

Targeting key proximal drivers of type 2 inflammation in disease

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Abstract | Systemic type 2 inflammation encompassing T helper 2 (T_H2)-type responses is emerging as a unifying feature of both classically defined allergic diseases, such as asthma, and a range of other inflammatory diseases. Rather than reducing inflammation with broad-acting immunosuppressants or narrowly targeting downstream products of the T_H2 pathway, such as immunoglobulin E (IgE), efforts to target the key proximal type 2 cytokines — interleukin-4 (IL-4), IL-5 and IL-13 — represent a promising strategy to achieve therapeutic benefit across multiple diseases. After several initial disappointing clinical results with therapies targeting IL-4, IL-5 or IL-13 in asthma, applying a personalized approach achieved therapeutic benefit in an asthma subtype exhibiting an ‘allergic’ phenotype. More recently, efficacy was extended into a broad population of people with asthma. This argues that the Type 2 inflammation is broadly relevant across the severe asthma population if the key upstream drivers are properly blocked. Moreover, the simultaneous inhibition of IL-4 and IL-13 has shown significant clinical activity in diseases that are often co-morbid with asthma — atopic dermatitis and chronic sinusitis with nasal polyps — supporting the hypothesis that targeting a central ‘driver pathway’ could benefit multiple allergic diseases.

Allergic diseases are increasingly becoming a global epidemic. Epidemiological studies have demonstrated the increasing prevalence of food allergies, rhinoconjunctivitis, atopic dermatitis and asthma^{1–3}. Allergy is a systemic type 2 inflammatory reaction (BOX 1) to an innocuous antigen (allergen) resulting from a complex interplay between genetic and environmental factors. This reaction ultimately leads to increases in immunoglobulin E (IgE) production and various associated inflammatory immune responses. Patients may present with a wide range of disease severity, from mild to life-threatening, involving single or multiple organ systems and tissues. Allergic diseases may appear disparate based on their distinct organ and tissue manifestations and are often treated by clinicians with different medical specialties. However, the tendency for diverse allergic diseases to present as co-morbidities or progressively (that is, the ‘atopic march’) suggests that these diseases may share common underlying drivers. Along these lines, it has long been recognized that the T helper 2 (T_H2)-mediated responses of type 2 inflammation are important in both asthma and atopic dermatitis, two highly prevalent chronic diseases with distinct tissue-specific manifestations in the lung and skin, respectively.

Although initial clinical studies using type 2 pathway modulators were somewhat disappointing, more recent clinical data in patients with allergic asthma (as identified by biomarkers) have provided support for the important roles of three particular type 2 cytokines: interleukin-5 (IL-5) and the sister cytokines IL-4 and IL-13, which share a common receptor (FIG. 1). Treatment with the IL-5-specific humanized monoclonal antibody (mAb) mepolizumab (developed by GlaxoSmithKline) demonstrated efficacy in a subset of patients with asthma and chronic sinusitis with nasal polyps (CSwNP). In addition, simultaneous blockade of IL-4 and IL-13 signalling using a fully human IL-4 receptor subunit alpha (IL-4R α)-blocking antibody (dupilumab; developed by Regeneron/Sanofi) has demonstrated clinical activity across three allergic diseases: atopic dermatitis, CSwNP, and asthma. These recent clinical data raise the intriguing possibility that targeting a key central ‘driver pathway’ could have a substantial therapeutic effect on allergic diseases characterized by diverse organ-specific clinical manifestations. Analogous to oncology — in which it is now recognized that it may be better to define cancers based on their causative mutations rather than

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Box 1 | Type 2 immunity

Type 2 immunity refers to a specialized immune response involving the innate and the adaptive arms of the immune system to promote barrier immunity on mucosal surfaces, in particular to eliminate parasitic pathogens. Type 2 immunity is characterized by T helper 2 (T_H2) CD4⁺ T cells and B cell production of the immunoglobulin E (IgE) antibody subclass. In addition, the innate cellular response includes group 2 innate lymphoid cells, eosinophils, basophils, mast cells and interleukin-4 (IL-4)- and/or IL-13-activated macrophages. The response is associated with several cytokine mediators such as IL-4, IL-5, IL-9 and IL-13. Epithelial-derived cytokines, thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 also propagate or initiate type 2 responses, but their functions are not limited to type 2 immune responses. This inflammatory response can also be initiated in response to allergens, leading to allergic diseases.

their tissue of origin for personalized treatment — it may be time to define and group allergic diseases and thereby tailor therapy based on common immunological pathways.

The predominance of type 2 inflammation is a key driver of allergic diseases^{4,5}. The type of antigen, in combination with environmental factors and underlying genetics, influences the release of an array of cytokines that results in the initiation or propagation of type 2 inflammatory processes by innate and lymphoid cells. At the barrier interface to environmental stimuli, epithelial-derived cytokines such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) serve to initiate type 2 immune responses or amplify existing type 2 inflammation. These upstream mediators stimulate innate cells to produce type 2 cytokines and also contribute to priming and polarizing naive T cells into CD4⁺ T_H2 cells^{6,7}. T_H lymphocyte subsets are classified based on the immune response associated with particular cytokines and inflammatory mediators specific to each subset. For example, interferon- γ (IFN γ) is produced by T_H1 cells, IL-4, IL-5 and IL-13 by T_H2 cells, IL-9 by T_H9 cells, IL-17A, IL-17F, IL-21 and IL-22 by T_H17 cells, IL-22 by T_H22 cells, and IL-10 by regulatory T cells. Among other roles, T_H2 cells induce B cell proliferation and the subsequent production of antibodies that undergo isotype switching, resulting in high levels of circulating IgE (FIG. 2a). Thus, IgE is a key downstream biomarker of T_H2 cell activation. IgE binds to the high-affinity IgE receptor (Fc ϵ RI) found on basophils and mast cells, and the crosslinking of IgE on these cells leads to cellular activation and the degranulation of several inflammatory mediators. These inflammatory mediators include histamine, prostaglandins and other pro-inflammatory cytokines (for example, IL-4, IL-5 and IL-13), thus amplifying the type 2 response. In the lower airways, this type 2 inflammatory milieu results in eosinophilia, mucus production and smooth muscle contractility (FIG. 2b). These processes are important protective immune functions for eliminating parasitic infections but are pathological in response to innocuous antigens or allergens, leading to allergy.

In addition to the classically defined allergic diseases (including asthma, atopic dermatitis, food allergy, allergic rhinitis and conjunctivitis), a diverse range of diseases with less clear aetiologies are also characterized by type 2 clinical hallmarks (most notably eosinophilia

and/or high serum IgE levels). Such diseases include chronic idiopathic urticaria, CSwNP and eosinophilic oesophagitis. Here, we provide a review of recent milestone developments in clinical research that have led to a deeper understanding of the pathobiology of allergic diseases and provided support for the notion that the type 2 inflammatory cascade is a unifying feature of multiple diseases.

Unmet need in severe allergic diseases

Although some allergic diseases (such as allergic rhinitis) are well served with antihistamines and specific immunotherapy, non-specific immunosuppression is the mainstay of therapy for more-severe allergic diseases such as atopic dermatitis and asthma. In both these diseases, aberrant inflammatory responses exacerbate and propagate the disease symptoms. Reducing inflammation by systemically administering broad-acting immunosuppressants such as oral or intravenous corticosteroids, cyclosporin A, methotrexate, azathioprine or mycophenolate mofetil effectively alleviates symptoms of severe disease⁸. The immunosuppressive activity of these agents results from targeting downstream mediators such as transcription factors. For example, corticosteroids bind glucocorticoid receptors and suppress the expression of key transcription factors that drive inflammation, such as nuclear factor- κ B⁹. Cyclosporin A is a calcineurin inhibitor that prevents the production of IL-2 (via the transcription factor nuclear factor of activated T cells (NFAT)), which is required for T cell activation and proliferation¹⁰. However, owing to the broad mechanism of action of cyclosporin A and corticosteroids, systemic immunosuppressive therapies result in pleiotropic effects that lead to toxicities such as fluid retention, glucose intolerance, hypertension, muscle weakness, gastrointestinal intolerance, potential bone loss, suppression of the hypothalamic–pituitary–adrenal axis and increased susceptibility to infections^{11,12}. Local administration (that is, inhaled drugs, topically applied drops, nasal sprays or creams) reduces the side effects of these immunosuppressive agents; however, local immunosuppression is not sufficient to treat the more severe forms of these diseases. Thus, there remains a significant need for more-specific therapies.

Applying a brute force approach to suppressing inflammation does not provide insight into which immune pathways propagate disease. For example, the broad-acting drug cyclosporin A is effective in treating both psoriasis and atopic dermatitis. By contrast, specifically targeting tumour necrosis factor (TNF) in psoriasis and atopic dermatitis results in differential clinical efficacy, indicating that these two skin diseases have distinct driver pathways. TNF blockers are approved therapies for the treatment of psoriasis, but have not demonstrated sustained efficacy in atopic dermatitis, which is a type 2 inflammation-driven disease¹³.

