is enhanced by cytokines, such as TSLP.⁵⁹ Thus numerous components of type 2–related immune processes are now therapeutic targets for asthma/allergies.

Biomarkers: Type 2 molecular phenotypes

Biomarkers are needed to target type 2 therapies to the correct patients. Type 2 biomarkers identified to date include periostin, fraction of exhaled nitric oxide (FENO), and sputum/blood eosinophils. Early on, sputum eosinophil counts were determined

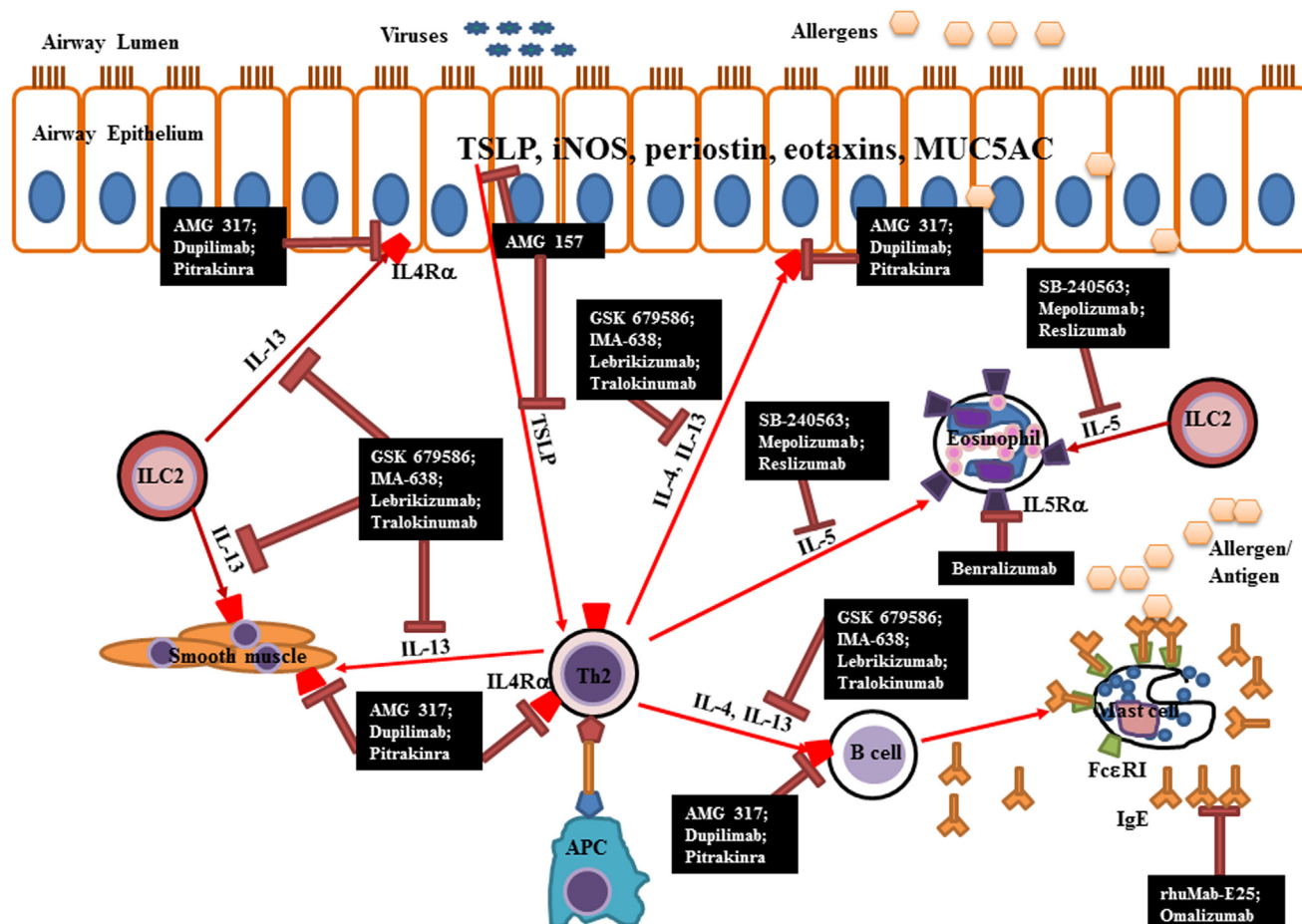


FIG 1. The matrix of type 2 inflammatory components involving resident and inflammatory cells in the human airway, highlighting type 2 cytokine and IgE pathways. The potential impact of investigative and approved biologic therapies on critical cell-cell interactions important to T_H2 /type 2-high asthma and allergic disease is indicated, specifically including biologic agents targeting IgE, IL-5 and its receptor, IL-13, IL-4 receptor α and TSLP. APC, Antigen-presenting cell; ILC2, type 2 innate lymphoid cell; iNOS, inducible nitric oxide synthase.

to predict response to systemic corticosteroids, with later studies showing their absence associated with poor corticosteroid responses.^{60,61} Although *in vitro*/animal studies supported a relationship of type 2 cytokine levels to eosinophil counts, this was not confirmed until anti-IL-5 antibodies almost totally depleted blood eosinophils.^{18-21,26,27} Interestingly, although blood eosinophil counts correlate reasonably well with sputum IL-13 mRNA levels, these counts are not dependent on IL-4/IL-13.^{28,29,62} When measured in serum, levels of periostin, which has been identified in airway epithelial brushings as a downstream signature of IL-13, predicted eosinophilic airway inflammation.^{23,63} FENO, which is generated primarily by inducible nitric oxide synthase in airway epithelial cells, is also strongly induced by IL-13.^{64,65} Although FENO levels correlate with lung eosinophilia,⁶⁵ FENO levels are independent of IL-5.²⁰ Thus several type 2-related biomarkers have emerged, although their specific utility across type 2 therapies remains to be determined.

Targeting IgE

Asthma. Allergic asthma. For years, asthma has been considered an allergic disease strongly associated with allergen-

specific IgE. Allergen challenge studies model both immediate and later delayed hypersensitivity allergic reactions. A recombinant humanized anti-IgE mAb (omalizumab) was developed to block interactions of IgE with high-affinity IgE receptors (Fc ϵ RI). As predicted, anti-IgE suppressed the early immediate hypersensitivity response to nebulized inhaled allergen in corticosteroid-naïve patients with mild asthma.³⁰ Surprisingly, subsequent studies reported decreases in both early and late asthmatic responses, sputum and tissue eosinophil counts, and submucosal IgE⁺ and Fc ϵ RI⁺ cell counts.^{31,32} Finally, omalizumab blunted the decrease in FEV₁ and symptom scores after cat chamber exposure in asthmatic patients with cat allergy.³³ Thus anti-IgE is consistently effective in allergic asthmatic reactions.

