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Abstract

Hypereosinophilic syndromes (HESs) are a diverse group of conditions characterized by clinical manifestations attributable to eosinophilia and eosinophilic infiltration of tissues. HESs are chronic disorders with significant morbidity and mortality. Although the availability of targeted chemotherapeutic agents, including imatinib, has improved quality of life and survival in some patients with HESs, additional agents with increased efficacy and decreased toxicity are sorely needed. The purpose of this review is to provide an overview of eosinophil biology with an emphasis on potential targets of pharmacotherapy and to provide a summary of potential eosinophil-targeting agents, including those in development, in clinical trials, or approved for other disorders.

Keywords

Hypereosinophilic syndromes; eosinophil-associated gastrointestinal disorders; eosinophilic esophagitis; Churg-Strauss syndrome; IL-5; mepolizumab; reslizumab

Characterized by marked eosinophilia in the peripheral blood, tissue, or both without a secondary cause, hypereosinophilic syndromes (HESs) are a heterogeneous group of disorders in which eosinophils are believed to play a primary role in disease pathogenesis, including idiopathic HESs, Churg-Strauss syndrome (CSS)–related vasculitis, and eosinophil-associated gastrointestinal disorders (EGID). Although recent data from a multicenter retrospective study of 188 patients with different HESs suggest that corticosteroids are effective initially in most patients with these disorders,[1] a majority of patients become corticosteroid refractory or experience significant corticosteroid toxicity. Conventional second-line agents, including hydroxyurea and IFN-α, are only effective in approximately 30% of patients and have undesirable side effect profiles. Thus better agents are clearly needed to treat patients with these disorders.

Recent advances in drug design have led to the creation of a wide variety of agents that target specific molecules involved in disease pathogenesis. For example, imatinib, a tyrosine kinase inhibitor developed for the treatment of chronic myelogenous leukemia, has revolutionized the treatment of patients with *PDGFRA*-associated myeloproliferative neoplasms (MPNs; a myeloproliferative form of HES) and became the first US Food and

Drug Administration–approved treatment for HESs. However, development of clinical trials for these rare eosinophilic disorders is challenging because of the paucity of potential study subjects at any single center. For instance, the participation of 22 centers in Europe, Australia, and the United States was required to provide the 84 patients necessary for a placebo-controlled, randomized trial that demonstrated that mepolizumab (an mAb against IL-5 initially developed for the treatment of asthma) is effective and well tolerated in the treatment of corticosteroid-responsive HESs.[2] As more and more targeted agents become available, it will become increasingly difficult to design studies with adequate numbers of patients to determine their safety and efficacy in the treatment of rare eosinophilic disorders.

On the basis of a workshop cosponsored by the National Institutes of Health and industry partners that was held in conjunction with the biennial symposium of the International Eosinophil Society, the purpose of this review is to provide an overview of eosinophil biology with emphasis on potential targets of pharmacotherapy and to provide a summary of potential eosinophil-targeting agents, including those in development, in clinical trials, or approved for other disorders. Although the main focus of the review is on therapeutic approaches in HESs, the concepts discussed are applicable to other disorders in which eosinophils are partially or entirely responsible for disease pathogenesis.