Omalizumab (Xolair; Novartis/Genentech) induces more specific immune suppression in allergic diseases by targeting an end mediator of type 2 inflammation and potent trigger of mast cell and basophil degranulation: IgE. This IgE-specific humanized mAb was the first mAb therapy that received regulatory approval for asthma,

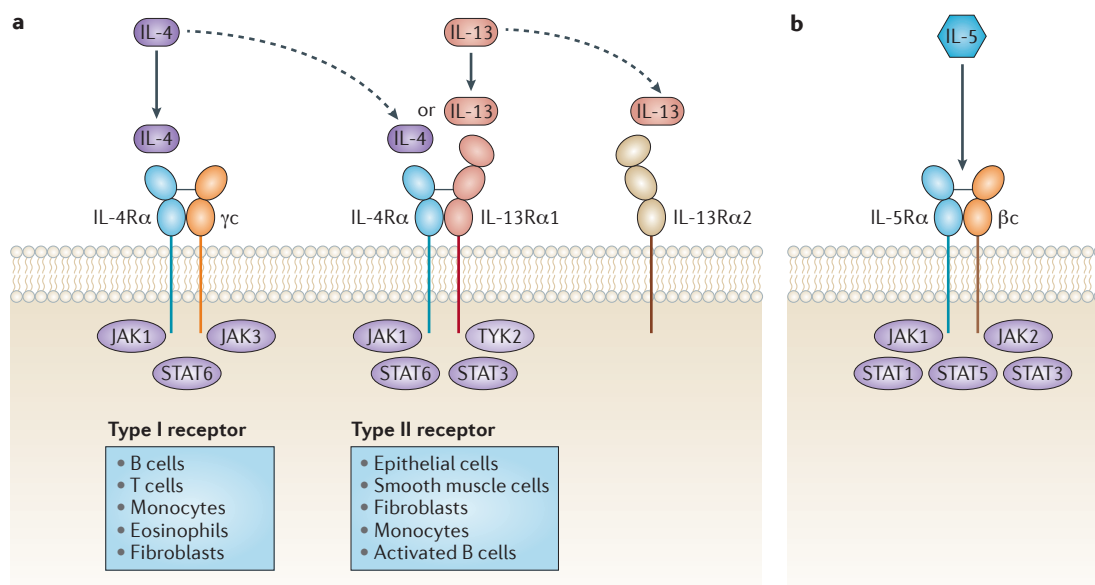


Figure 1 | Receptor systems for IL-4, IL-13 and IL-5. **a** | The distinct and overlapping functions of interleukin-4 (IL-4) and IL-13 are partly due to their shared receptor moiety, IL-4 receptor subunit alpha (IL-4Rα). IL-4Rα forms two distinct heterodimeric receptor complexes to mediate the biological functions of IL-4 and IL-13. The type I receptor, comprising IL-4Rα and common cytokine receptor γ-chain (γc), only binds IL-4. The type II receptor complex, formed between IL-4Rα and IL-13Rα1, is the primary receptor for IL-13 but also binds IL-4. In addition, IL-13 will bind to a second receptor, IL-13Rα2, the function of which is unclear but postulated to be a decoy receptor. The expression of the accessory chains on distinct cell lineages dictates the formation of type I or type II receptor complexes and, subsequently, which cytokine or cytokines act on specific cell types. **b** | IL-5 binds to IL-5Rα, which forms a complex with βc; this signalling subunit is also required for granulocyte–macrophage colony-stimulating factor and IL-3 signalling. JAK, Janus kinase; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2.

but is not effective in atopic dermatitis. Omalizumab reduced free serum IgE levels¹⁴, and with this novel anti-inflammatory mechanism it demonstrated efficacy in reducing exacerbations but with minimal improvements to the forced expiratory volume in the first second of a forced expiration (FEV1), a measure of lung function^{15,16}. Omalizumab also improved symptoms of co-morbid CSwNP¹⁷. It was recently approved for the treatment of chronic idiopathic urticaria refractory to antihistamine use; in pivotal trials, omalizumab demonstrated significant improvement on pruritus and disease activity end points^{18–20}. By contrast, in atopic dermatitis, a reduction in free serum IgE levels is not sufficient to achieve good clinical response. Patients with moderate atopic dermatitis treated with 16 weeks of omalizumab did not experience improvements in disease end points (eczema area and severity index (EASI), and investigator global assessment)²¹ (BOX 2). Moreover, treated patients experienced slight worsening of pruritus scores compared with the placebo group²¹. These results suggest that even among type 2 inflammation-driven diseases, the end product of the T_H2 pathway, IgE, is not a unifying pathogenic mediator of disease.

Targeting key proximal drivers of the type 2 inflammatory pathway, rather than the end mediators, may be required to achieve optimal therapeutic efficacy across multiple allergic diseases (FIG. 3). Towards this end, the three key type 2 cytokines IL-4, IL-5 and IL-13, are promising candidates for a common node among allergic inflammatory diseases.

Type 2 pathway activation in disease

Hallmarks of type 2 inflammation, most notably eosinophilia and IgE production, provide evidence of activation of this pathway in disease. Historically, allergic diseases were classified by disease trigger — intrinsic (non-allergic) versus extrinsic (allergic) — with extrinsic forms of disease characterized by high serum IgE levels. The intrinsic form is not as well understood as the extrinsic form, but manifests with similar clinical symptoms without the association of other atopic diseases or allergen-specific IgE^{22,23}. Eosinophilia, hyperplasia and excess mucus production are also well-recognized type 2 disease features associated with type 2 pathway activation. Additionally, family history or co-morbidity with other atopic diseases (such as allergic rhinitis) is a criterion commonly associated with extrinsic disease²⁴.

Asthma is a chronic inflammatory disease of the airways characterized by airway hyperresponsiveness, acute and chronic bronchoconstriction, airway oedema and mucus plugging. The inflammatory component of asthma involves many cell types, including mast cells, eosinophils, T lymphocytes, neutrophils, group 2 innate lymphoid cells and epithelial cells, and their biological products. This diversity of cell types present in inflammatory infiltrates suggests that the asthma population may be heterogeneous. However, there is increasing interest in identifying distinct phenotypes because targeted therapy is more likely to be successful in patients with similar underlying pathobiological features. In particular, numerous therapeutic approaches in asthma

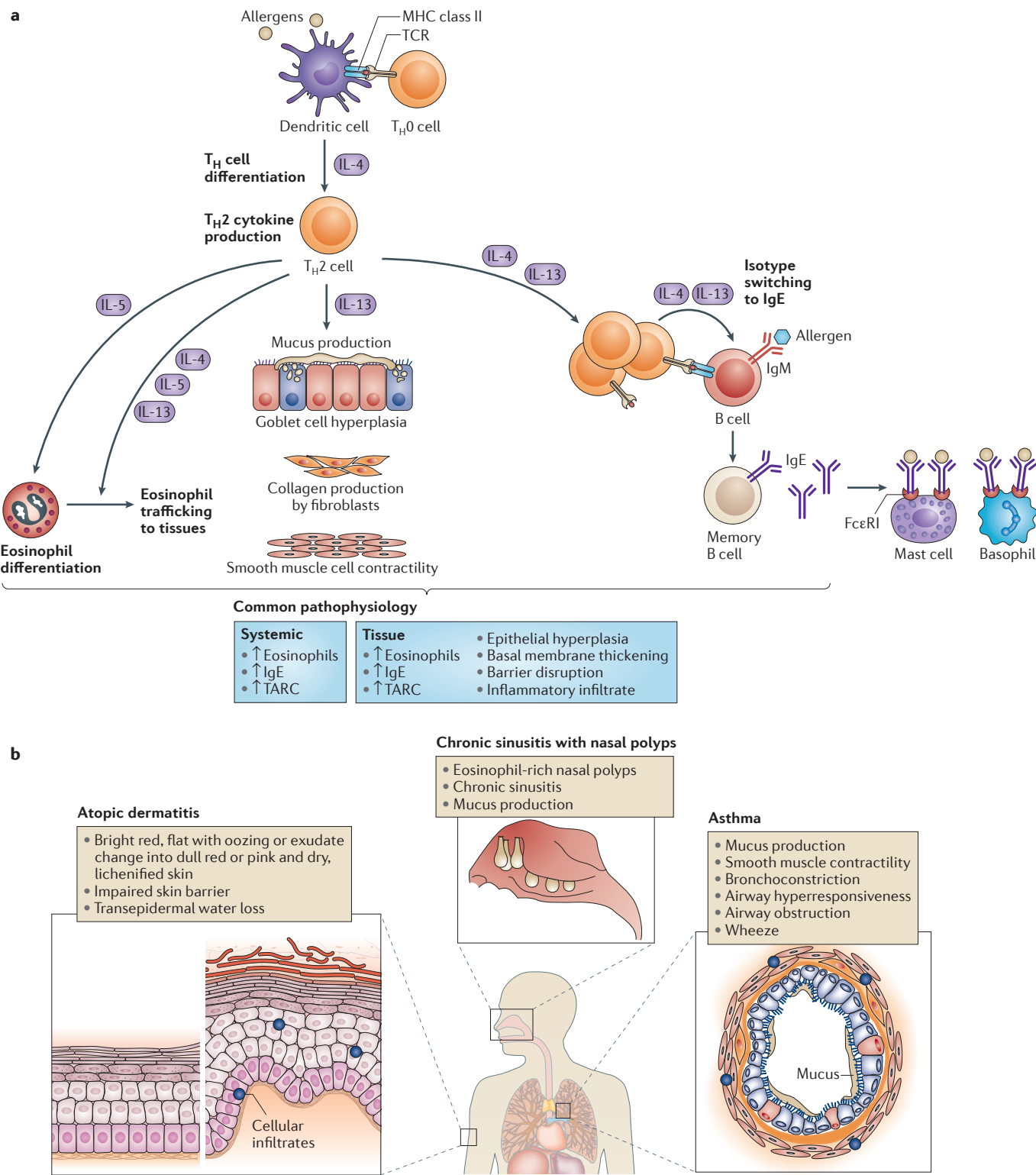


Figure 2 | Type 2 inflammation in severe asthma, atopic dermatitis and chronic sinusitis with nasal polyps.