Further omalizumab studies in patients with chronic asthma also targeted an allergic phenotype, which was loosely defined by atopy (presence of specific IgE measured by using skin or serum tests) and a total serum IgE level of between 30 and 700 IU/mL. Early studies of patients with moderate allergic asthma taking ICSs demonstrated reductions in asthma exacerbations and corticosteroid requirements.^{34,35} In 2 large trials of adults with moderate allergic asthma, subcutaneous

TABLE I. Summary of DBPC trials using biologic medications in patients with T_H2/type 2–high asthma

Target	Biologic therapies used	Type of study	Major outcome	References
IgE	Anti-IgE mAb (rhuMAb-E25, omalizumab)	Allergen challenge: mild-to-moderate allergic asthma	↓ Early and late asthmatic response, ↓ serum free IgE	30-33
		Chronic moderate-to-severe allergic asthma	↓ Asthma exacerbations, ↓ serum free IgE	34-42, 44
		Chronic severe allergic asthma	↓ Asthma exacerbations greater when subanalyzed by type 2–high phenotypes (↑ FENO levels, blood eosinophil counts, or serum periostin levels)	43
IL-4 and IL-13	Mutant IL-4 (pitakinra); IL-13 antibody (IMA-638)	Allergen challenge: mild allergic asthma	↓ Late asthmatic response	48, 49
	IL-4Rα mAb (AMG 317); mutant IL-4 (pitakinra)	Chronic moderate-to-severe asthma	No effect on prespecified clinical asthma outcomes in “all comers,” + SNPs of IL-4Rα gene associated with clinical response (pitakinra)	50, 84
	IL-13 mAb (lebrikizumab, tralokinumab)	Chronic moderate-to-severe asthma	↑ FEV ₁ ; greatest clinical benefit when subanalyzed by type 2–high phenotypes (↑ periostin and sputum IL-13 ⁺)	29, 83
	IL-13 mAb (GSK679586)	Very severe asthma	No effect on prespecified clinical asthma outcomes	99
	IL-4Rα mAb (dupilumab)	Chronic moderate-to-severe asthma with type 2–high phenotype (blood eosinophils ≥300 cells/μL or sputum eosinophils ≥3%)	↓ Asthma exacerbations, ↓ FENO, ↓ β-agonist use, ↑ FEV ₁	28
IL-5	Anti-IL-5 (SB-240563)	Allergen challenge: mild allergic asthma	No effect on clinical asthma outcomes despite ↓ in blood and sputum eosinophil counts	18
	Anti-IL-5 (mepolizumab)	Mild-to-moderate allergic asthma	No effect on clinical asthma outcomes despite ↓ in blood, sputum, bone marrow, and airway eosinophil counts	19, 26
	Anti-IL-5 (mepolizumab; reslizumab)	Chronic, refractory, severe asthma with type 2–high phenotype (sputum eosinophils >3%, or ↑ blood eosinophil counts or FENO levels)	↓ Asthma exacerbations, ↓ blood/sputum eosinophils, ↑ FEV ₁	20, 21, 27, 54, 89, 90
	Anti-IL-5Rα (benralizumab)	Chronic eosinophilic asthma with type 2–high phenotype (sputum eosinophils ≥2.5%)	↓ Eosinophils in airway mucosa/submucosa, sputum, bone marrow, and blood; clinical measures not evaluated	91
TSLP	Anti-TSLP mAb (AMG 157)	Allergen challenge: mild allergic asthma	↓ Late asthmatic response, ↓ in blood/sputum eosinophil counts, ↓ FENO levels	97

SNP, Single nucleotide polymorphism.

omalizumab reduced asthma exacerbations during a stable steroid phase (16 weeks) and a subsequent steroid dose-reduction phase (12 weeks) compared with placebo.^{36,37} Effects on symptoms and lung function were minimal, despite decreases in exacerbations.

Given the expense of treatment with biologic agents, subsequent studies targeted patients meeting published definitions of severe asthma, including high-dose ICS treatment alone or in combination with a second controller. In patients with severe atopic asthma taking ICSs and long-acting β-agonists (LABAs), omalizumab reduced ICS requirements at 32 weeks (primary end point) compared with placebo after a 16-week fluticasone reduction phase.³⁸ In contrast, in a 28-week severe asthma trial, add-on omalizumab did not significantly reduce asthma exacerbations.³⁹ In the patients with the most severe asthma studied (inadequately controlled despite ICSs/LABAs, with 17% taking oral corticosteroids [OCSs]), omalizumab reduced asthma exacerbations over 48 weeks compared with placebo. Quality of life and symptom scores improved but not

FEV₁.⁴⁰ Although omalizumab is not US Food and Drug Administration (FDA) approved for use in children younger than 12 years, 3 trials have demonstrated efficacy in reducing asthma exacerbations.^{35,41,42}

Anti-IgE treatment most consistently affects asthma exacerbations. Although the effect on allergic reactions suggests some reductions are related to allergic exacerbations, a study of inner-city children (6-20 years of age) with persistent asthma (with or without controller use) reported that omalizumab significantly decreased school-related, likely viral exacerbations, but the mechanisms for this are not clear.⁴¹

Although anti-IgE was developed for allergic asthma, as defined by the presence of atopy/minimally increased total IgE levels, it is not effective in all “qualified” patients. Additionally, use of omalizumab has been limited by its expense, multiple injections and injection-site reactions, a black box warning on anaphylaxis, and new warnings on cardiovascular risk. Thus an improved ability to predict responsive patients with high certainty is important. Although omalizumab has not

been prospectively studied in patients identified based on other type 2 biomarkers, a retrospective analysis of the study by Hanania et al⁴⁰ divided patients into those with and without type 2 inflammation based on median splits of blood eosinophil counts and serum periostin and FENO levels. Patients with biomarker levels greater than the median had greater reductions in asthma exacerbations with omalizumab therapy compared with those with levels less than the median.⁴³ Although no other outcomes were affected, this approach should be prospectively validated.

Allergic disease. Because omalizumab reduces allergic asthmatic responses, other allergic diseases involving IgE cross-linking on mast cells/basophils have also been targeted.

Allergic rhinitis. Although not approved for use in allergic rhinitis, omalizumab has been extensively studied, with a meta-analysis of 11 trials showing consistent reductions in nasal symptom severity and daily nasal rescue medication scores.⁶⁶ Subcutaneous omalizumab improved rhinitis symptoms, rescue antihistamine use, and quality of life when given before and/or during the ragweed or birch pollen season compared with placebo.^{67,68} It also improved rhinitis in asthmatic patients.^{33,44} When used in association with allergen immunotherapy, omalizumab reduced side effects (including anaphylaxis) and led to greater symptom improvement and quality of life compared with immunotherapy alone.⁶⁹⁻⁷¹ Despite this efficacy, omalizumab is not approved for the treatment of allergic rhinitis, likely because of its overall cost and side-effect profile.

Food allergy. A dose-ranging trial of anti-IgE (TNX-901) increased the tolerated dose on oral challenge from half a peanut (at baseline) to almost 9 peanuts.⁷² Similarly, omalizumab treatment increased the amount of peanut flour tolerated on oral challenge compared with placebo.⁷³ When added to peanut desensitization, omalizumab-treated patients were more likely to achieve the maximum maintenance peanut dose compared with those receiving placebo and were able to continue at this tolerance level despite stopping omalizumab.⁷⁴ Although these small studies support targeting IgE in patients with food allergy, anti-IgE therapy is not FDA approved for this indication.