EOSINOPHIL BIOLOGY AND TARGETS FOR EOSINOPHIL-MEDIATED DISORDERS

Eosinophil ontogeny

As is true of all hematopoietic cells, eosinophils differentiate from $CD34^+$ multipotential myeloid progenitors in the bone marrow. These myeloid progenitors give rise to $CD34^+$ cells that also express GATA-1 and IL-5 receptor (IL-5R) α.[3,4] The $CD34^+$ IL-5Rα$^+$ eosinophil-committed progenitors undergo further development in response to IL-3, GM-CSF, and IL-5, the most lineage-specific of the cytokines involved in eosinophil hematopoiesis.[3,5] It is because of this lineage specificity that therapies targeting IL-5 and IL-5R have received the most attention to date (see below). The presence of distinct sets of transcription factors at key time points is also necessary for eosinophil maturation.[6,7] For example, commitment and terminal differentiation of eosinophils from myeloid progenitors requires concomitant expression of C/EBPα, PU.1, and a low-to-moderate level of GATA-1, with no expression of FOG-1. The transcription factor interferon consensus sequence binding protein has also been shown to play a role in eosinophil differentiation, as evidenced by decreased numbers of eosinophil progenitors in interferon consensus sequence binding protein–deficient mice.[8] Once a progenitor is committed to the eosinophil lineage, C/EBPε is required for terminal differentiation and functional maturation.[9] Survival of mature eosinophils can be influenced both positively and negatively by a variety of cytokines and other mediators, including IL-5, CCR3, and other molecules for which targeted therapies are currently in development (see below).[6,10]

Eosinophil surface phenotype

Beyond their unique granular, nuclear, and tinctorial properties, eosinophils can be distinguished from other granulocytes by a variety of cell-surface markers, including the potential therapeutic targets CD16, CD28, CD49d, (very late antigen [VLA] 4α chain), IL-5Rα (CD125), Siglec-8, EMR1, and FcεRIα (Fig. 1).[11] Cell-surface markers expressed on eosinophils that regulate cell recruitment and activation include CD193 (CCR3), C3a receptor, cysteinyl leukotriene type I receptors, platelet-activating factor receptor, and DP2, the type 2 prostaglandin D_2 receptor otherwise known as CRTH2. A variety of inhibitory receptors that regulate eosinophil survival and activation have also been described. These include Siglec-8, CD300a, killer activating receptor, potassium inwardly rectifying channel,

delta-like notch ligand 3, FcγRIIb, signal-regulatory protein α, paired immunoglobulin-like receptor B, and CD85a/leukocyte immunoglobulin-like receptor 3.[12] An extensive comparative list of receptors on eosinophils and other cells has been published previously.[13]

Eosinophil mediators and functions

Human eosinophils are sources of a multitude of mediators of inflammation and immune responses. Lipid mediators produced by eosinophils include leukotriene C_4, platelet-activating factor, and eoxins.[14,15] Eosinophil granules contain 4 principal cationic proteins, major basic protein, eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase, which can be secreted, causing significant tissue damage.[16] Eosinophils are also notable for their content of preformed cytokine and chemokine proteins,[17] which are stored within eosinophil granules and secretory vesicles.[18] Among the more than 3 dozen cytokines known to be produced by eosinophils are those with T_H2 (eg, IL-4 and IL-13), T_H1 (eg, IFN-γ), and immunomodulatory (eg, TGF-β) activities.[18] The majority of these mediators and cytokines are released in response to eosinophil activation. Consequently, any therapeutic intervention that reduces the number of eosinophils, their state of activation, or both has the potential to limit disease pathogenesis. This approach opens the door to less lineage-specific modalities, such as anti-IgE and alemtuzumab (anti-CD52), which might have an indirect effect on eosinophilia and eosinophil activation.

Therapeutic approach to eosinophilic disorders

In recent years, the concept of HESs has expanded beyond the previously defined "idiopathic HESs" to include a diversity of disorders in which eosinophils and eosinophil activation are believed to play a primary role in disease pathogenesis (Table I).[19,20] These include (1) forms of HESs previously considered idiopathic for which the causes are now known, including *PDGFRA*-associated MPNs[21] and lymphocytic variants of HESs, in which the eosinophilia is driven by eosinophilopoietic cytokine production by T cells that are clonal and/or express an aberrant surface phenotype, most commonly $CD3^{-}CD4^{+}$[22]; (2) eosinophilic syndromes restricted to specific organs, such as EGIDs and eosinophilic pneumonias; (3) defined syndromes in which eosinophilia and eosinophil-associated pathogenesis are central to the diagnosis, such as CSS; and (4) hereditary disorders characterized by eosinophilia.