a | The type 2 cytokine interleukin-4 (IL-4) drives the differentiation and clonal expansion of T helper (T_H) cells into T_H2 cells and further production of cytokines IL-4, IL-5 and IL-13. Hallmarks of type 2 pathway activation include immunoglobulin E (IgE) production and eosinophilia. In particular, IL-5 primarily drives the differentiation of eosinophils in the bone marrow, and IL-4, IL-5 and IL-13 have roles in tissue-specific trafficking. IL-4 and IL-13 are primarily responsible for isotype class switching of B cells to produce IgE. IL-13 is implicated in tissue effects such as mucus secretion, smooth muscle contractility and hyperplasia. **b** | Common underlying pathological type 2 pathway activation results in different allergic diseases based on the tissue affected. FcεRI, high-affinity IgE receptor; TARC, thymus- and activation-regulated chemokine.

have recently focused on trying to control the T_H2 -type responses. Evidence of type 2 immune activation is represented both in the lung tissue and systemically. IL-4 and IL-5 are highly expressed in the bronchial mucosa²⁵ and IgE is present in serum. Histological changes to the bronchial epithelium include oedema, sub-basement membrane thickening, and goblet cell hyperplasia, indicative of an increase in IL-13 activity. Eosinophils are often observed both peripherally and in the sputum. In addition, suppression of eosinophils tracks with disease activity and response to corticosteroid treatment²⁶. Historical observations have documented inflammatory infiltrates that included T cells ($CD4^+$ and $CD8^+$), mast cells, neutrophils and eosinophils^{25,27} in both extrinsic and intrinsic types of disease.

In atopic dermatitis, evidence of type 2 inflammation is found systemically and in both acute and chronic skin lesions, regardless of extrinsic or intrinsic classification. The majority (80%) of patients with atopic dermatitis are generally classified as having extrinsic (allergic) disease based on the detection of high serum IgE levels²³. However, regardless of the differences in serum IgE levels, biopsy samples of lesions taken from patients with intrinsic or extrinsic disease and with comparable disease severity showed the presence of several type 2 cytokines (for example, IL-5 and IL-13)^{22,23}. Additionally, both forms were associated with an increase in activation of genes associated with several T cell subsets (T_H1 , T_H2 , T_H22 and T_H17)²³. The major discernible difference between the intrinsic and extrinsic-type lesions was that patients categorized as having intrinsic atopic dermatitis had overall greater immune activation than patients with the extrinsic form²³.

A recent analysis of acute versus chronic atopic dermatitis lesions has shed light on the temporal role of T_H2 cell mediators in this disease²⁸. IL-4 expression is observed in both acute and chronic skin lesions; however, as red, flat and oozing acute lesions transition to thick, lichenified chronic lesions, IFN γ expression increases relative to IL-4. Consequently, chronic lesions were initially thought to have a strong T_H1 component^{29,30}. However, it was subsequently shown that in the lichenified chronic lesions the entire inflammatory response becomes more intense, with increased expression of T_H1 -, T_H2 - and T_H22 -associated cytokines and an increase in T cell and dendritic cell infiltration, as well as activated eosinophils^{28,29}. Reasons for the sequential activation of other T_H cell subtypes in the skin are poorly understood. However, this inflammation coincides with pathological changes such as skin barrier breakdown as a consequence of the itch-scratch cycle, *Staphylococcus aureus* infection and epidermal hyperplasia.

Several lines of evidence have also implicated systemic type 2 inflammation as an underlying disease feature of atopic dermatitis. Patients with atopic dermatitis have eosinophils present in the peripheral blood³¹, and there is molecular evidence of T_H2 pathway activation in the non-lesional (unaffected) skin of patients with atopic dermatitis compared with normal skin from healthy controls³². Moreover, patients with atopic dermatitis have increased serum levels of thymus- and

activation-regulated chemokine (TARC; also known as CCL17), an IL-4- and IL-13-induced chemokine that functions as a selective chemoattractant for T cells, compared with healthy controls^{33–36}. Broad-acting therapies (for example, topical corticosteroids and oral cyclosporin A) induce a rapid decline in TARC levels, which closely correlates with atopic dermatitis severity³³, thus providing further evidence of an underlying type 2 inflammation-driven response in this disease.

Close examination revealed that a similar type 2 inflammatory profile is present in CSwNP, both in the tissue and systemically. Chronic sinusitis is a persistent inflammatory disorder of the paranasal sinuses. Chronic sinusitis occurring with bilateral nasal polyps is associated with intense eosinophil-dominated inflammation, and these patients present with several type 2 clinical hallmarks, including high serum IgE levels, blood and tissue eosinophilia, and goblet cell hyperplasia of the mucosal lining^{37–39}. Notably, it was hypothesized that nasal polyps, which have been described as ‘bags of eosinophils’, could serve as a visual biomarker to identify patients with type 2 inflammation^{40,41}.

Defining key drivers of type 2 inflammation. IL-4 is the key differentiation factor driving a T_H2 -type response. IL-4 initiates T cell differentiation towards the T_H2 subtype and induces production of type 2-associated cytokines and chemokines, such as IL-5 (REF. 42), IL-9, IL-13 (REFS 43,44), TARC^{45,46} and eotaxins^{47,48}. In B cells, IL-4 induces isotype class switching to IgE. *In vitro*, IL-4 promotes differentiation of T_H naive T cells into type 2 cytokine-producing effectors⁴⁹. *In vivo*, the lack of IL-4 leads to impaired type 2 cytokine production in response to parasite infection^{43,44}. In turn, IL-4 negatively regulates the T_H1 -type response associated with IFN γ production^{49–51} and macrophage activation⁵², serving to maintain immune polarization towards a type 2 response.

IL-4 and IL-13 are potent mediators of type 2 immunity with both overlapping and distinct functions related to their receptor expression patterns. Although IL-4 and IL-13 only share 25% amino acid homology⁵³, these cytokines share a common receptor moiety, IL-4R α , which partners with distinct accessory chains to induce signalling. IL-4R α is expressed on both haematopoietic cells and non-haematopoietic cells; however, the differential expression of the accessory chains in different cell types has shed light on their functional differences (FIG. 1). The type 1 complex consists of IL-4R α partnering with the common γ -chain, which is found exclusively on haematopoietic cells. The type 2 receptor complex consists of IL-4R α partnering with IL-13R $\alpha1$, which is found on many non-haematopoietic cells, such as keratinocytes, hair follicles, epidermal sebaceous and sweat glands, nasal and bronchial epithelial cells, smooth muscle cells and fibroblasts⁵⁴. IL-4 signals through both the type 1 and type 2 receptor complexes, whereas IL-13 signals only through the type 2 complex. This is because IL-13 binds to its own primary binding chain (IL-13R $\alpha1$) whereas IL-4 primarily binds to IL-4R α ⁵⁵. In addition, the two cytokines have different potencies and signalling kinetics. IL-4 binds to IL-4R α with high

Box 2 | Common clinical symptoms and end points of atopic dermatitis

There are numerous symptoms that are associated with atopic dermatitis:

- Inflamed, red, itchy lesions, epidermal hyperplasia (thickening) and lichenified skin; lesions can vary in severity and with varying distribution over the body (including visible surfaces), commonly recurring at the same site
- Intense intractable pruritus (itch) leads to loss of sleep and affects adult productivity and children's education
- New or acute lesions (<72 hours) appear bright red, flat with oozing or exudates, whereas chronic lesions (>72 hours) appear dull red or pink and dry, with thick, lichenified skin
- Impaired skin barrier protection, heightened transepidermal water loss and generalized dry skin

Scoring atopic dermatitis (SCORAD)

- SCORAD is a composite measure of affected area and disease severity
- Extent of body surface area (BSA) involvement is divided into nine sections, assigning 9% each to head, each arm, each leg and the anterior and posterior trunk, and 1% to the perineum
- Disease severity is assessed on five signs of skin inflammation: erythema, oedema or papulation, oozing or crusts, excoriations, lichenification, and dryness; scored from 0–3 (0 = absent; 1 = mild; 2 = moderate and 3 = severe)
- The sum of BSA is divided by 5 and added to 3.5 times the total severity score, giving a maximum score of 83 (score <15 = mild, score of 15 to 40 = moderate, score >40 = severe)^{135,136}
- Patient-scoring by visual analogue scales of subjective measure of disease, pruritus and loss of sleep, can be incorporated for a maximum score of 103
- A SCORAD score reduction of 50% is considered clinically meaningful; the responder rate (SCORAD50) indicates the proportion of patients that achieved 50% reduction in symptoms