Atopic dermatitis. Two small double-blind, placebo-controlled (DBPC) trials of subcutaneous anti-IgE in patients with atopic dermatitis have been reported.^{75,76} In one study, although atopy patch test results improved, clinical disease parameters were not significantly altered.⁷⁵ In the other trial omalizumab-treated patients had decreased levels of T_H2 cytokines (TSLP and thymus and activation-regulated chemokine), but clinical improvements were similar to those seen in placebo-treated patients.⁷⁶ Although small case studies have reported possible clinical improvements,⁷⁷⁻⁷⁹ its efficacy is unknown, and current use of this drug to treat atopic dermatitis is considered investigational.⁸⁰

Urticaria. Although little is understood regarding the pathobiology of chronic urticaria, recent trials with omalizumab support a role for IgE. Like asthma, chronic urticaria consists of multiple phenotypes, but little is known about which phenotype is targeted by anti-IgE. In 90 symptomatic patients with chronic idiopathic urticaria, a single dose of omalizumab improved hive and itch scores compared with placebo.⁴⁵ In 2 more trials in patients with chronic symptomatic urticaria treated with high-dose antihistamines, leukotriene receptor antagonists, or both,^{46,81}

monthly omalizumab injections (300 mg) significantly decreased the itch score compared with placebo.^{46,81} For chronic idiopathic urticaria, the FDA approved a dose of 150 or 300 mg administered subcutaneously every 4 weeks for patients 12 years or older; this is not dependent on serum IgE level or body weight, and length of treatment has not been defined.⁴⁷ Interestingly, many responders had low/undetectable IgE levels, such that the mechanism for efficacy is unclear.

Targeting the canonical type 2 cytokines IL-4 and IL-13

IL-4 and IL-13 are type 2 cytokines long believed important in allergic airway inflammation.¹⁴⁻¹⁶ Yet studies of molecules targeting these pathways were not broadly successful until linked with type 2 biomarkers beyond atopy/IgE.

Asthma. Allergic asthma. Human allergen challenge models replicate animal models of allergic asthma in which the importance of T_H2 cytokines (particularly IL-13) has long been established. Pitrakinra is a mutant IL-4 molecule that blocks the ability of human IL-4 or IL-13 to bind to IL-4R α . Nebulized pitrakinra for 4 weeks in patients with mild atopic asthma reduced the late asthmatic response 3.7-fold compared with placebo.⁴⁸ Similarly, an IL-13 antibody (IMA-638) decreased both early and late asthmatic responses.⁴⁹ Although both approaches improved lung function after challenge, they did not affect methacholine hyperresponsiveness or sputum eosinophil counts. However, FENO levels decreased over the 4-week baseline compared with placebo, linking it to IL-4/IL-13 pathways and suggesting its use as a type 2 biomarker.⁴⁸ Thus the importance of IL-4/IL-13 blockade to allergic airway reactions was confirmed.

Studies of patients with nonphenotyped chronic asthma. Earlier studies targeting IL-4 in patients with chronic asthma did not target specific asthma phenotypes. Although a small pilot steroid-tapering study supported the efficacy of blocking IL-4 in patients with moderate asthma, no subsequent studies were published.⁸² In fact, many years passed before IL-4 and IL-13 were again targeted in patients with chronic asthma. In a large trial of adults with moderate-to-severe asthma whose symptoms were inadequately controlled despite mid- to high-dose ICS/LABA treatment, subcutaneous lebrikizumab, a humanized IgG₄ mAb to IL-13, administered for 6 months improved FEV₁ compared with placebo, although the difference was small, and other outcomes were not affected.²⁹ Twelve weeks of tralokinumab (another humanized IgG₄ mAb to IL-13) also reported only modest improvement in FEV₁ and some decrease in β -agonist use.⁸³

Two studies evaluated a broader blockade, inhibiting IL-4R α without any additional benefit. Pitrakinra did not decrease induced asthma exacerbations compared with placebo when background medication was withdrawn in a population with moderate-to-severe asthma.⁵⁰ Similarly, a study of a humanized mAb to IL-4R α (AMG 317) for 12 weeks had no effect on asthma outcomes, although it did reduce serum IgE levels, suggesting some biologic activity.⁸⁴ Thus in patients with nonphenotyped asthma, blockade of IL-13 alone or in combination with IL-4 demonstrated only modest clinically insignificant improvements.

Studies in patients with increased type 2 biomarker asthma phenotypes. Two of the studies listed above

prespecified additional analysis in the context of type 2 biomarkers.^{29,83} In the lebrikizumab study described previously, preplanned analyses identified patients with “T_H2-high” asthma by different means. T_H2-high asthma was initially identified by using serum IgE and blood eosinophil measurements, which only modestly improved the efficacy of lebrikizumab on exacerbations.²⁹ However, type 2–high asthma was also prespecified by serum periostin levels, using a median split to define the “high” and “low” groups. Patients with high periostin levels treated with lebrikizumab had a more marked improvement in FEV₁ (8.2%) compared with those receiving placebo (with a tendency toward decrease exacerbations), whereas those with low levels had no improvement.²⁹ High FENO levels (by median split) also predicted a responsive phenotype, whereas high levels of both markers were even more predictive of an FEV₁ response.²⁹ Similar to pitrakinra, lebrikizumab also decreased FENO levels, which is supportive of biologic effect on active type 2–associated pathways. In contrast, blood eosinophil counts increased for unclear reasons. Tralokinumab (anti-IL-13) also performed better in asthmatic patients with measurable type 2 signatures, as defined by detectable IL-13 in sputum, with greater improvement in FEV₁ and Asthma Control Questionnaire (ACQ) 6 scores at week 13 compared with those in the sputum IL-13–negative tralokinumab and placebo groups.⁸³

The first study to prospectively target a type 2–high phenotype, as defined by blood (≥ 300 cells/ μ L) or sputum eosinophilia ($\geq 3\%$ at screening), used dupilumab, a humanized mAb to IL-4R α .²⁸ In a small 12-week study of patients with moderate-to-severe asthma whose symptoms were not well controlled with moderate- to high-dose ICS/LABA treatment (approximately 80% on high dose combination), dupilumab was associated with fewer “induced” asthma exacerbations (87% reduction compared with placebo) when LABAs and then ICSs were successively withdrawn.²⁸ In this eosinophilic/type 2–high population dupilumab improved ACQ scores, symptoms, and FEV₁ on top of combination ICS/LABA treatment when background therapy was withdrawn and even when no background therapy remained. There were also significant improvements in upper airway symptoms.²⁸ Multiple type 2 biomarkers, including FENO, thymus and activation-regulated chemokine (CCL17), eotaxin-3 (a type 2 eosinophilic CC chemokine), and IgE levels, also decreased. In fact, the change in FENO levels inversely correlated with the change in FEV₁, which was highly supportive of a clinically meaningful effect of dupilumab on its target biologic pathway.²⁸ Similar to lebrikizumab, however, blood eosinophil counts did not decrease.

Pharmacogenetics and IL-4/IL-13 targets. Polymorphisms in type 2 cytokine pathway genes have been strongly linked to asthma.⁸⁵ Single nucleotide polymorphisms in *IL4RA* are associated with both asthma and severe asthma, including increased tissue mast cell numbers.⁸⁶ When the allergen challenge response was evaluated in the context of *IL4RA* polymorphisms, greater improvement in response to pitrakinra was seen in patients expressing certain *IL4RA* alleles.⁸⁷ Perhaps more importantly, in the 12-week pitrakinra study that did not show efficacy in nonselected asthmatic patients, those homozygous for the common alleles at rs8832, rs1029489, or rs8832 who were randomized to pitrakinra had a dose response–related decrease in asthma exacerbations, nocturnal awakenings, and activity limitation compared with those

receiving placebo.^{50,87} These studies suggest that a type 2 genetic background might also play a role in predicting response to type 2–directed interventions.