As has been the case with imatinib-responsive HESs, in which specific targeting of abnormal eosinophils and eosinophil precursors in *PDGFRA*-associated MPNs has dramatically altered the prognosis of patients with this disorder, therapeutic approaches specifically targeting eosinophil production, activation, migration, and/or survival have the potential to prevent end-organ manifestations and improve the quality of life of patients with a wide variety of eosinophilic disorders. Below we provide an overview of currently available targeted therapies with potential activity in eosinophilic disorders, as well as those in clinical trials and pre-clinical development (Fig. 1).

AGENTS CURRENTLY AVAILABLE OR IN CLINICAL TRIALS

IL-5–related targets

Targeting IL-5 (or IL-5R) is an appealing approach to the treatment of patients with all types of HESs, given the specificity of this cytokine for the eosinophil lineage and the assumption that tissue damage in patients with HESs is directly related to the presence of activated eosinophils. Anti–IL-5 antibodies target eosinophils by binding to IL-5, interfering with its ligation to IL-5Rα expressed on the eosinophil membrane. Two different humanized anti–

IL-5 antibodies, mepolizumab and reslizumab, have been developed and have shown efficacy in clinical trials for asthma[23–25] and HESs.[2,26]

Mepolizumab—Mepolizumab (anti–IL-5; GlaxoSmithKline, Research Triangle Park, NC) is a humanized mAb (IgG_1) constructed by grafting complementarity-determining regions from a parent murine anti–IL-5 mAb into human heavy and light chain frameworks. Mepolizumab has been investigated for the treatment of asthma, atopic dermatitis (AD), *FIP1L1/PDGFRA*-negative HESs, eosinophilic esophagitis (EoE), nasal polyposis, and CSS.

The therapeutic efficacy of mepolizumab in patients with HESs was first evaluated in the setting of small compassionate-use (open-label) studies. Three patients with corticosteroid-refractory eosinophilic dermatitis with marked peripheral eosinophilia (2 of whom met the criteria for an HES based on the absolute eosinophil count in peripheral blood) experienced clinical improvement (in both skin lesions and pruritus) and reduction in blood eosinophil levels after a 750-mg mepolizumab infusion.[27] The improvement persisted for 17 months after a second infusion in one patient, with significant clearance of skin-infiltrating eosinophils.

In another open-label study[28] mepolizumab was administered (10 mg/kg every 4 weeks) to 3 patients with HESs and 1 with severe refractory EoE after an 8-week run-in period during which maintenance therapy (including systemic corticosteroids in all patients combined with other agents in 3 cases) was carefully tapered to allow disease to begin to flare. In all 3 patients with HESs, mepolizumab was effective in reducing eosinophilia on a background of reduced doses of maintenance therapy. Moreover, eosinophil-mediated clinical complications involving various tissues regressed (including dermatitis, nasal polyposis and associated congestion, and constitutional symptoms) and lung function improved in all 3 cases. The patient with EoE demonstrated reduced dysphagia and vomiting in association with reductions in both blood and esophageal tissue eosinophil counts.

Given the encouraging results of these pilot studies, a double-blind, placebo-controlled clinical trial was designed to evaluate the efficacy of mepolizumab as a corticosteroid-sparing agent in patients with *FIP1L1/PDGFRA*-negative HESs.[2] Eighty-five subjects with stable symptoms and eosinophil levels receiving daily corticosteroid monotherapy (prednisone at 20–60 mg/d) were randomized to receive 750 mg administered intravenously or placebo every 4 weeks for 36 weeks. Daily prednisone doses were progressively tapered according to a predefined algorithm based on both eosinophil levels and clinical manifestations. The primary end point (ie, maintenance of disease control with ≤10 mg/d prednisone for a period of ≥8 consecutive weeks) was achieved in a significantly higher proportion of subjects who received mepolizumab compared with those receiving placebo (84% vs 43%, $P < .001$). This steroid-sparing benefit was also supported by additional exploratory analyses that showed a significant reduction in the mean dose of prednisone at the end of the study (6.2 ± 1.9 mg in the mepolizumab group vs 21.8 ± 1.9 mg in the placebo group, $P < .001$) and more subjects able to discontinue prednisone until study's end (47% on mepolizumab vs 5% in the placebo group, $P < .001$). Importantly, mepolizumab was well tolerated and effective with repeated dosing over 9 months. Long-term safety was demonstrated in an open extension of this clinical trial.[29] Two subsequent open-label studies in patients with CSS corroborated mepolizumab's efficacy by demonstrating safe reduction of corticosteroid dosing and reduction in CSS exacerbations.[30,31]

