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Immune modulation for treatment of allergic disease

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Summary: Immune modulation for treatment of allergic diseases aims to decrease the pathologic immune response rather than to cause a return to an immunologically naive or unresponsive state. Our expanding knowledge of innate and adaptive immune responses at the molecular level has led to development of immunomodulators for several allergic diseases, particularly asthma, allergic rhinitis, and eosinophilic esophagitis. Although successful immune modulation in mouse models of allergic disease have often failed to translate into significant results in human clinical trials, much has been learned about the pleotropic nature of cytokines and their effector mechanisms and of the varied phenotypes of allergic disease. We examine strategies of immune modulation in allergic diseases that have undergone human clinical trials, all with the common goal of decreasing the T-helper 2 (Th2) response, but through different mechanisms: blocking critical Th2 cytokines, inhibiting Th2 cytokine synthesis, blocking critical Th2 effector molecules, inhibiting important cells in the Th2 response, and stimulating Th1 responses. Therapies directed against specific effector molecules, such as immunoglobulin E and prostaglandin D2, hold promise in immune modulation of allergic disease, as do targeting the IL-4/IL-13 receptor and augmenting Th1/Th2 balance with Toll-like receptor agonists.

Keywords: immune modulator, cytokine, asthma, allergic rhinitis, immunotherapy, Toll-like receptor

Introduction

Immune modulation for treatment of allergic diseases holds promise to provide newer avenues for controlling and optimally reversing key pathologic processes important in allergic diseases. Our expanding knowledge of the biology of immune responses has led to development of new therapeutic targets for allergic diseases, particularly asthma. Although successful immune modulation has been demonstrated in mouse models of allergic disease, these same strategies have often failed to translate into positive results in human clinical trials.

In this review, we examine strategies of immune modulation in allergic diseases that have undergone human clinical trials, all with the common goal of decreasing the T-helper 2 (Th2) response, but through different mechanisms: blocking critical Th2 cytokines, inhibiting Th2 cytokine synthesis, blocking critical Th2 effector molecules, inhibiting important

cells in the Th2 response, and stimulating Th1 responses (Fig. 1, Table 1).

Blocking critical Th2 cytokines

Th2 cytokines, particularly interleukin-4 (IL-4), IL-5, IL-13, and IL-9, play critical roles in allergic diseases and have been targeted for immune modulation by monoclonal antibodies (mAbs) directed against the cytokines themselves or their receptors. Cytokine blocking therapies must be evaluated carefully, as redundancy of effector mechanisms may render single cytokine-directed therapies ineffective. However, inhibition of pleiotropic cytokines may lead to undesired and/or unexpected side effects and risks that outweigh potential benefits in patients, as has been previously demonstrated with agents directed against IL-2 and tumor necrosis factor- α (TNF- α). Anti-IL-5 therapies are discussed in a later section on antieosinophil therapies.

IL-4 antagonists

IL-4 induces immunoglobulin (Ig) isotype switching to IgE and promotes differentiation of naive T lymphocytes to the Th2 lineage, leading to subsequent production of IL-4, IL-5, and IL-13. Altrakincept is a nebulized, soluble recombinant human IL-4 receptor (Amgen, Thousand Oaks, CA, USA) that binds IL-4 without mediating cellular activation because it

consists of only the extracellular receptor component. Although an initial study showed some promise, a subsequent study was not as favorable, and this molecule is no longer under development for asthma (1).

Clinical trials of pascolizumab (SB 240683), a fully humanized anti-IL-4 mAb, and a similar mAb made by PDL BioPharma (Incline Village, NV, USA), had disappointing results in asthma clinical trials and are being developed further. Targeting IL-4 alone may be ineffective because of redundant mechanisms shared with other cytokines, particularly IL-13.

IL-13 antagonists

Blockade of IL-13 may be an important strategy in the treatment of allergic diseases, particularly asthma, due to its effects on IgE production, airway hyperresponsiveness, mucus production, and airway remodeling in animal and *in vitro* models. IL-13 blockade reduced lung inflammation in monkey and mouse studies (2, 3). There are several anti-IL-13 mAbs that are undergoing or have completed Phase II human clinical trials, although results of many of these studies are not published at this time.

QAX576 (Novartis, Basel, Switzerland) is an intravenously administered anti-IL-13 mAb being developed for several allergic diseases. QAX576 failed to attenuate nasal symptoms and eosinophilia in subjects undergoing nasal antigen challenge with Timothy grass pollen, although nasal IL-13 was inhibited (4). A Phase II study of QAX576 in moderate-to-severe atopic asthmatics was withdrawn due to complexity of design and lack of enrollment (5). However, this molecule is still under development for asthma. Finally, there is an ongoing Phase II clinical trial evaluating its effectiveness in eosinophilic esophagitis (6).

Anrukinzumab (IMA-638, Pfizer, New York, NY, USA) is a humanized anti-IL-13 mAb administered subcutaneously. Although an initial Phase II clinical trial in mild atopic asthmatics demonstrated attenuation of postallergen early and late asthmatic responses, a subsequent clinical trial in persistent asthmatics did not meet its clinical efficacy endpoint and its development was halted in May 2009; however, Pfizer is developing another anti-IL-13 mAb, IMA-026, which completed a Phase II asthma clinical trial in 2009 (7–9).

Talokinumab (CAT-354, MedImmune, Gaithersburg, MD, USA) is a humanized IgG4 anti-IL-13 mAb in development for asthma. Intraperitoneal injection of talokinumab attenuated intratracheal human IL-13-induced airway hyperresponsiveness as well as airway and esophageal eosinophilia in a murine model of asthma (3). Tolerability and safety have been

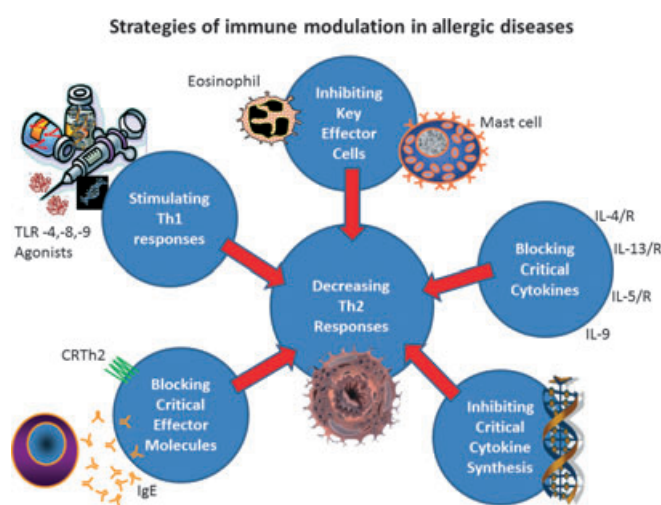


Fig. 1. Strategies of immune modulation in allergic diseases. Immune modulation in allergic diseases involves several strategies, but all with one common goal: to decrease Th2 responses that lead to allergic disease. In this review, we discuss the examples of immune modulation strategies in all five of these categories that have undergone human clinical trials. CRTh2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes; IgE, immunoglobulin E; IL, interleukin; Th1, T-helper 1; Th2, T-helper 2; TLR, Toll-like receptor.