Allergic disease. Atopic dermatitis. Only 3 early-phase studies involving 207 patients have been reported on IL-4/IL-13 blockade in patients with atopic dermatitis.⁵¹ A 4-week trial of subcutaneous dupilumab in adults with moderate-to-severe atopic dermatitis despite treatment with topical glucocorticoids and calcineurin inhibitors resulted in dose-dependent clinical improvement and reduced pruritus scores.⁵¹ In a longer trial 85% of dupilumab (alone)–treated patients achieved 50% or greater improvement in Eczema Area and Severity Index scores versus 35% with placebo. Forty percent of dupilumab-treated patients cleared or nearly cleared their skin lesions compared with 7% receiving placebo.⁵¹ When combined with topical glucocorticoids, 100% of the dupilumab group met the 50% or greater improvement in Eczema Area and Severity Index score criteria, whereas only 50% of the placebo group did so.⁵¹ Dupilumab-treated patients were also able to decrease topical glucocorticoid use. Similar to the asthma study, dupilumab treatment was associated with type 2 biomarker reduction compared with baseline and placebo values, including decreased keratin 16 mRNA on skin biopsy specimens.^{28,51} Thus like type 2–high asthma, inhibition of type 2 inflammation in patients with atopic dermatitis improved clinical outcomes.

Targeting the proeosinophilic type 2 cytokine IL-5

IL-5, also a type 2 cytokine produced by T_H2 and type 2 innate lymphoid cells, is the most potent eosinophilic cytokine known, with its receptor (IL-5 receptor α [IL-5R α]) found on eosinophils and some basophils.^{58,52} IL-5 blockade selectively affects the eosinophil (and perhaps basophil) and not other “T_H2-like” inflammatory elements. Eosinophilia can be present in the early-onset/allergic asthma phenotype but is not always associated with IgE-mediated allergy.^{4,5,12,53} In addition to traditional allergic asthma, phenotypic approaches have identified a severe, adult-onset, highly eosinophilic asthma phenotype often present despite high ICS doses and often requiring systemic corticosteroids. This phenotype, although not associated with traditional allergic biomarkers or symptoms, is associated with sinus disease, nasal polyps, higher urinary leukotrienes, and more aspirin-sensitive disease.^{4,5,11,12,53} Although the clinical characteristics of these 2 asthma phenotypes are discordant, the eosinophil was a logical target, with anti-IL-5 the logical pathway.

Asthma. Studies in a type 2–associated allergen challenge model. Given the association of eosinophils (and T_H2 inflammation) with allergen challenge, it is not surprising that anti-IL-5 was initially studied in an allergen challenge. In contrast to the studies of IL-4/IL-13 blockers, anti-IL-5 therapy did not inhibit early or late asthmatic responses or airway hyperreactivity, despite profoundly decreasing blood and sputum eosinophil counts.¹⁸ This study might suggest eosinophils (and IL-5) are not as critical to traditional allergic asthma phenotypes and their allergic reactions.

Studies in patients with nonphenotyped asthma. Two subsequent studies with 3 intravenous doses of anti-IL-5 (mepolizumab) in patients with mild (no ICSs) atopic asthma

and those with moderate persistent asthma (with ICSs) yielded similar negative clinical results.^{19,26} Eosinophil counts were decreased in the airway, bone marrow, and blood, although there was a suggestion that airway eosinophil counts were only reduced by 50%. In both studies there were no effects on FEV₁ or other clinical asthma measures.^{19,26} Thus anti-IL-5, similar to treatment with antibodies to the IL-4/IL-13 pathway, was not effective in nonselected asthmatic patients and, in contrast to IL-4/IL-13, was not effective in an allergic model of asthma.

Studies targeting patients with eosinophilic asthma.

Two pioneering single-center studies were crucial to the overall phenotype approach to asthma therapy. In both studies eosinophilic asthma was defined as persistent sputum eosinophilia of greater than 3% at least once in the previous year.^{20,21} One study targeted patients with moderate-to-severe asthma,²⁰ and the other targeted OCS-dependent patients.²¹ In the larger trial intravenous mepolizumab for 1 year reduced asthma exacerbations by approximately 48% compared with placebo, with a modest effect on asthma quality of life, accompanied by a marked decrease in blood and sputum eosinophil counts.²⁰ FEV₁, symptoms, and FENO levels were not affected. However, on computed tomographic imaging, there was a decrease in airway wall thickening.²⁰ In a smaller study of systemic corticosteroid-dependent patients, mepolizumab was associated with significant reductions in OCS dose compared with placebo and baseline, small improvements in symptoms, and reductions in sputum eosinophil counts.²¹ In both trials the majority of patients appeared to have late-onset eosinophilic asthma compared with early-onset “more allergic” asthma.

Because sputum eosinophil measurement is not generally available, subsequent studies identified “eosinophilic (type 2–high) asthma” in relation to blood eosinophil counts. A very large mepolizumab trial in patients with severe asthma receiving high-dose ICS/LABA treatment with evidence of eosinophilic/type 2 inflammation (≥ 1 in the prior year: eosinophils $\geq 3\%$ sputum, $\geq 300/\text{mL}$ peripheral blood, or FENO ≥ 50 ppb) identified blood eosinophil counts of 300/mL or greater as a highly predictive biomarker of treatment response.²⁷ Three different doses of mepolizumab were equally effective in decreasing clinically significant asthma exacerbations compared with placebo, with the greatest reductions seen in those with the highest blood eosinophil counts and greatest prior exacerbation history. Once again, there was no effect on other asthma outcomes, including symptoms and FEV₁.²⁷ Mepolizumab appeared to be equally effective in patients with eosinophilic asthma, irrespective of whether they were treated with systemic corticosteroids.⁸⁸

In a recent large study of patients with eosinophilic asthma (based on peripheral blood) with recurrent exacerbations despite high-dose ICSs, monthly intravenous or subcutaneous mepolizumab decreased exacerbations by 47% to 53% and in this study increased FEV₁ and modestly affected symptom and ACQ-5 scores compared with placebo.⁵⁴ Finally, following up on the original small systemic corticosteroid-sparing study, mepolizumab was studied in patients with systemic corticosteroid-dependent severe asthma, generally of late onset with persistent blood eosinophilia. Subcutaneous mepolizumab for 20 weeks was more effective than placebo in decreasing

daily corticosteroid doses. In addition, in these patients with very severe eosinophilic asthma, mepolizumab improved asthma control and quality of life and marginally improved FEV₁, despite decreased OCS use.⁸⁹ Forty percent of mepolizumab-treated patients reduced their OCS dose by greater than 75%.⁸⁹

The anti-IL-5 antibody reslizumab was similarly studied in patients with poorly controlled asthma taking high-dose ICSs and additional controllers with persistent sputum eosinophils ($\geq 3\%$ on 2 occasions).⁹⁰ Like mepolizumab, intravenous reslizumab decreased both blood and sputum eosinophil counts with marginal increases in FEV₁ and ACQ scores compared with placebo.⁹⁰ In contrast to mepolizumab and reslizumab, benralizumab is a humanized mAb targeting IL-5R α . When engaged, benralizumab activates opsonization pathways to destroy eosinophils and basophils expressing the receptor.⁹¹ In a small trial of adult asthmatic patients, benralizumab decreased airway, sputum, bone marrow, and blood eosinophil counts, but clinical measures were not evaluated.⁹²

Overall, approaches targeting the IL-5 pathway have been efficacious, with prominent effects on exacerbations but with growing numbers of studies suggesting effects on symptoms and lung function. It remains unclear whether approaches to block IL-5R α (and opsonize the eosinophil) will differ in efficacy or target phenotype from those targeting IL-5.