Eczema area and severity index (EASI)

- EASI is a composite measure of affected area and disease severity
- BSA is divided into four regions (head, trunk, upper limbs and lower limbs); each region is scored from 1 to 6 in 10% increments, where 1 = >10% BSA, and 6 = 90–100% BSA
- Four signs of inflammation (that is, erythema, infiltration and papulation, excoriation, and lichenification) are scored from 0 to 3 (none, mild, moderate and severe)
- The sum of all signs of inflammation (erythema + infiltration + excoriation + lichenification) is multiplied by the region score and a defined constant value that allows for weighting of the body region, for a maximum of 72 (REFS 137, 138)
- A 50% EASI score reduction is considered clinically meaningful; EASI50 is reported as the responder rate
- This composite end point for atopic dermatitis excludes subjective measures and was adapted from the psoriasis area and severity index (PASI, the benchmark scoring for psoriasis)

Investigator global assessment (IGA)

- IGA provides the overall severity of atopic dermatitis on a 6-point scale: 0 = clear or 1 = almost clear to 5 = very severe atopic dermatitis with severe erythema, severe papulation or infiltration with oozing or crusting¹³⁸
- The proportion of patients who achieved IGA of 0 or 1 demonstrates high level of efficacy of therapy and can be used to provide a responder rate

Pruritus numerical rating scale (NRS)

- A validated patient-reported measure of itch severity¹³⁹
- The intensity of itch is scored on a numerical scale from 0 to 10; 0 = no symptoms, 10 = worst imaginable symptoms

affinity (subnanomolar dissociation constant (K_d) values) independent of γ -chain or IL-13R α 1 association, whereas the presence of IL-4R α increases the affinity of IL-13 binding to IL-13R α 1 (a change in K_d from 10 mM to 30 pM)⁵⁶. Moreover, the time course of intracellular signalling activated by type 2 receptor engagement is more rapid with IL-4 than IL-13 (REF 57). The physiological differences of IL-4 and IL-13 have been dissected through cytokine knockout and overexpression phenotypes in mouse models of parasitic infection and allergy, as discussed below. Careful examination of these phenotypes supports the hypothesis that IL-4 is the central mediator of T_H2 cell differentiation, growth of B cells, initiation of isotype class switching (especially to IgE) and recruitment of eosinophils^{6,58}. Although IL-13 has some redundancy in these pro-inflammatory processes, IL-13 has additional roles in mediating goblet cell hyperplasia

and smooth muscle contractility, potentially related to the distinct expression pattern of the type 2 receptor complex and local cytokine production.

IL-4 and IL-13 both mediate aspects of IgE production through activity on B cells. Knocking out either IL-4 or IL-13 in mice results in a significant defect in IgE production in response to allergen challenge^{55,59–63}. Although IL-4 initiates and promotes isotype class switching and B cell growth, there is evidence to indicate that IL-13 can also bind to activated human B cells^{53,64}, which suggests that IL-13 contributes towards the propagation of IgE production and explains the phenotype observed in knockout mice.

IL-4, IL-5 and IL-13 promote the tissue and blood eosinophilia characteristic of type 2 inflammation. IL-5 is a potent eosinophil cytokine; it is responsible for growth and differentiation in the bone marrow, survival

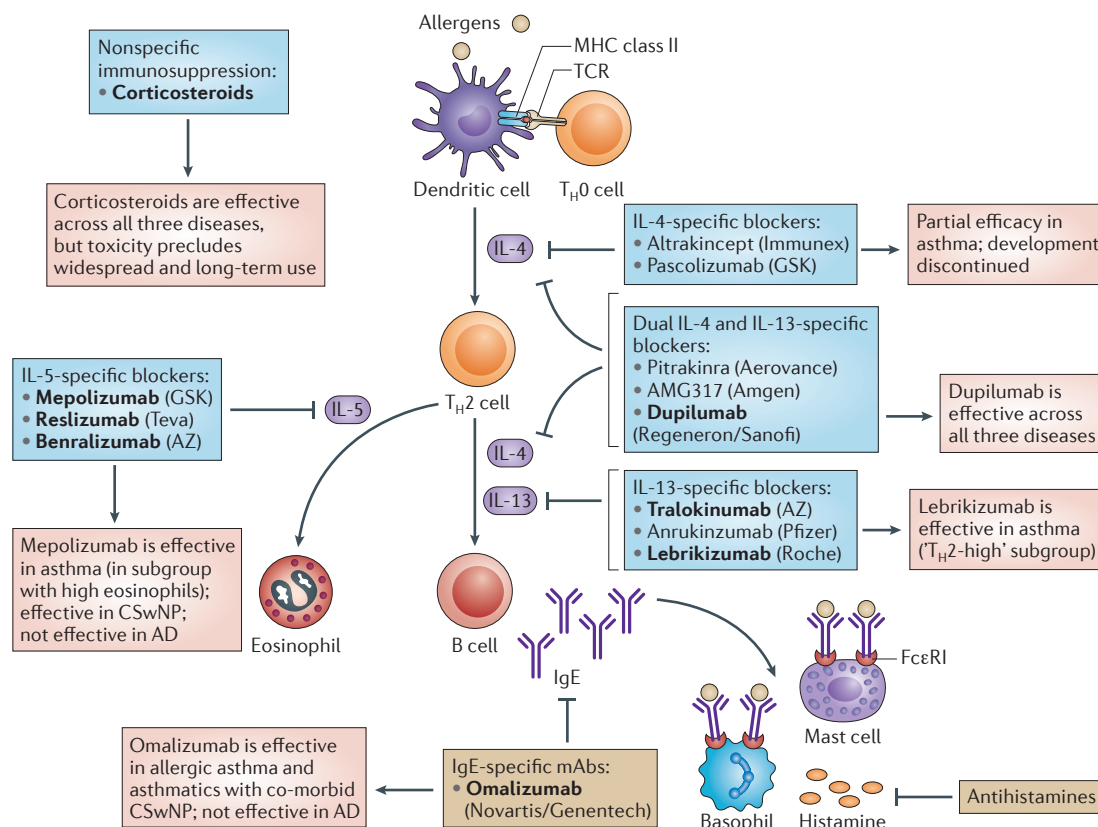


Figure 3 | Targeting key proximal drivers of the type-2 pathway versus end-product mediators. Different clinical effects are achieved in asthma, atopic dermatitis (AD) and chronic sinusitis with nasal polyps (CSwNP) by blocking different steps in the type 2 inflammation pathway. Targeting the end products of this pathway, such as immunoglobulin E (IgE) or histamines, has not demonstrated robust efficacy in asthma. Conversely, systemically reducing inflammation via broad-acting corticosteroids is associated with toxicities owing to their pleiotropic effects. Interleukin-5 (IL-5), IL-4 and/or IL-13 represent proximal targets in the type 2 pathway and have demonstrated efficacy in type 2-driven allergic inflammatory diseases. In particular, the IL-5 blocker mepolizumab has shown efficacy in two diseases with features of high eosinophilia: a subset of asthma with high eosinophil levels in sputum, and CSwNP. Blocking individual cytokines, for example, blocking IL-13 with lebrikizumab, has shown efficacy in asthma. Dual blockade of both IL-4 and IL-13 with dupilumab, which blocks their shared receptor subunit alpha (IL-4Ra), has shown efficacy across three diseases: asthma, CSwNP and AD. These clinical results provide evidence to support the notion that type 2 inflammation underlies all three diseases. Drug names in bold are still in development or approved, drug names not in bold have been discontinued. AZ, AstraZeneca; FcεRI, high-affinity IgE receptor; GSK, GlaxoSmithKline; TCR, T cell receptor; T_H, T helper.

and mobilization, as well as emigration from bone marrow to the blood. IL-5 binds to the cytokine-specific subunit receptor IL-5Rα, which forms a complex with a shared signalling subunit, the β-chain; granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 also require β-chain signalling⁶⁵ (FIG. 1b). IL-5Rα is highly expressed on eosinophil and eosinophil progenitors, and is also present on basophils^{65,66}. In the absence of IL-5 (in a genetic knockout or after treatment with an IL-5-specific antibody), the blood and tissue eosinophil response in response to allergen challenge is abrogated⁶⁷.

IL-4 and IL-13 stimulate eosinophil tissue trafficking from the periphery to the site of inflammation by inducing the production of eosinophil-promoting factors, including IL-5 and eosinophil chemokines (such as eotaxin 1)^{48,68–71}. Mice lacking IL-4Ra have a marked reduction in the number of tissue, but not

blood, eosinophils in response to allergen challenge^{70,72}. Dissecting the predominant role of IL-4 in tissue eosinophilia, IL-4 knockout mice do not develop significant lung eosinophilia in response to allergen challenge⁷³, whereas IL-13 knockout mice can still develop significant eosinophilic lung inflammation^{70,73,74}. In addition to IL-4 promoting eosinophilia by inducing IL-5 production, IL-4 may also propagate eosinophilia, as human eosinophils express IL-4Ra⁵². Thus, the synergy of several type 2 factors is required to induce blood and subsequently tissue eosinophilia.