Table 1. Overview of immunomodulators

Name	Target/mechanism	Route	Development
Pascolizumab (SB 240683)	Anti-IL-4 mAb	IV	Phase II
Altrakinecept	IL-4 decoy receptor	Nebulized	Phase II
QAX576	Anti-IL-13 mAb	IV	Phase II
Anrukinzumab (IMA-638)	Anti-IL-13 mAb	SC	Phase II
IMA-026	Anti-IL-13 mAb	SC	Phase I
Tralokinumab (CAT-354)	Anti-IL-13 mAb	SC	Phase I/II
Lebrikizumab	Anti-IL-13 mAb	SC	Phase II
AMG 317	Anti-IL-4R α mAb	SC	Phase II
AIR 645	Anti-IL-4R α antisense OGN	Nebulized	Phase I/II
Pitrakinra	IL-4 mutein	SC, Nebulized	Phase II
MEDI-528	Anti-IL-9 mAb	SC	Phase II
AVP-13358	Th2 synthesis inhibitor	Oral	Phase I
Suplatast	Th2 synthesis inhibitor	Oral	Approved only in Japan
Rosiglitazone, Pioglitazone	PPAR γ agonists	Oral	Phase II
R0498991	Anti-OX40L mAb	SC, IV	Phase I/II
Omalizumab	Anti-IgE mAb	SC	FDA-approved
Lumiliximab (IDEC-I52)	Anti-CD23 mAb	SC	Phase I
AMG 853	CRTh2/DPI antagonist	Oral	Phase II
ACT-129968	CRTh2 antagonist	Oral	Phase II
ARRY-005/006	CRTh2 antagonist	Oral	Murine studies
OC000459	CRTh2 antagonist	Oral	Phase II
TPI-ASM8	CCR3 antisense OGN common β chain OGN	Nebulized	Phase II
RI12/R343	Syk inhibitor	Oral, Nebulized	Phase I/II
Mepolizumab	Anti-IL-5 mAb	IV	Phase II
Reslizumab	Anti-IL-5 mAb	IV	Phase II
Benralizumab (MEDI-563)	Anti-IL-5R α mAb	IV	Phase I/II
Tolamba	TLR9 agonist	SC	Phase II
CYT003-QbG10	TLR9 agonist	SC	Phase II
Pollinex Quattro	TLR4 agonist	SC	Phase II
VTX-1463	TLR8 agonist	Intranasal	Proof of concept

IV, intravenous; SC, subcutaneous; OGN, oligonucleotide; TLR, Toll-like receptor.

Includes target or mechanism of action, method of administration, and current status of development.

demonstrated in Phase I clinical trials (10). A Phase II clinical trial has been completed, although the results have not yet been published (11).

Lebrikizumab (Genentech, South San Francisco, CA, USA) is a humanized anti-IL-13 mAb currently undergoing Phase II clinical trials in asthmatics inadequately controlled on only inhaled corticosteroids (ICS) (12).

IL-4 receptor antagonists

IL-4 has two cognate receptors that are heterodimers consisting of one IL-4R α subunit and either the common γ chain (Type I) or the IL-13R α 1 subunit (Type II). Binding of the type II IL-4 receptor by either IL-4 or IL-13 results in common pleotropic effects, mediated via the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, to promote Th2 cytokine production, eosinophil transmigration into tissue, and immunoglobulin class switching to IgE. Several therapies targeting IL-4R α in allergic diseases have been evaluated in human clinical trials.

AMG 317 (Amgen, Thousand Oaks, CA, USA) is a fully human IgG2 anti-IL-4R α mAb being developed for allergic asthma. A Phase II clinical trial of weekly subcutaneous AMG 317 in asthmatics did not significantly improve Asthma Control Questionnaire Scores, its primary endpoint, but did decrease frequency of exacerbations and increase time to exacerbations in subjects in the higher dose groups. Subjects with more asthma symptoms at baseline were more likely to respond to AMG 317, which was also well tolerated (13).

AIR 645 (Altair Therapeutics, San Diego, CA, USA) is a 2'-O-methoxyethyl '2-MOE Gapmer' antisense oligonucleotide that targets the mRNA encoding IL-4R α and prevents its translation into a functional protein. A Phase I clinical trial of nebulized AIR 645 in healthy volunteers demonstrated low systemic absorption and no severe adverse events (14). A Phase IIa clinical trial in mild allergic asthmatics was recently completed, although no results have been reported yet (15).

Pitrakinra (Aerovant or AER 001, Aerovance, Berkeley, CA, USA) is a recombinant IL-4 mutein that interferes with IL-4R α binding and is being developed for allergic asthma. Two Phase

Ila clinical trials were conducted in atopic asthmatics receiving pitrakinra, either as daily subcutaneous injection or twice daily nebulization versus placebo (16). Late phase asthmatic response after allergen challenge, measured by maximum percentage decrease in FEV₁, was decreased in both treatment groups, although statistically significant only in the nebulized pitrakinra group. There were significantly fewer asthma-related adverse events only in the subcutaneous pitrakinra group compared with placebo. A Phase IIb clinical trial of an inhaled dry powder formulation is currently underway. Aerovance is also developing Aeroderm (AER 003), a PEGylated pitrakinra mutein as a weekly or every 2-week subcutaneous injection for severe atopic dermatitis. A Phase IIa study of subjects with moderate-to-severe atopic dermatitis receiving subcutaneous Aeroderm twice daily demonstrated significant reductions in symptom score at 28 days and also fewer exacerbations compared with placebo (17).