Allergic disease. Only one study has been reported in patients with nonasthmatic allergic disease. Two intravenous doses of 750 mg of mepolizumab were compared with placebo in 40 patients with atopic dermatitis.⁹³ A reduction in peripheral blood eosinophil counts was accompanied by modest improvement in physician-assessed clinical parameters but no differences for validated dermatitis scoring measures.⁹³ Thus unlike IL-4/IL-13 approaches, the utility of approaches blocking IL-5/IL-5R α in patients with “allergic” type 2 disorders remains unclear.

Treatment in patients with other eosinophilic disorders. Hypereosinophilic syndromes are rare diseases classified by increased peripheral blood eosinophil counts of greater than 1500/mL for more than 6 months and end-organ involvement. Intravenous mepolizumab for 36 weeks in 85 corticosteroid-dependent hypereosinophilic patients allowed tapering of systemic corticosteroids (50% completely tapered off) and decreased peripheral blood eosinophil counts and serum eosinophil-derived neurotoxin levels compared with placebo.⁹⁴ Nasal polyposis is also frequently associated with eosinophilic inflammation, although less with allergy. In a small study of eosinophilic nasal polyps, reslizumab-treated responder patients (improved nasal polyp scores) had significantly higher baseline nasal secretion IL-5 levels compared with the nonresponders.⁵⁵ Similarly, in adults with severe nasal polyposis refractory to corticosteroid therapy, mepolizumab decreased nasal polyp size (on computed tomographic scans) in 12 of 20 patients treated with mepolizumab compared with 1 of 10 patients treated with placebo.⁹⁵ In contrast, in patients with eosinophilic esophagitis, mepolizumab reduced esophageal eosinophilia but did not lead to clinical improvements.⁵⁶ These mixed results with anti-IL-5 in patients with nonasthmatic eosinophilic disorders suggest differences in the contributions of eosinophils, type 2 inflammation, and even allergy to these disorders.

Targeting TSLP in asthmatic patients

TSLP is a recently described innate “alarmin” capable of regulating type 2 responses through suppression of dendritic cell–derived IL-12 production, thus skewing T_H0 cells toward T_H2. TSLP levels are reported to be increased in human asthmatic airways (mRNA and protein) compared with those of healthy control subjects, making it a potential type 2 target.⁹⁶ Using the T_H2-associated allergen challenge model, AMG 157 (human anti-TSLP monoclonal IgG_{2A}) significantly attenuated allergen-induced late asthmatic responses and tended to attenuate the early response in corticosteroid-naïve patients with mild asthma.⁹⁷ There were associated decreases in blood and sputum eosinophil counts before and after the allergen challenge, as well as decreased FENO levels.⁹⁷ Thus TSLP blockade in an allergen challenge model appeared to more broadly affect physiologic and inflammatory pathways than targeting either IL-4/IL-13 or IL-5. Whether similar effects will be observed in patients with more severe corticosteroid-treated asthma, likely with a type 2 (perhaps allergic) phenotype, awaits further study.

Targeting type 2 pathways in patients with increasingly severe (complex) asthma

Recent clustering, molecular studies, and treatment trials have begun to suggest that type 2 asthma itself represents several different molecular phenotypes. Data are emerging that late-onset, less allergic, but highly eosinophilic asthma might respond better to IL-5–targeted therapies than earlier-onset (allergic) asthma, whereas in allergic models of asthma, anti-IL-4/IL-13 approaches appear superior.^{20,27,28} However, nonresponders to these treatments also exist, with 40% to 60% of patients in the recent corticosteroid-sparing study of mepolizumab failing to respond better than placebo.⁸⁹ This heterogeneity of type 2 inflammation is supported by recent clustering studies, which suggest type 2 biomarkers (including eosinophil counts and FENO levels) are present across a range of asthma severities, clinical features, and even gene signatures.^{11,98} Furthermore, expression of type 2 biomarkers can be discrepant, with one biomarker present while another is not. An epithelial gene-profiling study of 155 asthmatic patients and healthy control subjects identified 588 genes highly correlated with the type 2 biomarker FENO.⁹⁸ By using these genes, 5 distinct subject clusters emerged, including 3 with high FENO levels, each with distinct gene expression profiles. Although all associated with expression of type 2 signature genes, the 3 FENO-high clusters differed substantially in relation to other genes.⁹⁸ Whether type 2–targeted therapies will work equally well in all 3 type 2–high clusters is not yet clear but might help explain why a humanized mAb to IL-13 (GSK679586) that inhibited IL-13 binding to IL-13 receptor α 1 and α 2 was not efficacious in patients with very severe asthma (16% receiving OCSs), even when evaluated in those with high blood eosinophil counts.⁹⁹ Although lack of efficacy could be related to the molecule itself, it is also conceivable that blocking type 2 immunity in these very refractory patients might only be inhibiting a small portion of the complex immunoinflammatory process.

In summary, the successful evolution of type 2–targeted therapies has been strongly influenced by the recognition that only a portion of asthmatic patients manifest type 2 inflammatory

processes. To date, these biologic approaches have been reported to be safe and well tolerated, although with greater numbers of patients exposed for more prolonged periods of time, adverse aspects to their use are likely to emerge. Remaining questions include those related to long-term safety, whether one of these approaches will be more successful than others or will be selectively more effective in some type 2 phenotypes than others, whether certain biomarkers will better identify treatment-responsive patients (or those less likely to have adverse events), and, importantly, whether any of these molecules will produce a disease-modifying effect.

NON-TYPE 2 BIOLOGIC APPROACHES

Approximately half of all asthmatic patients do not have evidence of type 2 inflammation.^{23,65,100} “Type 2–low asthma” is currently defined as the “apparent” absence of type 2 cytokines and their downstream signatures. This non–type 2 group is poorly defined, clinically heterogeneous, and without specific biomarkers, making molecular phenotyping and targeted therapy approaches difficult. Some might lack type 2 inflammation simply because corticosteroids have substantially reduced that pathway. Non–type 2 patients generally have adult-onset disease, often in association with obesity, postinfectious, neutrophilic, and smoking-related factors, and are less likely to be atopic/allergic.^{4,11,12,101}

Targeting TNF- α

TNF- α is a pluripotent cytokine identified in innate, type 1, and type 2 immunity.¹⁰² Blockade of TNF- α is highly effective in patients with the type 1–associated chronic inflammatory disease rheumatoid arthritis. In murine models inhalation of TNF- α contributes to neutrophilic inflammation and bronchial hyperresponsiveness.¹⁰³ A small study in patients with corticosteroid-refractory severe asthma reported that etanercept, a soluble TNF- α receptor inhibitor, increased postbronchodilator FEV₁ and decreased bronchial hyperresponsiveness compared with placebo.¹⁰⁴ However, in a larger follow-up study of patients with severe corticosteroid-refractory asthma, etanercept only marginally reduced ACQ scores and C-reactive protein levels compared with placebo, without an effect on any other outcomes.¹⁰⁵

Infliximab, an mAb against TNF- α , was also studied in patients with nonphenotyped asthma. In a small study of patients with moderate symptomatic asthma, infliximab treatment was associated with a decrease in diurnal peak expiratory flow variation at week 8 and decreased asthma exacerbations.¹⁰⁶ However, like etanercept, a larger follow-up DBPC trial of golimumab (a fully humanized mAb against TNF- α) in patients with severe uncontrolled asthma, despite high-dose ICS/LABA treatment (32% receiving OCSs), did not improve lung function or decrease exacerbations.¹⁰⁷ Patients with adult-onset disease, FEV₁ reversibility of 12% or greater, and/or chronic sinusitis had a modestly lower risk of asthma exacerbations with golimumab compared with placebo. However, golimumab was associated with increases in systemic infections and cancer, such that the trial was stopped prematurely.¹⁰⁷ This modest efficacy of anti-TNF- α approaches combined with an unacceptable safety profile stopped further development of these drugs in asthmatic patients.