Overall, these studies support a beneficial treatment effect of mepolizumab in patients with different forms of HESs and good tolerability with extended and repeated dosing. Of note, patients with both normal and increased serum IL-5 levels before treatment responded to mepolizumab.[27,28,32] Furthermore, a spectrum of HES disease variants were included in

these studies and might benefit from treatment with mepolizumab, including patients with truly idiopathic HESs, lymphocytic variant HESs, EoE, eosinophilic pneumonia, and eosinophilic gastrointestinal disease.[2,27,28,32–35] A response was even observed in 1 patient with a *FIP1L1/PDGFRA* rearrangement,[32] although it is unanimously agreed that imatinib should be first-line therapy for patients with *PDGFRA*-associated MPNs.

Not only do eosinophil counts decrease in response to mepolizumab treatment, but those eosinophils that remain are less activated, less able to respond to stimuli, or both. Indeed, blood eosinophils after treatment with mepolizumab undergo less shape change (a marker of activation) in response to eotaxins compared with eosinophils from the same patients obtained before mepolizumab treatment (the latter respond to eotaxins in a similar fashion to eosinophils from healthy control subjects).[32] Furthermore, serum ECP and EDN levels decrease after mepolizumab infusions.[2,27] These findings might account for the dramatic regression of both symptoms and numbers of tissue eosinophils in patients with EoE after mepolizumab.[35] Some have also speculated that anti–IL-5 therapy might interfere with an endogenous autoregulatory IL-5 pathway, as suggested by the observed increase in IL-5R expression on eosinophils after mepolizumab treatment and increased production of IL-5 by T cells *in vitro*.[32] Together with the increase in serum IL-5 levels that occurs in some patients during anti–IL-5 treatment,[26,32] these experimental findings have fueled concern that interruption of mepolizumab could result in uncontrolled IL-5–mediated inflammation. However, it has been shown that the IL-5 levels measured in patients' sera after mepolizumab treatment is actually part of a complex with anti–IL-5 antibodies,[32] the functional relevance of which remains unknown.

Reslizumab—Reslizumab (SCH55700, Cinquil; anti–IL-5; Teva Pharmaceuticals, Petah Tikva, Israel) is a humanized anti-human IL-5 mAb in clinical development for the treatment of eosinophilic inflammatory disorders, such as EoE and asthma. Reslizumab has high affinity for human IL-5 (dissociation constant = 20 pmol/L) and inhibits the IL-5–dependent proliferation of the human erythroleukemic cell line TF-1 (inhibitory concentration of 50% = 45 pmol/L). In experimental animal models reslizumab has been shown to inhibit the development of pulmonary eosinophilia, bronchoconstriction, cutaneous eosinophilia, and esophageal eosinophilia.[36] Reslizumab has been evaluated in randomized controlled clinical trials in patients with asthma,[25,37] nasal polyps,[38] and EoE, as well as a small open-label compassionate-use study in 8 subjects with treatment-refractory HESs or eosinophilic gastroenteritis with peripheral eosinophilia.[26,39] The terminal half-life of reslizumab in asthmatic patients was approximately 25 days, and reslizumab doses of 0.3 mg/kg or greater resulted in a rapid decrease in peripheral blood eosinophilia. Maximal suppression of blood eosinophilia was observed at a dose of 1.0 mg/kg and was sustained for at least 4 weeks after dosing. Blood eosinophil counts returned to baseline values within 5 to 6 months after the dose, without any evidence of rebound eosinophilia in the placebo-controlled studies. In all studies reslizumab was well tolerated, with an adverse event profile comparable with that of placebo.