IL-9 antagonist

IL-9 is implicated in asthma because it increases the number of mast cells and Th2 lymphocytes in the lung and leads to increased airway inflammation, airway hyperresponsiveness, and mucus production. Expression of IL-9 and its receptor are also higher in asthmatic airways, and the chromosomal region 5q31-33, where the IL-9 gene is located, has been linked to asthma in genome-wide association studies. Murine models of asthma also show that IL-9 induces airway eosinophilia and IgE production (18, 19). MEDI-528 (Medimmune LLC, Gaithersburg, MD, USA) is a humanized IgG1 anti-IL-9 mAb currently undergoing a Phase IIb clinical trial in adults with uncontrolled asthma receiving either biweekly subcutaneous MEDI-528 or placebo for 24 weeks (20). Recently, a pilot study reported that MEDI-528 may improve exercise-induced bronchospasm by reducing the maximum mean decrease in FEV₁ after exercise (21).

Inhibiting Th2 cytokine synthesis

In addition to blocking critical Th2 cytokines from binding to their cognate receptors, efforts at immune modulation of allergic diseases have also focused on inhibiting synthesis of these cytokines by oral agents, such as suplatast and peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists.

Suplatast

Suplatast tosilate (IPD-1151T, Taiho Pharmaceutical, Tokyo, Japan) is an oral dimethylsulphonium compound that selec-

tively inhibits Th2 type cytokine production and is taken three times daily, but is currently available only in Japan. In murine models of asthma, suplatast reduced levels of IL-4, IL-5, IL-13, eotaxin-1, and transforming growth factor- β (TGF- β) in bronchoalveolar lavage fluid and inhibited airway remodeling (22). Pretreatment with suplatast in rats and mice significantly suppressed allergen-induced nasal symptoms, mucus secretion, and nasal eosinophilia, whereas blunting the increase in mRNA expression of histamine H1 receptors and of IL-4 (23, 24).

In human asthmatics, suplatast inhibited monocyte-derived dendritic cells *in vitro* from stimulating CD4⁺ T cells to produce IL-5 and interferon- γ (IFN- γ) (25). Suplatast administered during the first 6 months of immunotherapy (IT) in adults with perennial allergic rhinitis to house dust mites (HDM) significantly decreased serum IL-4 and specific IgE compared with placebo and perhaps may improve efficacy and/or tolerability to IT (26). Moderate-to-severe asthmatics who received suplatast demonstrated significantly better FEV₁, peak expiratory flow and asthma symptom scores than placebo when their stable dose of ICS was reduced by half. Suplatast may be effective in mild asthmatics who do not respond to leukotriene receptor antagonist monotherapy (27, 28). Suplatast has also been shown to improve lung eosinophilia and airway hyperresponsiveness (29–31). Topical suplatast formulations inhibited expression of dermal IL-4 and IL-5 in a mouse model of atopic dermatitis and may be effective as an adjunctive, sparing agent to topical calcineurin inhibitors in humans (32, 33).

A Phase I clinical trial with AVP-13358, a novel, orally available 2-(substituted phenyl) benzimidazole derivative (Avanir Pharmaceuticals, Aliso Viejo, CA, USA) demonstrated safety and tolerability at all tested doses in 54 healthy volunteers and was able to suppress antigen-stimulated IL-4 and IL-5 responses, but is not currently in further development.

PPAR- γ agonists

GATA-3 is a key transcription factor of the Th2 cytokines IL-4, IL-5, and IL-13. Downregulation of GATA-3 using a dominant negative mutation in a murine model of asthma attenuated Th2-driven inflammatory responses, including mucus production, airway eosinophilia, and cytokine and IgE synthesis (34). PPARs, particularly the α and γ subtypes, can also downregulate GATA-3 with similar effects, but also inhibit *in vitro* eosinophil chemotaxis and antibody-dependent cellular cytotoxicity (35). PPAR- γ agonists include the thiazolidinediones, rosiglitazone and pioglitazone, which are oral hypoglycemic

agents used in non-insulin-dependent diabetes mellitus. PPAR- γ agonists can bind to and inhibit other transcription factors such as activator protein-1 (AP-1), STAT-1, nuclear factor κ B (NF κ B), and nuclear factor of activated T cells (NFAT), whereas attenuating production of reactive oxygen and nitrogen metabolites and cyclooxygenase-2 expression (36, 37).

Bronchodilatory effects of rosiglitazone were studied in 46 mild-to-moderate asthmatics that were current smokers, and participants were randomized to either rosiglitazone 8 mg daily or inhaled beclomethasone dipropionate 200 μ g daily for 4 weeks. At 28 days, subjects on rosiglitazone had significantly improved FEV₁, FEV₂₅₋₇₅, and forced vital capacity than those on beclomethasone, but did not have significant improvement in asthma symptoms (38). Results of an open-label pilot study were recently reported in 14 steroid-naïve asthmatics given rosiglitazone 4 mg daily for 8 weeks then 8 mg daily for four additional weeks. Rosiglitazone treatment significantly improved airway hyperresponsiveness, but no significant effects were demonstrated on FEV₁ (39).

Further studies of rosiglitazone in asthma are unlikely to take place, because the modest potential for benefit is largely outweighed by association of increased adverse cardiovascular events in patients already taking this drug, which has prompted its withdrawal in Europe and severe restrictions in the United States (US) for use only in diabetes not controlled on other medications. Pioglitazone, however, does not have such known associations and is currently being investigated in two Phase II clinical trials for asthma (40, 41).

OX40L antagonists

OX40L is expressed on antigen-presenting cells, such as macrophages, dendritic cells, endothelial cells, but also B and T lymphocytes. In the absence of IL-12, dendritic cells activated by thymic stromal lymphopoietin (TSLP) upregulate OX40L, which binds to OX40, and leads to Th2 cell differentiation and expansion of Th2 memory cells (42). OX40L-deficient transgenic mice had attenuated asthmatic responses to allergen challenge compared with wildtype, and administration of an OX40L antibody to wildtype mice during sensitization also prevented asthmatic responses (43). R0498991 (Hoffman-Roche Pharmaceuticals, Basel, Switzerland) is a humanized anti-OX40L mAb and Phase II clinical trials are underway to assess safety of a subcutaneous formulation in subjects with allergic rhinitis. Efficacy of an intravenous formulation to attenuate allergen-induced responses in asthmatics is under study (44, 45).