Neutrophil-targeted approaches

Neutrophil predominance in asthmatic patients has been associated with lower FEV₁ and higher ICS use.^{108,109} Sputum-based studies have identified 2 neutrophilic groups, one in which there is accompanying eosinophilia and one without, with different clinical characteristics. It is unclear whether the molecular mechanism behind the neutrophilia differs between the 2 groups.^{110,111} Only one published study has specifically targeted lung neutrophilia. Because CXCR2 is the receptor for IL-8, which mediates neutrophil migration to sites of inflammation, a CXCR2 receptor antagonist was targeted to neutrophilic asthma. Although sputum and blood neutrophil counts decreased, there was no clinical benefit.¹¹²

Similarly, IL-17 cytokine subtypes (IL-17A-D and IL-17F) associate with the third adaptive immune pathway (T_H17) and are related to bacterial host defense and subsequent neutrophilia.^{113,114} Brodalumab is an anti-IL-17 receptor antibody, which blocks receptor binding of IL-17A and IL-17F but also blocks binding of the type 2-associated cytokine IL-17E/IL-25.¹¹⁵ In patients with nonphenotyped moderately severe asthma, brodalumab was not effective compared with placebo.¹¹⁶ Subphenotyping by the presence of blood neutrophils or eosinophils did not better identify a responder group. However, a subgroup with 20% or greater postbronchodilator FEV₁ improvement had marginal improvement in ACQ scores and symptoms.¹¹⁶ Whether this large bronchodilator responsiveness (in the absence of inflammatory signals) will identify a T_H17-associated asthma phenotype responsive to targeted therapy awaits further study.

CONCLUSION

It is increasingly recognized that human asthma is a heterogeneous disease. Although the testing of biologic medications might begin in animal models, the immunologic response in human asthma is more complex. Because the results of the studies summarized in this article are varied even for antibodies directed toward the same biologic pathway, it is appreciated that the response to a biologic medication can be confounded by multiple factors, including treatment duration, dose, phenotype, and differing outcome measures assessed. Furthermore, the effects of smoking and interactions with biologic medications are unknown because current smokers have not been included in these DBPC trials. Moving forward, it will be critical to determine the optimal biomarkers necessary to determine which patients will derive the best therapeutic benefit from each specific targeted medication. Identification of proteomic and/or genomic biomarkers might enhance our ability to identify a subgroup of asthmatic patients who are responsive to biologic medications.

Biologic approaches targeted to type 2 inflammation are emerging as promising new therapies for a large portion of the asthmatic population. However, much remains to be understood regarding their long-term efficacy and safety, their comparative efficacy, and, finally, their cost-effectiveness. Further integration of treatment responses as related to current and potential future type 2 biomarkers has the potential to make these therapies among the first successful “precision” medicines. However, development of biologically targeted therapies in patients lacking type 2 biomarkers

remains in its infancy and will require greatly improved molecular understanding of their underlying pathology or pathologies.

What do we know?

- Asthma has been defined traditionally by using nonspecific clinical and physiologic variables that encompass multiple different phenotypes and treated with nonspecific anti-inflammatory therapies.
- Recent molecular and genetic studies have identified clinical and inflammatory phenotypes that associate with specific biomarkers.
- Biomarkers for type 2 (T_H2) inflammation, including FENO levels and blood/sputum eosinophilia and serum periostin levels, have helped identify a type 2 molecular phenotype of asthma.
- Treatment of type 2–high patients with biologic agents targeting IgE and the canonical type 2 cytokines IL-4, IL-5, and IL-13 are emerging as efficacious asthma therapies.

What is still unknown?

- Biomarkers to identify a type 2–low asthma phenotype and potentially to guide therapy are unknown.
- Although these targeted biologic agents are efficacious in treating some phenotypes of asthma and allergic disease, some patients might respond better to one biologic agent than another or not at all.
- The reasons for these differential responses are unknown.

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Table EI. Detailed Summary of Double Blind Placebo Controlled Trials using Biologic Medications in Asthma (A) and Allergic Disease (B) Treatment

A) Asthma studies

Mediator Targeted	Biologic Therapy Used	Type of Asthma Targeted	1° endpoint	Met 1° (Y/N)	Other Clinical Results	Investigators
IgE						
	Monoclonal anti-IgE antibody (rhuMAB-E25)	Allergic, moderate-severe, on ICS or OCS, 11-50 yrs of age	improvement in asthma symptom score at 12 weeks	Y	↓ oral CS use, ↓ free IgE in serum	[43]
	Monoclonal anti-IgE antibody (omalizumab)	Allergic, moderate-severe, on ICS, 6-12 yrs of age	evaluate safety and steroid-sparing effect	Y	↓ rescue medication use, ↓ asthma exacerbations	[44]
		Perennial allergy, total serum IgE ≥30 to ≤ 700IU/mL, moderate-severe	# of exacerbations during steroid reduction period and during stable steroid phase	Y	↓ ICS and rescue β agonist use, improved symptoms and lung function	[45]
		Perennial allergy, total serum IgE ≥30 to ≤ 700IU/mL, moderate-severe	# of exacerbations during steroid- stable phase and steroid- reduction phase	Y	↓ ICS use	[46]
		Perennial allergy, total serum IgE ≥30 to ≤ 1,300IU/mL, moderate-severe asthma with persistent allergic rhinitis	co-primary efficacy variables: incidence of asthma exacerbations & proportion of patients showing a response in both asthma and rhinitis QoL assessments	Y/Y		[56]
		Severe, allergic asthma on ICS/LABA, ICS reduction phase	ICS reduction	Y	↓ rescue medication use, improved asthma symptoms and quality of life	[47]
		Severe persistent asthma inadequately controlled on ICS/LABA	clinically significant asthma exacerbation rate	N (*rate ↓ but not significant)	significantly ↓ severe asthma exacerbation and emergency visit rate, improved AQLQ , morning peak expiratory flow and asthma symptom scores	[48]
		Perennial allergy, total serum IgE ≥30 to ≤ 1300 IU/mL, 6- <12 years of age , history of exacerbations and asthma symptoms despite mid-high dose ICS	rate of clinically significant asthma exacerbations over a period of 24 weeks	Y	↓ asthma exacerbations over 52 weeks, ↓ severe asthma exacerbations	[51]
		Perennial allergy, total serum IgE ≥30 to ≤ 1,300IU/mL, persistent, inner-city asthma, ages 6-20 years	# of days with symptoms during past 2 weeks	Y	↓ asthma exacerbations (esp., school related viral exacerbations), ↓ ICS & LABA use	[50]
		Perennial allergy, total serum IgE ≥30 to ≤ 700IU/mL, Severe persistent on ICS/LABA	rate of exacerbations during 48-weeks of treatment	Y	↓ rescue inhaler use, improved symptom and AQLQ scores	[49]
		*Subanalysis of above by Type-2 High Phenotype groups: ↑ FeNO , ↑ blood eosinophils or ↑ serum periostin	rate of exacerbations during 48-weeks of treatment	Y	↓ in exacerbations greater in Type-2High vs. low subgroups for all three biomarkers	[52]
IL4R (IL-4 & IL-13)						
	Recombinant human soluble IL-4R	Poorly controlled asthma, on ICS	FEV1 change from baseline	Y	Tendency to improve asthma symptom scores	[71]