The tissue-specific effects of IL-4 and IL-13 have been examined in preclinical asthma models. Studies comparing mice lacking responsiveness to IL-4 alone versus both IL-4 and IL-13 have revealed specific roles for IL-13 in goblet cell hyperplasia, mucus production, effects on smooth muscle and epithelium, and airway

hyperresponsiveness (AHR). AHR is an exaggerated bronchoconstriction response to normally innocuous stimuli resulting from chronic inflammation and associated with structural changes to airway smooth muscle cells^{70,73–76}.

In response to allergen challenge, wild-type mice develop asthma-like phenotypes, including lung eosinophilia, goblet cell hyperplasia, mucus production and AHR. Studies using various cytokine knockout mice and/or selective inhibitors have shown that IL-13 is critical for mucus production and AHR, but not tissue eosinophilia or inflammatory infiltrate^{77,78}. For example, *Il13* knockout mice still demonstrate tissue eosinophilia and intense airway inflammation; however, they do not develop AHR, suggesting that IL-13 is required for AHR⁷⁴. IL-13 also mediates two hallmarks of asthma: mucus hypersecretion and goblet cell hyperplasia. The lungs of transgenic mice overexpressing IL-13 showed a pronounced increase in goblet cell hyperplasia^{79,80} and an elevation in markers for fibrosis, such as collagen deposition and subepithelial basement membrane thickening⁸¹. *In vitro*, IL-13 contributes to smooth muscle contractility⁵⁷ and increases the expression of the mucus marker MUC5AC in cultured human bronchial epithelial cells⁸²; these physiological functions would be beneficial in eliminating a parasitic infection but contribute to lung pathology in asthma.

The production of type 2 proximal cytokines is propagated and potentially initiated by epithelial-derived cytokines, IL-25, IL-33 and TSLP, which are released at the barrier interface upon tissue damage or allergen exposure. The activity of these epithelial-derived cytokines on a number of innate cell types (for example, group 2 innate lymphoid cells and mast cells) induces the production of IL-4, IL-5 and IL-13 and also serves to promote T_H2-type responses^{6,7}. In particular, IL-33 acts as an 'alarmin' (signal of cell or tissue damage) that can polarize naive T cells to T_H2 cells and also amplify existing type 2 responses⁸³. IL-33 together with IL-25 also induces innate lymphoid cells to produce high levels of type 2 cytokines, particularly IL-5 and IL-13 (REF. 7). TSLP promotes cytokine production in basophils, monocytes and natural killer T cells, and also activates dendritic cells to prime and activate T_H2 cells⁸⁴.

Taken together, IL-4, IL-5 and IL-13 have pleiotropic roles to orchestrate the type 2 immune response and play distinct parts in driving the manifestation of the hallmarks of type 2 and T_H2 pathway activation: IgE production and eosinophilia. Preclinical data provided solid evidence that both asthma and atopic dermatitis are driven by these mediators of type 2 inflammation. Transgenic mice overexpressing all three type 2 cytokines, IL-4, IL-5 and IL-13, spontaneously develop asthma-like lung pathology and atopic dermatitis-like skin lesions characterized by hallmarks of an exaggerated type 2 response; that is, high serum IgE levels, extensive cellular infiltrations in skin and lung (including eosinophils and lymphocytes), dermal thickening and airway epithelial hypertrophy⁸⁵. Additional studies showed that IL-4 and IL-13 independently are sufficient to cause similar pathology^{80,86–88}. These preclinical data implicating IL-4 and IL-13 as key proximal disease drivers

are supported by human genetic evidence showing that polymorphisms in genes related to the IL-4 and IL-13 signalling pathway, including *IL4RA* and *IL13*, are associated with increased risk for asthma and atopy⁵⁸.

Given the hallmarks of eosinophilia, high IgE levels and increased type 2 cytokines in asthma and atopic dermatitis, modulating the cytokines driving this pathway was thought to be an attractive therapeutic strategy. However, biologic therapeutics targeting type 2 mediators initially had limited success in treating asthma and atopic dermatitis, and these early clinical failures led to scepticism about the importance of this pathway in these diseases.

Targeting type 2 cytokines in atopic diseases

Given the prominence of eosinophilia in asthma and atopic dermatitis, it was expected that reducing eosinophilic inflammation by targeting IL-5 would result in disease improvement. Mepolizumab is highly specific for IL-5 and potently depletes eosinophils from the blood and tissue in patients with asthma^{89–91} and atopic dermatitis⁹² (TABLE 1). However, two initial studies of mepolizumab in asthma failed to demonstrate efficacy. One was a bronchial provocation study in patients with mild allergic asthma^{91,93}, and the other a study of 362 patients with moderate persistent asthma (asthma that was refractory to inhaled corticosteroid (ICS) treatment). Twelve weeks of treatment did not result in improvement in any clinical outcome measures (peak expiratory flow rate, FEV₁, asthma symptom scores or exacerbation rate)⁹⁰ (see BOX 3 for a description of clinical end points for asthma).

In atopic dermatitis, reducing blood and skin eosinophil numbers with mepolizumab had no effect on disease endpoints. Two mepolizumab doses reduced eosinophil numbers in blood (after 2 days) and skin (by day 16) in a study of 43 patients with moderate to severe atopic dermatitis. However, there was no statistically significant improvement on SCORAD, a measure of skin lesion severity, or pruritus (see BOX 2 for a description of clinical end points for atopic dermatitis) compared with placebo at the 2-week time point^{92,94}. This was unexpected because cyclosporin A, an effective therapy, significantly reduces blood eosinophil numbers³¹, and suggests that other mediators contribute to disease pathology.

Given the well-characterized pleiotropic roles of IL-4 and IL-13 in orchestrating the type 2 responses in allergic and atopic diseases, the early failures of blocking these cytokines in asthma were unexpected. Heading into the clinic supported by extensive preclinical rationale, it was expected that blockade of these cytokines would lead to significant effects on lung function and asthma symptoms. Two IL-4 inhibitors (altrakinecept developed by Immunex and pascolizumab developed by GlaxoSmithKline) and several IL-13 inhibitors (including anrukinzumab (IMA-638) and IMA-026, both developed by Wyeth) generated some promising preclinical data as well as suggestive signals in early clinical studies, but these data were not reproduced in further studies. All of these early blockers had slightly different properties: altrakinecept, administered by a nebulizer, is a recombinant soluble IL-4Ra that competitively binds IL-4 (REFS 95,96);

Box 3 | Common clinical symptoms and end points of asthma**Lung function**

- Airway inflammation and obstruction, mucus production and smooth muscle constriction result in impaired lung function
- Expiratory lung capacity determined by forced expiratory volume in the first second of a forced expiration (FEV1) and peak expiratory flow, which are both objective, physiological measurements
- FEV1 is a commonly used end point for bronchodilators or combination agents
- Clinically meaningful improvement on FEV1 ranges from 7 to 10% (placebo-corrected changes)^{140,141}

Airway hyperresponsiveness (AHR)

- AHR is an exaggerated bronchoconstriction response to a normally innocuous airway stimulus, which is probably due to structural changes in the airways
- The provocative concentration of agents that directly induce smooth muscle contraction (such as histamine or methacholine) leading to a decline in lung function is determined via PC20 (the provocative concentration leading to 20% decline in FEV1)^{76,142}
- Tolerating a higher provocative dose is suggestive of improved AHR
- AHR is also assessed in preclinical models of asthma

Asthma Control Questionnaire (ACQ)

- The ACQ is a measure of asthma symptom control including cough, wheeze, chest tightness, shortness of breath and night waking
- It is a validated clinical instrument used to measure the level of asthma control^{120,143,144}
- It comprises seven components: five symptomatic elements (night waking, asthma symptoms, limitation on activities, shortness of breath and wheeze) and two objective elements (FEV1 score and short-acting bronchodilator use)
- Each component is equally weighted and scored from 0 to 6, with 0 indicating well controlled and 6 indicating extremely poorly controlled
- Uncontrolled asthma was defined as a score on the symptom-only version of the ACQ5 of 1.5 or higher, with higher scores indicating poorer control of asthma
- The minimal clinically important difference on the ACQ5 is 0.50 points^{120,144}

Exacerbations

- Episodic worsening of asthma symptoms is associated with decline in lung function and a need for more intense treatment¹⁴⁵
- Exacerbations are associated with inadequate asthma control and eosinophilic inflammation
- They can be classified as mild, moderate or severe exacerbations, or measured on a composite index
- Because episodes are infrequent, allergen provocation or steroid withdrawal clinical study designs are utilized

Allergen challenge or bronchoprovocation

- Mild asthmatics inhale a relevant allergen that mimics an exacerbation resulting in a drop in FEV1
- There is a biphasic allergic response. The early asthmatic response (EAR) is the initial drop in FEV1 lasting 10–30 minutes, following a short recovery, the late asthmatic response (LAR) is a second drop in lung function observed 3–7 hours after allergen administration
- Attenuating the lung function decline associated with LAR is suggestive of efficacy for interventions¹⁴⁶

Steroid withdrawal

- The controlled dose tapering of inhaled corticosteroids leads to destabilization of asthma control and subsequent exacerbation of asthma symptoms¹⁴⁶
- Efficacy is assessed if intervention can prevent frequency of exacerbations compared with placebo

paslizumab is a humanized IL-4-specific mAb; and the two humanized IL-13-specific mAbs anrakinzumab and IMA-026 bind to different epitopes of IL-13 (REF. 97). However, all of these early therapeutic candidates failed to demonstrate efficacy in larger Phase II studies and the programmes were discontinued (sponsor press releases)^{98,99} (TABLE 1).