Blocking critical Th2 effector molecules

Immune modulation targeting critical Th2 effector molecules has shown promise in allergic disease, particularly strategies directed against IgE, but also prostaglandin D2 (PGD2) and leukotriene D4 (LTD4). Targeting such molecules may provide better benefit-to-risk profiles over cytokine-directed therapies as they appear to be more specific to allergic driven processes.

Omalizumab

Omalizumab (Xolair, Genentech, South San Francisco, CA, USA) is a 95% humanized mAb that forms soluble immune complexes with free IgE at the same site (C ϵ 3) that normally binds the high-affinity IgE receptor, Fc ϵ RI, thus preventing Fc ϵ RI cross-linking and subsequent basophil and mast cell activation. Omalizumab can reduce serum-free IgE levels by 99% within 2 h of administration and, within 2 weeks of therapy initiation, can reduce nasal allergen challenge responses (46). Omalizumab also induces downregulation of Fc ϵ RI on basophils, dendritic cells, and monocytes within 7 days (46, 47). In subjects with allergic rhinitis, omalizumab reduced serum eosinophils. In asthmatic subjects, omalizumab reduced serum, tissue, and sputum eosinophilia, Fc ϵ RI⁺ cells and also CD4⁺ lymphocytes (48). Fig. 2 summarizes mechanisms of action of omalizumab.

Several studies have established the safety and efficacy of omalizumab, including three Phase III clinical trials conducted

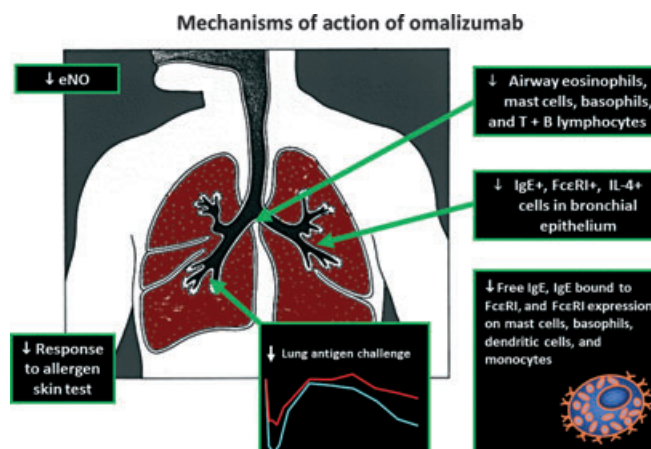


Fig. 2. Omalizumab mechanism of action. This figure illustrates the impact of adding omalizumab to the allergic inflammatory system. Omalizumab binds to free IgE, reducing cell-bound IgE. It reduces the number of high-affinity receptors on mast cells and basophils, which reduces mediator release. This leads to a reduction in asthma exacerbation and a reduction in symptoms. Adapted from Casale TB, Stokes J. Immunomodulation for allergic respiratory disorders. *J Allergy Clin Immunol* 2008;121:288–296.

on a total of 1405 subjects with moderate-to-severe persistent allergic asthma (49–51). All three studies demonstrated that omalizumab, compared with placebo, reduced asthma exacerbations and had a corticosteroid-sparing effect. A significantly greater number of omalizumab-treated subjects were able to reduce their ICS dose by 50% and/or 100%, compared with placebo (39.1% versus 19.1%, $P < 0.01$) (49). Omalizumab was also associated with decreased rescue medication use, fewer asthma symptoms, and improved quality of life (QOL) scores. Small but significant improvements in peak expiratory flow and FEV₁ were also observed (51). Omalizumab-treated subjects have also sought significantly fewer unscheduled outpatient and emergency department visits and required fewer hospitalizations (52). Fig. 3 highlights significant clinical effects of using omalizumab as an adjunctive treatment to ICS in asthma.

In light of the supporting evidence for omalizumab, the US Food and Drug Administration (FDA) approved it for the treatment of moderate-to-severe persistent allergic asthma in patients 12 years of age and older. Recent expert panel guidelines recommend considering omalizumab as adjunctive therapy in perennial allergic asthmatics who fail to achieve adequate control on medium-dose ICS in conjunction with a long-acting beta agonist. Subjects with greater disease severity appear to benefit more from therapeutic addition of omalizumab, although cost-benefit analysis is not favorable for all severe asthmatics (53). Omalizumab is administered subcuta-

neously every 2–4 weeks, with initial dosing determined by two factors: pretreatment serum total IgE, up to 700 IU/ml, and patient weight, up to 150 kg. Treatment effect is ascertained clinically by symptoms and objectively by lung function, but not by routine serum IgE measurements, as there is no commercially available assay to detect free IgE.

Subjects with seasonal allergic rhinitis also demonstrate improvement on omalizumab, including improved rhinitis QOL scores, reduced nasal symptom scores and antihistamine use, compared with placebo (54). Omalizumab was effective in attenuating responses to nasal allergen challenge in allergic rhinitis subjects and has been shown to be effective in treating perennial allergic rhinitis in adults and adolescents (55, 56). There are several Phase II clinical trials underway investigating the effects of omalizumab on chronic rhinosinusitis (57, 58).

Omalizumab may also be an effective adjunctive therapy to IT, improving its tolerability during build-up and effectiveness to reach maintenance dose. Omalizumab added as adjunctive therapy to subcutaneous IT in children allergic to birch tree and grass was shown to reduce seasonal allergic rhinitis symptoms, rescue medication use, and symptom load (59). Pretreatment of omalizumab in ragweed allergic subjects undergoing rush immunotherapy resulted in a fivefold decrease in risk of anaphylaxis due to IT and also improved severity scores during the ragweed season compared with those on IT alone (60). In a study of 248 allergic asthmatics undergoing IT to at least one of three perennial