Mutant IL-4 molecule (pitracinra)	Allergen challenge: Mild, stable, atopic asthma	average % ↓ in FEV1 over 4–10 h after allergen challenge	Y	↓ β agonist use after challenge, ↓ FeNO; Certain +SNPs of IL-4R α gene subgroups associated greater response	[69,77]
Humanized monoclonal antibody to IL4R α (AMG 317)	Moderate, ICS treated asthma	Change from baseline in ACQ at week 12	N	↓ serum IgE, <i>Subgroup effect</i> : worst asthma control modest benefit	[74]
Mutant IL-4 molecule (pitracinra)	Moderate-severe	↓ Asthma exacerbations	N	<i>Subgroup effect</i> : Certain +SNPs of IL-4R α gene associated with clinical response: ↓ asthma exacerbations, nocturnal awakenings, activity limitation	[73]
Humanized monoclonal antibody to IL4R α (dupilumab)	Moderate-severe asthma, mid-high dose ICS/LABA with Type-2 High Phenotype : Blood eosinophils ≥ 300cells/ul or sputum eosinophils ≥ 3%	↓ occurrence of asthma exacerbation	Y	↓ β agonist use, ↓ upper airway symptoms, ↓ FeNO (@ week 12), ↑ FEV1, ↑ asthma control	[34]

IL-13

Humanized IL13 antibody (IMA-638)	Allergen challenge: Mild, stable atopic asthma, no ICS	late-phase area under the curve (AUC)	Y (@ Day 14); N (@ Day 35)	↓ early asthma response	[70]
Monoclonal IL13 antibody (lebrikizumab)	Moderate-severe, not controlled by mid-high dose ICS/LABA; Subgrouped by Th2/Type-2 “High” Phenotype (Total serum IgE > 100IU/mL and blood eosinophils > 140cells/ul or high serum periostin or high FeNO)	↑ prebronchodilator FEV1 from baseline to Week 12	Y	<i>Subgroup effect</i> : high-periostin group had greatest ↑ FEV1, ↓ FeNO, trend to ↓ exacerbation	[35]
Monoclonal IL13 antibody (tralokinumab)	Moderate-severe uncontrolled asthma, Subgrouped with Th2/Type-2 “High” Phenotype (measurable IL-13 in sputum)	change from baseline in mean ACQ-6 at week 13	N	<i>Subgroup effect</i> : sputum IL-13+ group had ↓ β-agonist use, trend to ↑ FEV1, greater improvement in ACQ-6	[72]
Monoclonal IL13 antibody (GSK679586)	very severe asthma symptomatic despite high dose ICS, 16% on OCS	Change from baseline in ACQ-7 scores over 12 weeks	N	<i>No Subgroup effect</i> : Analysis by ↑ serum IgE, blood eosinophils, or both also negative	[95]

IL-5

Anti-IL5 (SB-240563)	Allergen challenge: Mild, allergic asthmatic males, no ICS use	Effect on late asthmatic response (max % change in FEV1 from the dose at 4 to 10 h after allergen challenge)	N	↓ eosinophils in blood and sputum, no effect on airway hyperreactivity	[18]
Anti-IL5 (mepolizumab)	Mild, stable, atopic asthma, no ICS	Depletion of airway tissue eosinophils and their products	N	↓ eosinophils in airway, bone marrow, and blood, no effect on FEV1 or other clinical measures of asthma	[19]
	Moderate, persistent asthma on ICS	change from baseline in morning peak expiratory flow (PEF) at Weeks 12 and 20	N	↓ blood/sputum eosinophils, trend to ↓ exacerbations, no other effect on clinical measures of asthma	[31]

		Severe refractory asthma, hx. of exacerbations and Th2/Type-2 “High” Phenotype (sputum eosinophilia >3%)	↓ # of severe exacerbations per subject during the 50-week treatment phase	Y	↓ blood and sputum eosinophils, improved quality of life, ↓ airway wall thickening	[20]
		Severe, prednisone dependent asthma, Th2/Type-2 “High” Phenotype (sputum eosinophilia >3%)	↓ Proportion of patients with exacerbations, and mean ↓ in prednisone dose	Y/Y	↓ sputum and blood eosinophils, ↑ asthma control and FEV1 maintained for 8 weeks s/p last infusion	[21]
		Severe, refractory asthma on high dose ICS/LABA with Th2/Type-2 “High” Phenotype (≥1 in the prior year: eosinophils ≥3% sputum or ≥300/mL peripheral blood, or FeNO ≥50ppb)	↓ rate of clinically significant asthma exacerbations	Y	↓ sputum and blood eosinophils	[32]
		Severe, eosinophilic persistent asthma with recurrent exacerbations despite high dose ICS +/- OCS, Th2/Type-2 “High” Phenotype (peripheral blood eosinophils ≥300/mL during prior 1 yr or ≥150/mL at screening)	↓ rate of exacerbations	Y	↑ FEV1, modestly improved symptom scores and ACQ-5	[82]
		Severe, systemic CS dependent asthma, generally of late onset, Th2/Type-2 “High” Phenotype (peripheral blood eosinophils ≥300/mL during prior 1 yr or ≥150/mL at optimization phase)	↓ daily systemic CS	Y	↓ exacerbation, ↑ quality of life and marginal ↑ in FEV1 and symptoms despite ↓ OCS use	[83]
	Anti-IL5 (reslizumab)	Poorly controlled asthma on high dose ICS + additional controller and Th2/Type-2 “High” Phenotype (sputum eosinophils ≥ 3%)	change in ACQ score from baseline to end of therapy (Week 15 or early withdrawal)	Y (trend)	↓ sputum eosinophils, trended to ↑ FEV1 and asthma control, results best in nasal polyp subgroup	[84]
	Anti-IL-5Rα (benralizumab)	Chronic eosinophilic asthma and Th2/Type-2 “High” Phenotype (sputum eosinophils ≥ 2.5%)	safety profile; and effect of benralizumab on eosinophil counts in airway mucosal/submucosal biopsy 28 days after dosing	Y	Also ↓ sputum, bone marrow and blood eosinophils; clinical measures not evaluated	[86]
TSLP	human anti-TSLP monoclonal antibody (AMG 157)	Allergen challenge: mild allergic asthma, no ICS	↓ late asthmatic response (measured 3-7 hours s/p allergen challenge)	Y	↓ in blood and sputum eosinophils pre/post allergen challenge, ↓ FeNO, tended to ↓ early response	[93]
TNF- α						
	soluble TNF- α inhibitor (etanercept)	Refractory asthma on ICS	Difference in change in PC20 from 0-10 weeks; and difference in change in asthma quality-of-life score from 0-10 weeks	Y/Y	↑ post-bronchodilator FEV1	[100]
		Severe, CS-refractory asthma	change from pretreatment baseline in AQLQ and ACQ scores @ 12 weeks	N (AQLQ) ; Y (ACQ)	↓ CRP	[101]
	Monoclonal antibody against TNF-α (infliximab)	Moderate symptomatic asthma, on ICS	change in morning PEF at Days 50–56 compared with the last 7 d of the run-in	N	↓ mean diurnal PEF variation @ Week 8; ↓ # exacerbations; ↓ sputum TNF- α, ↓ IL-1 α, IL-6 and IL-8, IP-10	[102]