Initial asthma studies demonstrated no improvement in FEV_1 or other pulmonary function test parameters in response to reslizumab. However, these studies did not select patients based on the presence of eosinophils in the blood, sputum, or any other tissue compartment. *Post hoc* analyses of subjects with baseline sputum eosinophil levels of 3% or greater did demonstrate a mean increase in FEV_1 of 0.29 L in subjects receiving 1.0 mg/kg reslizumab compared with a decrease of 0.04 L in subjects receiving placebo ($P < .05$). In subjects with baseline eosinophil levels of less than 3%, there was no difference in the change in FEV_1 in the 1.0 mg/kg reslizumab group versus the placebo group.[37]

The *post hoc* analysis of patients with increased baseline sputum eosinophil counts suggests that further clinical trials of reslizumab should be focused on patients with documented end-organ eosinophilia. One such trial, a phase 2 study evaluating the safety and efficacy of reslizumab in subjects with severe asthma and sputum eosinophil levels of 3% or greater, recently demonstrated significantly greater reductions in sputum eosinophil counts, improvements in airway function, and a trend toward greater asthma control in patients receiving reslizumab compared with those receiving placebo.[25] These findings have prompted multiple phase 3 asthma studies that are currently underway.

In a small open-label study of HESs and eosinophilic gastroenteritis, a single 1 mg/kg dose of reslizumab was effective in suppressing eosinophilia and clinical symptoms for up to 12 weeks in 2 of 4 subjects with treatment-refractory HESs, one of whom was subsequently found to have the *FIP1L1/PDGFRA* fusion gene,[39] and in 4 of 4 subjects with eosinophilic gastroenteritis and peripheral eosinophilia.[26] In a recently reported phase 2 dose-ranging study in children with EoE, reslizumab significantly reduced intraepithelial esophageal eosinophil counts. However, improvements in symptoms were observed in all treatment groups (including the placebo group) and were not associated with changes in esophageal eosinophil counts, perhaps related to limitations in reporting patient-related outcomes.

Benralizumab—Benralizumab (MEDI-563; MedImmune, Gaithersburg, Md) is a humanized mAb ($IgG_1\kappa$) that binds to human IL-5Rα, resulting in inhibition of IL-5–mediated receptor activation. The binding site of benralizumab on IL-5Rα is in proximity to the IL-5 binding site, further explaining its neutralizing activity.[40] Benralizumab is produced in Chinese hamster ovary cells deficient in the enzyme α 1,6 fucosyltransferase (FUT8)[41]; as a result, benralizumab is not fucosylated. This enhances the binding of benralizumab to human FcγRIIIa, leading to enhanced antibody-dependent cell-mediated cytotoxicity (ADCC). Benralizumab, when tested *in vitro* using natural killer (NK) cells as effector cells and purified eosinophils or basophils as target cells, induces apoptosis of both target cell types with approximately 1 pmol/L potency. The fucosylated parent anti-IL-5Rα control antibody did not induce ADCC of eosinophils or basophils to greater than background levels. Benralizumab-induced eosinophil apoptosis was not associated with eosinophil degranulation, as measured by the release of EDN or ECP.[40]

In a phase 1 clinical trial in patients with mild asthma receiving benralizumab, mean peripheral eosinophil counts decreased in a dose-dependent fashion.[42] Eosinopenia lasted greater than 12 weeks in the highest-dose groups. Serum ECP levels were reduced 24 hours after the dose. The most frequently reported adverse events were reduced white blood cell counts, nasopharyngitis, and increased blood creatinine phosphokinase levels. The pharmacokinetics of benralizumab were dose proportional at doses of 0.03 to 3 mg/kg. Single escalating doses of benralizumab had an acceptable safety profile and resulted in marked reduction in eosinophil counts within 24 hours after dosing.