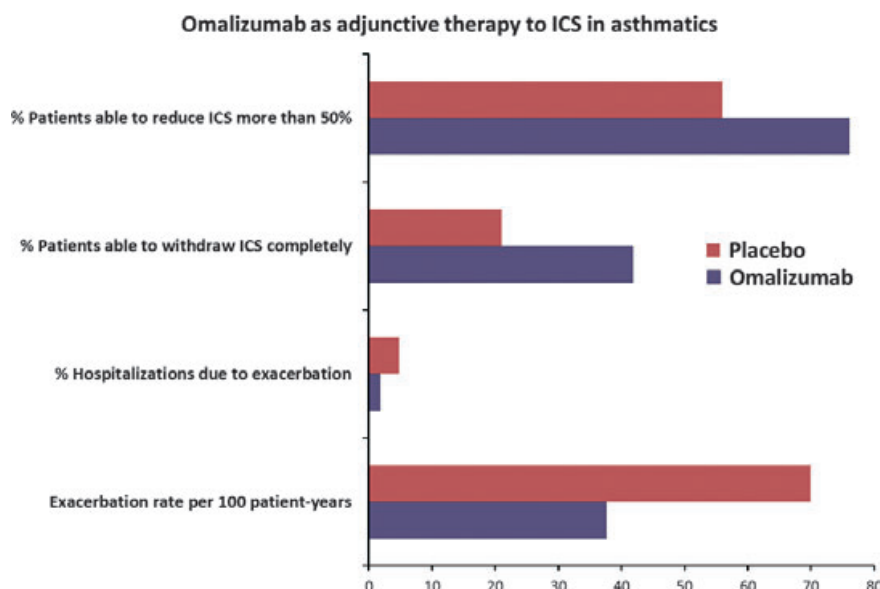


Fig. 3. Omalizumab as adjunctive therapy to inhaled corticosteroids in asthmatics. A recent meta-analysis demonstrated that omalizumab improved clinical parameters in asthma, particularly regarding asthma exacerbations and ability to reduce inhaled corticosteroid (ICS) dose. Adapted from Rodrigo GJ, Neffen H, Castro-Rodriguez JA. Efficacy and safety of subcutaneous omalizumab versus placebo as add-on therapy to corticosteroids for children and adults with asthma: a systematic review, *Chest* 2011;139:28–35.

aeroallergens, omalizumab added as pretreatment significantly reduced systemic allergic reactions, especially respiratory-related, and more subjects were able to achieve maintenance IT dose (61).

The use of omalizumab in several other allergic disorders is currently being investigated, including eosinophilic esophagitis, allergic bronchopulmonary aspergillosis, hyper-IgE syndrome, idiopathic anaphylaxis, and peanut allergy (62–67). There have been few case reports of omalizumab used with varying success in atopic dermatitis and chronic urticaria, for which there is now an upcoming Phase III clinical trial (68–70).

Omalizumab is generally well-tolerated with the most common adverse events noted as local reactions at the injection site. There was a higher rate of malignancy in omalizumab-treated subjects reported during initial clinical trials compared with controls, but causal association is unclear and is being closely monitored during postmarketing surveillance, including the Phase IV prospective observational cohort study, Xolair to Evaluate Effectiveness and Long-Term Safety in Patients With Moderate-to-Severe Asthma (EXCELS). The EXCELS study has also identified the potential association between omalizumab and cardiovascular events, but final conclusions await adjudication of events in relationship to patient characteristics. In 2007, the FDA ordered a black box warning to be placed on omalizumab due to possible association with increased risk of anaphylaxis. A review of data from approximately 57 000 patients treated with omalizumab have indicated that anaphylaxis can occur at any dose, and there can be delayed onset of symptoms beyond 2 h and also a protracted course, with signs and symptoms lasting many hours (71). Mechanisms of omalizumab-associated anaphylaxis and predisposing risk factors have yet to be elucidated. Current recommendations are to observe patients for 2 h after each of their first three injections, and then for 30 min following each subsequent injection, which would have captured 75% of anaphylactic reactions that were reviewed by the Joint Task Force of the American Academy of Allergy, Asthma and Immunology and American College of Allergy, Asthma and Immunology (72). There may also be an association between omalizumab and Churg–Strauss syndrome, although causation is questionable because this effect may result from better asthma control leading to reduction of oral steroids that unmasks the underlying disease (73).

Genentech, which manufactures and markets omalizumab under the trade name Xolair, also is conducting a Phase I clinical trial in asthma for anti-M1 prime (RG7449), a humanized mAb that binds to the M1 prime segment of membrane IgE

and targets B lymphocytes before they produce IgE, rather than neutralizing existing IgE.

CD23 antagonist

CD23 is the low affinity IgE receptor, FcεRII, and its expression on monocytes, alveolar macrophages, and B lymphocytes is increased in allergic patients. *In vitro* studies have demonstrated that cross-linking CD23 downregulates IgE synthesis. Lumiliximab (IDEC-152, Biogen Idec Pharmaceuticals, Westin, MA, USA) is a primatized, IgG1 anti-CD23 mAb that was studied in peripheral blood mononuclear cells (PBMCs) of atopic subjects and reduced allergen-induced PBMC proliferation and levels of IL-1β, IL-5, and TNF-α (74). Anti-CD23 mAb has not been shown to be effective for allergic rhinitis or asthma despite a significant effect on IgE levels, albeit much less than omalizumab (75, 76). Currently, no additional clinical trials have been planned for lumiliximab in allergic diseases.

PGD2 receptor antagonists

PGD2 is a potent prostanoid released upon mast cell activation linking early and late phase allergen responses. Chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTh2) is the biologically relevant G-protein coupled receptor for PGD2 and its metabolites, as is the D prostanoid receptor, DP1 (77). Currently, several CRTh2 antagonists are in active development for allergic diseases and have completed or are undergoing clinical trials.

OC000459 (Oxagen Ltd, Oxfordshire, UK, USA), an oral CRTh2 antagonist, was evaluated in 132 moderate-persistent asthmatics randomized to receive OC000459 or placebo twice daily for 28 days. The treatment group had significant improvement in FEV₁ (7.4%) compared with placebo, and continued to improve even at the study endpoint. QOL significantly improved on OC000459, and there was a significant reduction in total serum IgE and trend toward reduced sputum eosinophilia (78). OC000459 has now completed Phase II clinical trials in both asthma and allergic rhinitis with enrollment of over 600 patients and has been well-tolerated. In addition, a Phase II proof-of-concept clinical trial for use in eosinophilic esophagitis is currently under way (79).