	Monoclonal antibody against TNF- α (golimumab)	Severe, persistent, uncontrolled asthma despite high dose ICS/LABA	Co-primary: change from baseline- Week 24 in prebronchodilator FEV1%p; and # of severe asthma exacerbations through Week 24	N/N	<i>Subgroup effect:</i> Pts w/adult onset disease, FEV1 reversibility $\geq 12\%$, and/or chronic sinusitis had modest $\downarrow$ risk of exacerbations; early discontinuation of study due to $\uparrow$ systemic infections and cancer profile	[103]
Neutrophils	CXCR2 receptor antagonist (SCH527123)	Severe asthma, $\uparrow$ sputum neutrophilia ($> 40\%$)	Safety; $\downarrow$ sputum and blood neutrophil counts	Y,Y,N	$\downarrow$ mild exacerbations, trend to improvement in ACQ, no effect on FEV1	[108]
IL-17	Anti-IL17 receptor antibody (brodalumab)	Moderate-severe asthma on ICS	change in ACQ score from baseline to Week 12	N	<i>Subgroup effect:</i> Marginal ACQ change in high-reversibility group ($\geq 20\%$ bronchodilator response)	[112]

Abbreviations used: AUC (area under the curve), AQLQ (Asthma related Quality of Life Questionnaire; ACQ: Asthma Control Questionnaire; EAR (Early Asthmatic Response); FeNO (fraction of exhaled nitric oxide); ICS: inhaled corticosteroids, LAR (Late Asthmatic Response); SNPs (single nucleotide polymorphisms); TSLP (thymic stromal lymphopoietin); TNF- α (tumor necrosis factor- α)

B) Allergic Diseases treated

Mediator Targeted	Biologic Therapy Used	Type of Allergic Disease Targeted	1 ^o endpoint	Met 1 ^o (Y/N)	Other Clinical Results	Investigators
IgE	Monoclonal anti-IgE antibody (rhuMab-E25)	Allergen challenge in Asthma : Allergy to house dust mite, cat pelt, or Ryegrass; stable, mild, no ICS	Change in early- and late-phase asthmatic responses to inhaled allergen	Y	$\downarrow$ serum-free IgE	[40]
		Allergen challenge in Asthma : Allergic, stable, mild, no ICS	Difference between log allergen PC15 on Day 77 and Day -1	Y	$\downarrow$ serum-free IgE	[39]
	Monoclonal anti-IgE antibody (omalizumab)	Allergen challenge in Asthma : Allergic to dust mite, stable, mild, no ICS	Early and late asthmatic responses	Y	$\downarrow$ sputum/bronchial mucosa eosinophils, $\downarrow$ submucosal high affinity IgE receptor+ and CD4+ cells	[41]
		Allergen challenge in Asthma : Allergic to cat, moderate, +ICS	% $\downarrow$ in FEV1 from pre-challenge FEV1 over 1-hour cat chamber exposure at week 16 expressed as AUC	Y	Suppresses late asthmatic response, $\downarrow$ chest and naso-ocular symptoms induced by cat challenge	[42]
		Allergic rhinitis to ragweed or birch: ≥ 2 yr. hx of moderate-severe seasonal allergic rhinitis due to ragweed/birch	Average daily nasal symptom severity score	Y	$\downarrow$ IgE, $\downarrow$ rescue antihistamine use, $\uparrow$ rhinitis quality of life	[54,55]
		Persistent allergic rhinitis in moderate-severe allergic asthma	co-primary efficacy variables: $\downarrow$ incidence of asthma exacerbations & # patients showing a response in both asthma and rhinitis QoL assessments	Y/Y		[56]

		Add on with specific allergen Immunotherapy: ≥2 yr. hx of moderate-severe seasonal allergic rhinitis; immunotherapy to ragweed or birch and grass	Improved allergy severity scores	Y	↑ quality of life, ↓ rescue medication use compared to immunotherapy alone	[57,58]
		Add on with specific allergen Immunotherapy: pediatric with moderate-severe seasonal allergic rhinitis; immunotherapy to birch and grass	↓ Local and systemic side effects vs. immunotherapy alone	Y		[59]
IgE	Monoclonal anti-IgE antibody (TNX-901/ omalizumab)	Pts w/ hx of immediate hypersensitivity to peanut; undergoing oral food challenge to peanut	↑ from baseline in threshold dose that induced hypersensitivity to peanut	Y		[60,61]
		Use in Peanut-allergic children undergoing oral peanut desensitization	Reaching 500 mg peanut flour dose on 1st day	Y	able to continue at tolerance level despite stopping omalizumab	[62]
IgE	Monoclonal anti-IgE antibody (omalizumab)	Chronic urticaria: symptomatic despite H1-antihistamine treatment;	Change from baseline to week 4 in urticaria activity scores over 7 days (UAS7) after single dose	Y (for 300mg and 600mg doses)	improved weekly hive and itch scores; onset of effect by 1 to 2 weeks	[66]
		Chronic urticaria: symptomatic despite antihistamine treatment, leukotriene receptor antagonists or both	change from baseline in weekly itch-severity score	Y	significantly ↓ itch severity score at week 12, benefits sustained to week 24	[67,68]
IL-4 & IL-13						
	Humanized monoclonal antibody to IL4R α (dupilumab)	Adults with moderate-to-severe atopic dermatitis despite treatment with topical CSs and calcineurin inhibitors, evaluated as monotherapy for 4 and 12 weeks	clinical efficacy	Y	↓ pruritus score; 85% of dupilumab patients achieved EASI-50, 40% cleared or nearly cleared their skin lesions; ↓ keratin 16 mRNA on skin-biopsy (Type 2 marker)	[78]
		Adults with moderate-to-severe atopic dermatitis despite treatment with topical CSs and calcineurin inhibitors, evaluated as add-on in combination with topical CSs for 4-weeks	Incidence and severity of adverse events	Y	100% of the dupilumab group met EASI-50 criteria, Dupilumab patients also able to ↓ topical glucocorticoid use	[78]
IL-5						
	Anti-IL5 (mepolizumab)	Moderate-severe atopic dermatitis	% of patients with at least “marked improvement” after 2 weeks as assessed by the Physicians Global Assessment of Improvement	N	↓ peripheral blood eosinophils; modest improvement in physician assessed clinical parameters in treatment group	[87]
		Clinically stable Hypereosinophilic syndrome patients, on prednisone monotherapy to blood eosinophil count <1000/uL	↓ of prednisone dose to ≤10 mg per day for 8 or more consecutive weeks	Y	50% completely tapered off CS; ↓ peripheral blood eosinophils, ↓ serum eosinophil-derived neurotoxin levels	[88]
		Adults with active eosinophilic esophagitis (>20 peak eosinophil number/high power field) and dysphagia	Proportion of responders (patients with <5 eosinophils per high power field) following treatment	N	↓ peripheral blood eosinophils, no clinical improvement	[91]
	Anti-IL5 (reslizumab)	Bilateral nasal polyps	safety and pharmacokinetics of reslizumab	Y	↓ blood eosinophils, ↓ eosinophil cationic protein up to 8 weeks after treatment in serum and nasal secretions; <i>Subgroup effect:</i> ↑baseline IL-5 in nasal secretions in reslizumab “responders” (improved nasal polyp scores)	[89]

Severe nasal polyposis (grade 3 or 4 or recurrent after surgery), refractory to CS therapy	↓ in nasal polyp scores at 8 weeks after 1 st dosing	Y	↓ nasal polyp size on CT scan	[90]
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Abbreviations used: AUC (area under the curve), CS (corticosteroids), (EASI-50) $\geq$ 50% improvement in Eczema Area and

Severity score