With the failed attempts of individually targeting IL-4 and IL-13, dual cytokine blockade by targeting the shared IL-4Rα represented a novel approach to modulating type 2 inflammation. Targeting both IL-4 and IL-13 also demonstrated early encouraging data: IL-4/IL-13

Dual Trap (Regeneron), generated as a fusion protein of IL-4Rα and IL-13Rα using proprietary Trap technology¹⁰⁰, reduced type 2 biomarkers in early clinical studies but was not pursued owing to lack of funding. Pitrakinra (also known as AER001 and BAY-16-9996; developed by Aerovance), a recombinant IL-4 variant that binds IL-4Rα with high affinity, also acts as a functional antagonist for both IL-4 and IL-13, and demonstrated efficacy in improving the late-phase FEV1 in an asthma allergen challenge study¹⁰¹ (TABLE 1). A polyethylene glycol (PEG)-modified form of pitrakinra (called Aeroderm) was also the first dual cytokine blocker tested in atopic

Table 1 | Summary of clinical and pharmacodynamics findings

Drug (company)	Patient population	Pharmacodynamic effects	Asthma	AD	CSwNP
IgE					
Omalizumab (Novartis/Genentech)	- Asthma: moderate to severe allergic asthma^{15,16} - AD: moderate AD - CSwNP: NP with asthma¹⁷	↓ Free serum IgE^{15,16,21,147}	- Decreased exacerbations - No effect on FEV1 (REFS 15,16) - Phase III	- No improvement in disease end points²¹ - Phase II	- Reduced polyp size - Improved CT scan and CS symptoms¹⁷ - Investigator-initiated study
IL-5					
Mepolizumab (GlaxoSmithKline)	- Asthma: severe eosinophilic asthma^{113,114} - AD: moderate AD⁹⁴ - CSwNP: severe NP	↓ Blood and tissue eosinophils^{92,94,113,114}	- Decreased exacerbations in subgroup with high sputum eosinophils - Modest effect on FEV1 or ACQ¹¹⁴ - Phase III	- No improvement in disease end points⁹⁴ - Phase II	- Reduced polyp size - Improved CT scan and CS symptoms (two doses)¹⁴⁸ - Phase II
IL-4					
Altrakcept (Immunex)	Asthma: moderate, persistent asthma⁹⁵	No change in blood eosinophils or serum IgE⁹⁵	- Improved FEV1 and asthma symptoms⁹⁵ - Phase I–II	Not tested	Not tested
IL-13					
Anrakinumab (Wyeth)	Asthma: mild stable allergic asthma⁹⁷	↑ IL-13 - No change in blood or sputum eosinophils or serum IgE⁹⁷	- Improved FEV1 and ACQ in allergen challenge study⁹⁷ - Phase II	Not tested	Not tested
Lebrikizumab (Roche)	Asthma: moderate to severe uncontrolled asthma¹¹⁷	↓ Serum IgE ↓ FeNO ↓ TARC and MCP4 ↑ Blood eosinophils¹¹⁷	- Improved FEV1 in a periostin high subgroup - Decreased exacerbations in 'T_H2-high' subgroup¹¹⁷ - Phase II	Not tested	Not tested
IL-4 and IL-13					
Pitrakinra* (Aerovance)	- Asthma: mild allergic or moderate to severe asthma¹⁰¹ - AD: moderate to severe AD¹⁴⁹	↓ Serum IgE¹⁴⁹ ↓ FeNO¹⁰¹ - No change in blood or sputum eosinophils or serum IgE¹⁰¹	- Improved FEV1, decreased exacerbations and improved asthma symptoms in subgroup with high blood eosinophils¹⁰¹ - Phase II	- Trends towards improvement in EASI¹⁴⁹ - Phase II	Not tested
AMG317 (Amgen)	Asthma: moderate to severe uncontrolled asthma¹⁰³	↓ Modest serum IgE¹⁰³	- No significant improvement in ACQ¹⁰³ - Phase II	Unknown	Not tested
Dupilumab (Regeneron/Sanofi)	- Asthma: moderate to severe with high blood eosinophils¹⁷ and broader population¹²² - AD: moderate to severe AD¹²⁶ - CSwNP: moderate to severe	↓ Serum IgE ↓ Serum TARC¹²⁶ ↓ FeNO - No change in blood eosinophils¹²¹	- Decreased exacerbations, improved FEV1 and ACQ¹²¹ - Phase II	- Significant improvements in EASI, IGA, and NRS (pruritus)¹²⁶ - Phase II	- Reduced polyp size - Improved CT scan and CS symptoms¹³⁴ - Phase II

ACQ, asthma control questionnaire; AD, atopic dermatitis; CS, chronic sinusitis; CSwNP, chronic sinusitis with nasal polyps; EASI, eczema area and severity index; FeNO, fractional nitric oxide concentration in exhaled breath; FEV1, forced expiratory volume in the first second; IGA, investigator global assessment; IgE, immunoglobulin E; MCP4, monocyte chemoattractant protein 4; NRS, pruritus numerical rating scale; TARC, thymus- and activation-regulated chemokine; T_H2: T helper 2. *A PEGylated form called Aeroderm is used in AD.

dermatitis. Aeroderm showed trends towards symptom improvement, but did not achieve statistical significance on disease end points¹⁰² (TABLE 1).

These encouraging findings were followed by negative results with AMG317 (Amgen), another dual IL-4 and IL-13 inhibitor. AMG317 is a fully human mAb that binds human IL-4Rα and inhibits the activity of both IL-4 and IL-13. Efficacy of 12 weeks of AMG317 treatment was evaluated in a placebo-controlled study in

patients with moderate to severe atopic asthma ($n = 300$) (TABLE 1). AMG317 failed to improve clinical measures of asthma, including the primary end point asthma control questionnaire (ACQ) (see BOX 3 for a description of ACQ)¹⁰³ and the programme was discontinued (sponsor press release).

Based on available data, there are several potential explanations for the early failures of these type 2 cytokine blockers, including the molecular properties of the

blockers, route of administration, bioavailability and/or incorrect study patient population. For example, in preliminary asthma clinical studies using highly similar patient populations and study designs, anrukinzumab and IMA-026 demonstrated differential efficacy: anrukinzumab, but not IMA-026, attenuated late-phase FEV1 induced by allergen challenge⁹⁷. These data highlight the importance of epitope binding: anrukinzumab binds an epitope on IL-13 that blocks IL-13 binding to IL-4R α , whereas IMA-026 binds at a site on IL-13 that blocks IL-13 binding to IL-13R α 1 and IL-13R α 2 (REF. 97). In the case of pitrakinra, 28 days of nebulized treatment did not affect systemic biomarkers of serum IgE or blood eosinophils; however, 28 days of the subcutaneous formulation of pitrakinra significantly reduced serum IgE levels¹⁰¹. AMG317 demonstrated only modest reduction of serum IgE levels¹⁰³ and is relatively rapidly cleared compared with other fully human mAbs¹⁰⁴. These two examples suggest that the route of administration and suboptimal pharmacokinetics could have affected its efficacy. The failures of early dual IL-4 and IL-13 blockers could be attributed to the suboptimal activity in reducing systemic type 2 biomarkers, suggesting that the selected candidates did not sufficiently block both the IL-4 and IL-13 pathways in humans.

Insight into disease drivers in asthma. In an effort to optimize treatment, researchers and clinicians began attempting to identify patient subsets who had consistent patterns of disease beyond categories of trigger (for example, allergens, aspirin or exercise) or disease severity (for example, mild, moderate or severe disease). Greater granularity in asthma classifications was described based on clinical symptomatology¹⁰⁵ and cluster analysis identified subsets of patients with demographic similarities^{106,107}. Importantly, these efforts determined subsets of patients with similar patterns of inflammation, leading to insight into the diversity of disease pathophysiology and providing enrichment strategies for identifying a potentially responsive patient population when matched to the mechanism of therapeutic intervention. Taken together, the lack of an observable benefit in the early asthma studies with type 2 blockers may be partly attributable to a diluted treatment effect in an unselected patient population.