ARRY-005 (Array BioPharma, Boulder, CO, USA) is an oral CRTh2 antagonist shown to dose-dependently inhibit early and late phase allergen responses in a murine model of allergic rhinitis and improve clinical scores, and in a murine model of atopic dermatitis, inhibit scratching behavior. ARRY-006, another candidate CRTh2 antagonist developed by Array

BioPharma, inhibited airway hyperresponsiveness and reduced cellular infiltration in bronchoalveolar lavage fluid after allergen challenge in ovalbumin-sensitized BALB/c mice (80).

ACT-129968 (Actelion Pharmaceuticals, South San Francisco, CA, USA) is another oral CRTH2 antagonist scheduled to undergo Phase II clinical trials in both asthma and allergic rhinitis. In a recently completed 28-day Phase II study in mild-moderate asthma patients, ACT-129968 improved airway hyperresponsiveness by 1–1.5 doubling doses, FEV₁ by 10–14% and Asthma QOL Questionnaire Scores by 0.7, and also decreased sputum eosinophils and IgE levels.

AMG 853 (Amgen Pharmaceuticals, Thousand Oaks, CA, USA) is an oral CRTH2 and DP1 antagonist that was tolerated well in a Phase I clinical trial and is currently being evaluated in a Phase II clinical trial in subjects with inadequately controlled asthma (81).

Inhibiting important cells in the Th2 response

Mast cells and eosinophils both play an important role in pathophysiology of allergic diseases, and their effector functions are triggered significantly by IgE and IL-5, respectively. Immune modulation of these target cells aim to block downstream events that are initiated by the release of their mediators.

Mast cells

Spleen tyrosine kinase (Syk) is an intracellular enzyme that is activated after allergen cross-links IgE bound to the high-affinity IgE receptor, FcεRI, on mast cells and transduces signals leading to the release of preformed mediators, such as histamine, production of proinflammatory cytokines, such as TNF-α, and synthesis of lipid mediators, including leukotriene C4. R112 (Rigel Pharmaceuticals, South San Francisco, CA, USA) is a potent, rapid-onset, reversible Syk inhibitor discovered by a human mast cell culture screen to inhibit all mast cell reactions mediated by FcεRI cross-linking (82). A Phase II clinical trial compared an intranasal R112 formulation administered twice daily to placebo in subjects with seasonal allergic rhinitis in a park setting over a 2-day period. R112 demonstrated significant symptomatic improvement with onset of action within 30–90 min and was well-tolerated (83). However, in a subsequent Phase II clinical trial comparing intranasal R112 and beclomethasone to placebo over a 7-day period in subjects with allergic rhinitis, R112 did not significantly improve symptoms over placebo, although beclomethasone did (84). Although the company has halted development of R112, it has brought R343, a dry

powder inhaler formulation similar to R112, to Phase I clinical trials for asthma.

Eosinophils

CCR3 and common β-chain antagonist

CCR3 is the chemokine receptor for eotaxin-3 and is involved in eosinophil trafficking. GM-CSF, IL-3, and IL-5 are critical to eosinophil development in the bone marrow. TPI-ASM8 (Pharmaxis, Frenchs Forest, Australia) is a nebulized drug containing two modified phosphorothioate antisense oligonucleotides: one targeting CCR3, the other targeting common β chain of the receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5. In mild asthmatics, TPI-ASM8 attenuated allergen-induced increases in mRNA of the common β chain and CCR3, and also significantly reduced the early asthmatic response and sputum eosinophilia (85). A subsequent study demonstrated dose-response relationships for these effects, significant attenuation of the late asthmatic response, and tolerability at all tested doses (86). In a rat model of asthma, TPI-ASM8 added to nebulized budesonide showed a synergistic, dose-dependent reduction of eosinophil recruitment induced by allergen challenge and decreased bronchoalveolar lavage fluid levels of monocyte chemoattractant protein-1 (MCP-1) and IL-13, suggesting that TPI-ASM8 may be an effective adjunctive treatment in asthma (87). A Phase II three-way crossover clinical trial of TPI-ASM8 in mild asthmatics started in late 2010 (88).

IL-5 antagonists

IL-5 is critical to the differentiation and maturation of eosinophils in the bone marrow and their subsequent egress to the circulation and may be important in the pathogenesis of several allergic disorders, including certain asthma phenotypes, eosinophilic esophagitis, and the hypereosinophilic syndrome. There are several anti-IL-5 mAbs in active development for allergic diseases, including Mepolizumab and Reslizumab. Initial data from animal models have been promising, but clinical trials thus far have been only able to demonstrate the decreases in eosinophilia with significant concurrent improvements in clinical outcomes only in subsets of subjects.

Mepolizumab (Bosatria, GlaxoSmithKline, Vienna, Austria) is a recombinant humanized anti-IL-5 mAb that has been studied in asthma, atopic dermatitis, and eosinophilic esophagitis. Two single intravenous infusions of Mepolizumab 750 mg given 1 week apart were studied in subjects with moderate-to-severe atopic dermatitis and resulted in significant decreases in peripheral blood eosinophilia, but not

clinically significant improvement in symptoms (89). In eosinophilic esophagitis, Mepolizumab improved mean esophageal eosinophilia histologically and decreased TGF- β 1 expression, but only resulted in limited improvement in clinical symptoms (90). In asthma, several initial clinical trials failed to demonstrate clinical outcome improvement despite reduction in sputum and blood eosinophilia. However, a recent clinical trial with Mepolizumab of 1 year duration demonstrated improvements in blood and sputum eosinophilia in subjects with refractory eosinophilic asthma and also in exacerbations and asthma QOL scores (91). Another clinical trial also demonstrated improved FEV₁, asthma control, and reductions in sputum and serum eosinophils in asthmatics with persistent sputum eosinophilia despite prednisone treatment, over a 5-month treatment period and up to 8 weeks after the last infusion (92) (Fig. 4).

Reslizumab (Cinquil or SCH55700, Ception Therapeutics, Frazer, PA, USA) is a humanized IgG2 anti-IL5 mAb that has been shown to block pulmonary eosinophilia induced by antigen challenge in monkeys and airway hyperresponsiveness and pulmonary eosinophilia in allergic guinea pigs (93). A Phase II clinical trial of subjects with eosinophilic asthma receiving monthly intravenous Reslizumab for 4 months was recently completed, but no results have been reported yet. Reslizumab has been granted orphan drug status by the US FDA for treatment of pediatric eosinophilic esophagitis, and a Phase II/III clinical trial was completed in early 2010 (94).

Benralizumab (MEDI-563, Medimmune LLC, Gaithersburg, MD, USA) is a humanized anti-IL-5 receptor α (anti-IL-5R α)

IgG1 mAb that induces apoptosis of eosinophils and basophils by antibody-dependent cell-mediated cytotoxicity (95). A Phase I clinical trial of intravenous Benralizumab demonstrated acceptable safety and tolerability, and Phase II trials in asthma are currently underway (95, 96).

Stimulating Th1 responses

A key strategy employed in immune modulation of allergic disease involves dampening Th2 responses by augmenting the Th1 response, particularly by Toll-like receptor (TLR) agonists. Allergen-specific subcutaneous IT is the only form of immune modulator that has been shown to induce immune tolerance, that is, long-lasting therapeutic effects that persist after discontinuation of therapy. In fact, it may also prevent further progression to multiple aeroallergen sensitization. However, with the benefits of IT come rare, but real risks of severe adverse reactions, including anaphylaxis and death. Using TLR agonists may help improve the immunogenicity of IT without increasing its allergenicity, thereby improving its benefit-to-risk profile.

TLRs

TLRs are cell surface or intracellular receptors that recognize pathogen-associated molecular patterns commonly conserved in bacteria, viruses, and some fungi. Binding of these TLRs activates antigen-presenting cells, such as macrophages and dendritic cells, to commence both innate and adaptive immune responses. Agents directed against TLRs may skew the cytokine balance from Th2 toward Th1, thereby affecting allergic diseases. To date, 11 TLRs have been identified in humans, and agonists for four TLRs, TLR-1, TLR-4, TLR-8, and TLR-9, have been studied in clinical trials for allergic diseases. Of these, we will focus on ligands for TLR-4, TLR-8, and TLR-9.

TLR9 agonists (immunostimulatory oligodeoxynucleotides)

TLR9 (CD289) recognizes unmethylated CpG DNA motifs from bacteria that are engulfed by endosomes, which are either exposed after proteolytic degradation or produced *de novo* if these pathogens persist intracellularly. In vertebrates, CpG sequences are often silenced by methylation. In humans, plasmacytoid dendritic cells (pDCs) and B cells express the highest concentrations of TLR9. Upon TLR9 activation, pDCs produce IFN- α leading to secondary activation of neutrophils, monocytes, and natural killer T cells, whereas B cells produce IL-6 and IL-10 and differentiate into plasma cells that produce

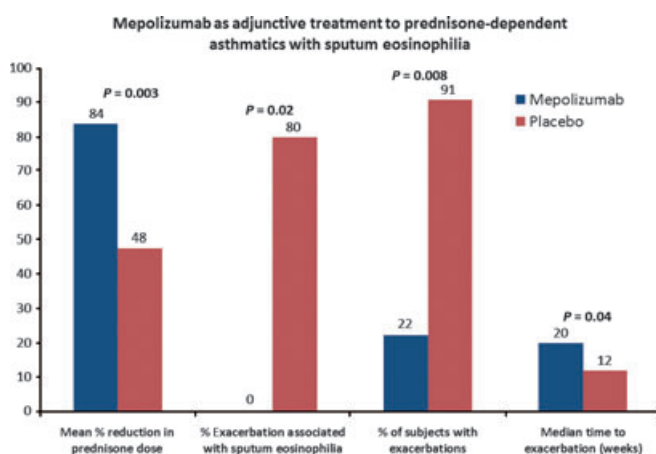


Fig. 4. Mepolizumab as adjunctive treatment to prednisone-dependent asthmatics with sputum eosinophilia. Mepolizumab-treated subjects had significantly fewer asthma exacerbations and longer time in between exacerbations. Adapted from Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, Hargreave FE, O'Byrne PM. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985–993.

IgG (97). TLR9 agonists act by these mechanisms to activate both innate and adaptive immune responses.

There are three known classes of immunostimulatory CpG oligodeoxynucleotides (ISS-ODNs) A, B, and C, which differ upon length, motif, and what immune responses each elicits, of which A and B have been studied as adjuvants in allergen IT, and B and C in infectious diseases and malignancy.

Tolamba (Dynavax Technologies, Berkeley, CA, USA) is a B-type ISS-ODN, ISS-1018, covalently linked to the major ragweed allergen Amb α 1. A Phase II clinical trial was conducted in 25 adult subjects with seasonal ragweed allergic rhinitis who were treated with six weekly subcutaneous injections of Tolamba or placebo before the ragweed season and then monitored for two ragweed seasons. Subjects receiving Tolamba reported better QOL and rhinitis symptoms than the placebo group and had suppression of seasonal increases in Amb α 1-specific IgE, which persisted over both ragweed seasons (98). A subsequent study demonstrated reduction in IL-4 and IFN- γ mRNA-positive cells and also eosinophils from nasal biopsies in Tolamba-treated subjects, but did not reproduce significant reductions in nasal symptoms (99). Questions about efficacy continued after the interim analysis of a large, multicenter Phase II clinical trial, the Dynavax Allergic Rhinitis Tolamba Trial (DARTT), failed to meet its primary endpoint difference in total nasal symptom score, possibly because symptom scores were too low in both study groups even during the ragweed season to detect a treatment effect (100). Further development of Tolamba has been placed on hold.

An inhaled form of ISS-1018 has been studied in atopic asthmatics (101). Although treatment significantly increased IFN- γ and IFN- γ -inducing genes, it did not attenuate early or late phase asthmatic responses to allergen challenge or were there significant reductions in sputum eosinophils or Th2-related gene expression.

A-type ISS-ODNs, with and without conjugation to allergen, have also been studied in allergen IT. A-type ISS-ODNs are more potent inducers of IFN- γ than B-type ISS-ODNs, but their unstable phosphodiester backbones made them less clinically useful until they were stabilized by association to virus-like particles, such as the bacteriophage Q β coat protein (Qb). A single center, open-label Phase II/IIa study of 20 perennial allergic rhinitis subjects with HDM allergy demonstrated that subcutaneous allergen IT of HDM extract conjugated to G10, an A-type ISS-ODN, contained in Qb, over a 10-week period was well-tolerated and still reduced symptoms and skin test responses at least 38 weeks after treatment completion (102). In a more recent Phase II clinical trial, CYT003-QbG10 (Cytos Biotechnology, Zurich, Switzerland) was used without aller-

gen in 80 subjects with mild-to-moderate perennial allergic rhinoconjunctivitis to HDM and/or cat allergens. Subjects received six weekly subcutaneous injections of CYT003-QbG10 or placebo, and after 8 weeks, the treatment group had significantly improved total rhinoconjunctivitis symptom scores and inhibition of nasal allergen provocation by 100-fold (103).