Inflammatory phenotypes of asthma are based on the presence or absence of immune cells in the lung or blood in patients with otherwise common clinical features. Insight into different inflammatory phenotypes was first observed by examining the differential patient response to corticosteroids. Eosinophil levels (in contrast to neutrophil levels) are often responsive to corticosteroids, yet some patients require very high doses of ICSs or systemic steroids to reduce eosinophilic inflammation¹⁰⁸. These patients were identified as eosinophilic asthma patients. Eosinophilic asthma is identified by the presence of eosinophils in sputum or blood, in contrast to patients with non-eosinophilic or neutrophilic asthma, who have low levels or an absence of eosinophils in lung or blood, or patients with paucigranulocytic asthma, who lack eosinophilic, neutrophilic and lymphocyte inflammation in

the lung^{105,109}. The high levels of eosinophils necessitated the high doses of corticosteroids to reduce eosinophil levels and improve symptoms for these patients.

Molecular evidence to support inflammatory phenotypes came from independent gene expression studies analysing airway epithelial cells from patients with asthma. Patients who were otherwise clinically indistinguishable with regards to lung function impairment and response to bronchodilators could be grouped by gene expression profiles: a subset of patients overexpressed three IL-4 and/or IL-13 inducible downstream genes: periostin (*POSTN*), chloride channel accessory 1 (*CLCA1*) and serpin B2 (*SERPINB2*)^{110,111}. Hierarchical cluster analysis of these three genes demonstrated that half of the patients clustered together with 'T_H2-high' gene expression, whereas the other half clustered with 'T_H2-low' gene expression along with healthy controls.

Further evaluation revealed that the T_H2-high subset of patients with asthma shared similarities in other biomarkers. Patients in the T_H2-high subtype had elevated blood and lung eosinophil numbers, significantly higher serum IgE levels, increased mucus production and evidence of airway structural changes compared with the T_H2-low group, consistent with T_H2-type biology. In addition, patients with T_H2-high asthma had greater AHR in response to methacholine than patients with T_H2-low asthma. Importantly, with ICS use, the T_H2-high group showed significant clinical improvements on FEV1 and molecular changes that were not observed in the T_H2-low group; after 1 week of ICS therapy, patients with baseline T_H2-high gene expression shifted to T_H2-low gene expression¹¹¹. This molecular profile defined a T_H2-high group corresponding to the clinically described eosinophilic asthma, with eosinophilia as a biomarker of this asthma subpopulation, providing the basis for a personalized medicine approach to asthma.

Taken together, the failures of type 2-cytokine-targeting therapeutics in clinical trials contributed to a growing understanding of the highly heterogeneous clinical and pathological nature of asthma, lending support to defining allergic inflammatory diseases, and thus tailoring therapy to the primary disease driver.

Recent advances in targeting IL-5 and IL-13 in asthma.

Targeting eosinophilia with anti-IL-5 therapy was revisited with a personalized medicine approach to asthma. Two groups independently hypothesized that patients with persistent eosinophilia, even with oral steroid treatment, may require a more potent drug to reduce eosinophil numbers and in turn their exacerbation burden. To test the effect of reducing eosinophil levels on exacerbations, two studies targeted IL-5 with mepolizumab using different study designs: one was a steroid withdrawal study design with 20 patients¹¹² and the other a 1-year treatment study of 61 patients¹⁰⁸. Although no significant improvement was attained in FEV1 or asthma symptoms, both studies demonstrated that mepolizumab significantly reduced exacerbations in an uncommon, severe subset of asthma associated with a predominance of eosinophils in sputum (TABLE 1). With renewed interest in anti-IL-5 therapies, the efficacy of mepolizumab was

further explored in eosinophilic-predominant asthma using broadened inclusion criteria to define eosinophilic inflammation. The two subsequent studies replicated the benefit of mepolizumab in reducing the rate of severe asthma exacerbations, and the most recent study also showed modest effects on FEV1 (REFS 113,114). These studies validated eosinophilia as a patient selection marker, and mepolizumab and other clinical programmes featuring similar therapeutic mechanisms have progressed into late-stage development.

Despite the previous failures in targeting IL-13 in asthma, a humanized IL-13-specific mAb, lebrikizumab (developed by Roche), has advanced in development by exploiting the use of type 2-associated biomarkers to enrich for treatment-responsive patients. Similar to anrunkizumab, lebrikizumab neutralizes IL-13 activity by blocking the binding of IL-13 to IL-4R α ¹¹⁵. In an allergen challenge study of 29 patients with mild allergic asthma, 12 weeks of lebrikizumab treatment improved late-phase FEV1 responses. Subset analysis revealed that patients with elevations in markers of type 2 inflammation (IgE, blood eosinophils, periostin (an IL-4 and IL-13 inducible protein)) had a greater response to therapy¹¹⁶ (TABLE 1). Taking this further, the Phase II proof-of-concept (MILLY) study was designed to evaluate the efficacy of lebrikizumab in patients with asthma who were not adequately controlled on ICSs; patients were stratified by T_H2-high status as determined by elevations in IgE and eosinophil levels. Three months of lebrikizumab treatment in addition to stable ICS or long-acting β -agonist (LABA) therapy significantly improved FEV1, the primary end point, in the intent-to-treat population. In addition, patients with type 2 markers showed a greater benefit from lebrikizumab: patients with elevated serum periostin and T_H2-high biomarkers (IgE and blood eosinophils) derived the most treatment benefit on the lung function end point, consistent with the smaller allergen challenge study and confirming the importance of the type 2 and T_H2-type biomarkers (TABLE 1). The combination of IgE and blood eosinophil levels, but not periostin, was also relevant for predicting response on the end point of exacerbation. A significant reduction (60%) in exacerbations was observed at 24 weeks in the T_H2-high subset of patients¹¹⁷. In the subsequent Phase IIb dose-ranging (MOLLY) study in patients not receiving ICSs, lebrikizumab demonstrated bioactivity; however, no clear dose-response effect was detected on the FEV1 end point. Although the drug appears less effective in this study, it is unclear whether this study population was enriched for patients who would be responsive to an IL-13 blocker¹¹⁸.

Recent advances with dual IL-4 and IL-13 blockade in asthma. Building on the lessons learnt from mepolizumab and lebrikizumab in defining asthma subtypes, blood eosinophilia also predicted patient response to pitrakinra (a dual IL-4 and IL-13 inhibitor) in a subset analysis of their Phase II study. Using an ICS and LABA withdrawal study design, 534 patients with moderate to severe asthma who were not fully controlled on current therapy were treated with inhaled dry powder pitrakinra or placebo for 12 weeks. Although there was no

statistically significant reduction in the primary end point of exacerbations in the intent-to-treat population, there was a 74% reduction in exacerbation frequency in a subset of patients with elevated blood eosinophils ($n = 125$). In addition, therapeutic benefits on other asthma clinical end points were noted in this subset (for example, ACQ, daily activities and night awakenings)¹¹⁹ (TABLE 1).

Taken together, the cumulative data showed that patients with asthma with particularly high biomarkers of type 2 inflammation respond particularly well to blockers of type 2 pathways. In particular, the subset analysis of the pitrakinra study data and the growing body of evidence from cluster analyses and gene expression profiling provided a strong rationale that elevated blood eosinophil levels are a marker for high type 2 inflammation in asthma, and that reducing type 2 inflammation with dual IL-4 and IL-13 cytokine blockade may lead to improved clinical benefit compared with blocking individual inflammatory mediators alone. This hypothesis was prospectively confirmed in a Phase II study with dupilumab, which is targeted at IL-4R α and therefore blocks the activity of both IL-4 and IL-13.

In a Phase IIa proof-of-concept study, dupilumab demonstrated significant biological activity in patients with moderate to severe asthma with elevated eosinophil levels, reducing several biomarkers relating to the type 2 pathway and significantly modulating multiple clinical outcomes, including exacerbations, ACQ and FEV1 (TABLE 1). In this steroid and LABA withdrawal study, dupilumab reduced exacerbations by 87% and consistently improved multiple asthma end points, including a clinically meaningful reduction on ACQ¹²⁰ and a significant FEV1 improvement of 10.7% relative to placebo at 12 weeks¹²¹. Benefit on FEV1 was observed with and without background therapy.

Interim results of the most recent and largest asthma Phase IIb study with dupilumab confirmed these results and also demonstrated benefit in the broader patient segment with lower or unelevated blood eosinophil levels. This double-blind, placebo-controlled, 24-week study enrolled 776 adult patients with moderate to severe uncontrolled asthma; approximately 40% of patients had high blood eosinophil numbers (≥ 300 cells per μ l) stratified across the dose groups. With the three highest doses, 12 weeks of dupilumab treatment in combination with standard-of-care therapy met the primary end point by improving lung function (FEV1) and also showed a reduction in severe exacerbations in patients with high blood eosinophil levels¹²². Significant clinical effects were also observed in the overall patient population. Interestingly, approximately 77% of the randomized patients had a history of atopic disease, which included atopic dermatitis, allergic conjunctivitis, allergic rhinitis, chronic rhinosinusitis, nasal polyposis, food allergy and/or hives¹²². A similar overall proportion of patients in the placebo and dupilumab groups experienced adverse events in both studies; the most common adverse events in the dupilumab-treated group were injection-site reactions, nasopharyngitis and headache, with a slightly higher rate of upper respiratory infections in the placebo group compared with the dupilumab group^{121,122}.

These clinical studies established the importance of type 2 inflammation in asthma and showed that the dual blockade of IL-4 and IL-13 results in robust therapeutic effects. The efficacy of targeting IL-4 and IL-13 on multiple disease symptoms raised the possibility that targeting the primary drivers of the type 2 response may modulate multiple downstream mediators, and therefore have an effect on a range of allergic diseases.

Dual IL-4 and IL-13 blockade in atopic dermatitis.

Probing the molecular underpinnings of atopic dermatitis revealed a convergence of all disease subtypes (for example, intrinsic or extrinsic) on the underlying type 2 inflammation. However, the lack of efficacy of single type 2 mediators targeting IgE or eosinophils in atopic dermatitis suggested that the root of disease pathology is driven by broader T_H2-type activation, and that, at least in atopic dermatitis, eosinophilia and IgE may be by-products of T_H2 cell activation rather than bona fide pathogenic mediators of disease. This hypothesis is being supported by recent results targeting IL-4 and/or IL-13.

Aeroderm showed trends toward disease improvement in a randomized placebo-controlled Phase II study of 25 patients with moderate to severe atopic dermatitis^{123,124} (TABLE 1). Although treatment with Aeroderm resulted in fewer episodes of worsening of existing atopic eczema symptoms and showed trends towards improvement in the primary end point of EASI score, these effects did not achieve statistical significance compared with placebo^{102,123,124}.

Dupilumab induced reductions in TARC levels in normal healthy volunteers in a Phase I study¹²⁵, providing a rationale for exploring its efficacy in patients with atopic dermatitis. Dupilumab was subsequently tested in four clinical studies in atopic dermatitis¹²⁶ (TABLE 1). In two Phase I studies in patients with moderate to severe atopic dermatitis not adequately controlled with topical medications, approximately 60% of patients achieved a 50% reduction in EASI score (EASI50) after 4 weeks of therapy with dupilumab compared with 19% of patients treated with placebo¹²⁶. When the treatment duration was extended to 12 weeks in a Phase IIa study, 85% of patients on dupilumab achieved EASI50 and 62% achieved EASI75 (compared with 35% and 15% for placebo, respectively). In both studies, a higher proportion of patients treated with dupilumab were clear or almost clear of disease (investigator global assessment score of 0–1)¹²⁶ (BOX 2). The severity of itch was also significantly affected, with a 30% reduction of pruritus numerical rating scale score within 2 weeks of dupilumab treatment (300 mg) in both the 4-week and 12-week studies; by 12 weeks the itch score was reduced further to 56% compared with 15% on placebo¹²⁶. Finally, patients on dupilumab for 12 weeks experienced fewer skin infections compared with patients on placebo, 3 (5%) versus 13 (24%), demonstrating skin barrier improvement¹²⁶. Adverse events occurred with a similar frequency in the placebo and dupilumab groups, and most were mild to moderate. Nasopharyngitis and headache were the most frequent adverse events with dupilumab, whereas skin infection occurred more frequently with placebo¹²⁶.

In a separate 4-week Phase IIa study, patients with moderate to severe disease ($n = 31$) who were concomitantly treated with topical corticosteroids (TCSs) and dupilumab achieved EASI50, and 62% of patients achieved EASI75 on combination therapy compared with placebo (50% and 40%, respectively). Similarly, substantial improvements in itch scores were observed with combination therapy: patients receiving TCSs in combination with dupilumab experienced a 71% reduction in pruritus compared with a 25% reduction with placebo. In addition, patients receiving dupilumab used 50% less TCSs than the placebo group¹²⁶. Preclinical data show that excess IL-4 impairs the cellular response to glucocorticoids^{127,128}. Hence, blocking IL-4 may enhance the efficacy of glucocorticoids, providing a mechanistic explanation of reduced use of TCSs in the dupilumab-treated group.

In response to dupilumab treatment, changes in serum type 2 biomarkers and skin lesional gene expression reflected improvements in clinical disease end points. Consistent with reports that TARC correlates with disease severity^{33–35}, a reduction in TARC levels corresponded with treatment response in all dupilumab studies in atopic dermatitis¹²⁶. Effects on serum IgE levels were slower, with the rate of IgE reduction increasing after 4 weeks of therapy¹²⁶. Thus, the onset of action of dupilumab was independent of IgE. Patients prospectively stratified by baseline IgE levels (reflecting intrinsic versus extrinsic disease) all responded similarly to dupilumab in the 12-week monotherapy study¹²⁶, providing pharmacological validation that IgE is not a pathogenic driver and that intrinsic and extrinsic forms of atopic dermatitis have similar molecular profiles. In addition, after 4 weeks of treatment, there was a downregulation of gene expression in lesional skin of patients treated with dupilumab, moving towards the gene expression profile of the non-lesional skin^{126,129}. The magnitude of these changes corresponded to the reduction in EASI¹²⁶. Taken together, these data provide clinical and molecular evidence that all atopic dermatitis is type 2 driven, and that atopic dermatitis pathology is independent of IgE. Lebrikizumab and tralokinumab (developed by AstraZeneca), which both target IL-13, are also being evaluated in atopic dermatitis, which will further discern the contribution of IL-13 in atopic dermatitis.

Dual IL-4 and IL-13 blockade in CSwNP. The principle of a personalized medicine approach using type 2 blockers has also been applied to patients with CSwNP. The currently limited treatment options include nasal steroids or aggressive treatment with radical sinus surgery. However, similar to other type 2 diseases, oral corticosteroids (OCSs) are also effective in reducing nasal polyposis and associated symptoms (facial pain, pressure, congestion and altered sense of smell)¹³⁰, demonstrating the systemic nature of the disease. However, because OCSs cannot be used long term, polyps often return shortly after therapy cessation¹³¹.

Mepolizumab, omalizumab and dupilumab have all demonstrated efficacy in CSwNP, supporting the hypothesis that type 2 activation is a disease driver and that nasal polyps, an outgrowth of sinonasal mucosa

containing eosinophilic inflammation, serve as visual biomarker of type 2 inflammation^{40,41}. In a chronic sinusitis patient population not selected by the presence of nasal polyps, 6 months of anti-IgE treatment was not effective in improving symptoms or measures of sinus inflammation¹³². However, in patients with CSwNP and co-morbid asthma, 16 weeks of anti-IgE therapy reduced nasal polyp size and improved several symptoms (that is, nasal congestion, loss of smell and dyspnoea) compared with placebo¹⁷, confirming that the eosinophil-rich polyps are a measurable and stable biomarker of type 2 inflammation. Targeting eosinophilic inflammation with anti-IL-5 agents is also a logical approach in this disease setting. Indeed, two doses of mepolizumab over 8 weeks led to significant improvement in reducing nasal polyp size in patients with severe nasal polyposis¹³³. Extending these results, simultaneous targeting of IL-4 and IL-13 with 16 weeks of dupilumab treatment significantly reduced nasal polyp size, as measured by endoscopic end points¹³⁴. Treatment also led to significant improvements in sinusitis as shown by objective measures, such as CT scan, and patient-reported symptoms (that is, sense of smell, congestion, postnasal drip, runny nose and sleep disturbance). The safety findings were consistent with previous dupilumab studies; the most common adverse events with dupilumab were injection-site reactions, nasopharyngitis, oropharyngeal pain, epistaxis, headaches and dizziness¹³⁴.

Conclusion

A paradigm shift in defining allergic inflammatory diseases is emerging. As in cancer therapy, focusing on the underlying pathway driving disease rather than apparent tissue manifestations can lead to tailored therapies

that curtail the root cause of pathology. Thus, treating immunological diseases by targeting their driver pathway should enable application across multiple diseases. Reflecting this approach, specific type 2 mediators have been targeted in several allergic inflammatory diseases that have disparate tissue-specific manifestations but share type 2 hallmarks, such as eosinophilia and/or high IgE. Despite the initial setbacks targeting type 2 cytokines in asthma, the therapeutic benefit of targeting individual type 2 mediators (that is, IgE, IL-5 and IL-13) has now been established in subsets of patients with asthma who match the therapeutic mechanism. Importantly, examining the contribution of type 2 mediators in a broader asthma population using a potent dual blocker of IL-4 and IL-13 revealed that type 2 inflammation is an underlying disease driver across the severe asthma population. In contrast to the early clinical failures targeting individual type 2 mediators in a broad asthmatic population, recent data with dual IL-4 and IL-13 blockade confirmed the presence of type 2-induced pathology in asthma. Beyond asthma, the efficacy of IL-4 and IL-13 blockade in two additional allergic inflammatory diseases — atopic dermatitis and CSwNP — supports the hypothesis that targeting the drivers of type 2 inflammation could have a therapeutic effect in multiple allergic diseases. This approach demands further investigation in additional allergic diseases such as chronic idiopathic urticaria, eosinophilic oesophagitis, eosinophilic predominant chronic obstructive pulmonary disease, food allergy, and allergic rhinitis. The advent of potent inhibitors with different or improved molecular properties may further optimize type 2-targeting therapeutics and provide new personalized treatment strategies to stem the tide of allergic disease.

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Competing interests statement

The authors declare **competing interests**: see Web version for details.