In another Phase II clinical trial, 63 persistent allergic asthmatics on ICS received either seven weekly subcutaneous injections of CYT003-QbG10 or placebo and were monitored over a 12-week period. During the run-in period, all subjects were stabilized on beclomethasone and then 4 weeks into the study, the ICS was reduced by 50%, and if possible, reduced to zero in another 4 weeks. From 6–12 weeks, the treatment group had significantly better combined symptom and medication scores than placebo and, at 12 weeks, had stable FEV₁ compared with placebo. A subgroup analysis in subjects who were able to come off ICS after week 8 suggests that CYT003-QbG10 may be as effective as an ICS in asthma (104).

TLR4 agonists

TLR4 (CD284) is expressed on the cell surface and, with the adapter molecule CD14, binds lipopolysaccharide (LPS) found on Gram-negative bacteria leading to proinflammatory Th1 responses. Monophosphoryl lipid A (MPLA) is derived from the active component of LPS of *Salmonella minnesota* R595 and has been used as an adjuvant in the anthrax vaccine used by the US Armed Forces and has been investigated in allergic rhinitis and IT.

CRX-675, an intranasal formulation of MPLA, was administered in subjects with ragweed allergic rhinitis at varying doses (2, 20, 100, 200 μ g) 24 h prior to ragweed intranasal challenge and was well-tolerated, but there was no inhibition of nasal allergen challenge responses compared with placebo. However, improvement in nasal symptom scores was observed at the 100 μ g dose (105).

Pollinex Quattro (Allergy Therapeutics, West Sussex, UK) is an allergy vaccine that contains pollen extract (available with grass, tree, parietaria, or ragweed) that is chemically modified by glutaraldehyde and adsorbed onto an L-tyrosine depot with an MPLA adjuvant. It has been used as a preseasonal ultrashort course IT consisting of four weekly subcutaneous injections in Europe and Canada. A large multicenter Phase III clinical trial of G301 (Pollinex Quattro-Grass) enrolled 1028 patients to receive ultrashort course IT or placebo for 4 weeks and demonstrated significant improvement in symptom plus medication scores during the peak (12%) and entire pollen season

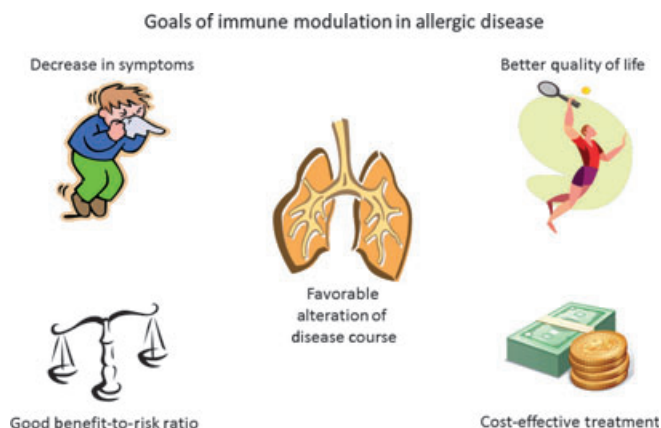


Fig. 5. Goals of immune modulation in allergic disease. Successful future immune modulators should favorably alter disease course, which leads to symptom reduction and improvement in quality of life, but must also have acceptable benefit-to-risk ratios and cost-effectiveness.

(13%) and improved QOL scores over placebo. Active treatment was well-tolerated. A prospective study of 34 subjects with seasonal grass pollen allergic rhinitis was conducted with Pollinex Quattro-G given over two seasons. Specific grass IgG and IgG4 levels increased slightly the first season, but only significantly after the second season along with an increase in FoxP3⁺ regulatory T cells, suggesting that at least two seasonal IT courses are needed to establish tolerance (106). A controlled field study of 29 parietaria weed seasonal allergic subjects who underwent MPLA-adjuvanted IT compared with 28 who refused, demonstrated persistent improvement in rhinoconjunctivitis and asthma symptoms, although no effect on lung function, after 3 years of IT and 5 years after discontinuation in the treated group (107). Although Pollinex Quattro appeared to be well-tolerated and effective in adults and children (108), clinical trials in the US were halted due to a reported serious adverse event of transverse myelitis in one subject. A recent study has demonstrated the potential of using

MPLA to augment the clinical and biologic responses to sublingual immunotherapy (SLIT) in grass-allergic patients (109).

TLR8 agonists

TLR8 (CD288) is an endosomal TLR that recognizes single-stranded viral RNA and certain imidazoquinoline drugs. An intranasal TLR8 agonist (VentiRx Pharmaceuticals, San Diego, CA, USA) recently underwent a proof-of-concept clinical trial in 80 grass-allergic subjects. VTX-1463 was administered as a weekly intranasal injection of four puffs each time, and subjects tested in Vienna Challenge Chamber had significantly better total nasal symptom scores than placebo (110).

Conclusions

Although many novel immune modulators in allergic diseases have failed to reproduce the successful results from *in vitro* or animal model studies into positive human clinical trial data, these agents have helped us learn about the pleiotropic effects of cytokines and the redundancies of their effector mechanisms and also of the heterogeneity of allergic disease phenotypes, especially asthma. Targeting Th2 effector molecules, such as IgE, via omalizumab, and PGD2, via CRTh2 antagonism, may be safer strategies as they are upregulated specifically during allergic processes; however, there is also promise in altering the Th1/Th2 balance using TLR agonists, particularly in allergen immunotherapy. Regardless of the mechanism of action, the goals of new immune modulator therapies in allergic disease remain the same: to decrease symptoms and improve quality of life, to favorably alter the course of disease, and achieve these goals with a good benefit-to-risk ratio and yet be cost-effective (Fig. 5).

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