Current studies with benralizumab are focused on asthma. Ongoing clinical trials are investigating the ability of benralizumab to deplete airway eosinophils and the safety characteristics of multiple subcutaneous doses. The efficacy and safety profiles from these studies will be an important prelude to the future use of benralizumab in HESs. Whereas anti–IL-5 therapy for HESs can be effective by “starving” eosinophils by neutralizing an important growth factor,[2,27,39] benralizumab directly targets eosinophils for ADCC. Therefore benralizumab is expected to also deplete eosinophils with relatively low levels of IL-5Rα expression and those that have become independent of IL-5 as a survival factor. This raises the intriguing possibility that benralizumab will be more effective in depleting tissue eosinophils through ADCC compared with passive depletion by means of IL-5

inhibition, which might result in better efficacy in diseases characterized by eosinophilic inflammation, such as asthma and HESs.

T-cell targets

Eosinophilia caused by activated T cells and their mediators, particularly IL-5, is common and can be observed in patients with allergic diseases, autoimmune diseases, lymphoproliferative forms of HESs, and T-cell lymphomas.[43] Therefore targeting T cells might be a promising therapeutic strategy in a subgroup of eosinophilic disorders.

Alemtuzumab—Alemtuzumab (Campath-1H; anti-CD52; Bayer, Leverkusen, Germany) is an $IgG_1\kappa$ mAb reactive with CD52, a 21- to 24-kd cell-surface glycoprotein present on T and B cells, most monocytes, macrophages, NK cells, and eosinophils. It is currently approved for the treatment of B-cell chronic lymphocytic leukemia. Potential mechanisms of action of alemtuzumab in patients with HESs include direct destruction of eosinophils by means of ADCC or opsonization with removal by the reticuloendothelial system, indirect reduction of eosinophilia through its effect on lymphocytes, or both. Alemtuzumab carries a US Food and Drug Administration boxed warning because of severe cytopenias, potentially fatal infusion reactions, and an increased risk of severe and/or opportunistic infections.

Published data on the use of alemtuzumab for the treatment of eosinophilic disorders are limited to individual case reports and one series of 11 patients. The first case report was of a 39-year-old woman with eosinophilia, a pruritic rash, fever, and malaise.[44] This patient underwent bone marrow transplantation, and as a consequence, alemtuzumab, 30 mg administered subcutaneously every 3 weeks, was found to control eosinophilia. This beneficial effect was maintained for 2½ years. A second case report described the effectiveness of alemtuzumab for control of lymphocytic variant HESs associated with a $CD3^-CD4^+$ lymphocyte clone.[45] A third case was a patient with HESs and multiple myeloma who responded to alemtuzumab for 2 years before becoming resistant.[46] Lastly, 2 brief recent reports comment on the ability of alemtuzumab to reverse encephalopathy associated with HESs and to resolve cardiac wall thickening and tethering of the mitral valve associated with HESs (as determined by using electrocardiography-gated cardiac magnetic resonance imaging).[47,48]

The case series describes 11 patients with eosinophilia of greater than 1.5×10^9/L who were treated with alemtuzumab; 2 of these 11 patients had karyotypic abnormalities and qualified for the diagnosis of chronic eosinophilic leukemia.[49] Seven were men with a median age of 64 years. These patients had received glucocorticoids, imatinib, IFN-α, dasatinib, and nilotinib previously and were treated with alemtuzumab by using intravenous or subcutaneous regimens. Ten of the 11 patients achieved complete hematologic remission at a median time of 2 weeks, and this was maintained for a median time of 3 months, with a range from 1.5 to 17 months. In 1 patient bone marrow aspirates before and after alemtuzumab showed a striking difference in cells staining with CD52 and CD123. Of the 10 patients achieving complete hematologic remission, 7 relapsed; subsequently, complete hematologic remission was achieved in 2 of these patients after retreatment. Three of the 11 patients had mild transfusion reactions, 2 had cytomegalovirus reactivation requiring treatment, and 1 had lymphoma. Five of 11 died during follow-up, including 2 who achieved complete hematologic remission, one with renal failure and one with thyroid cancer, and 3 who relapsed off treatment, one with fungal pneumonia, another with intractable diarrhea, and a third with complications of other treatments.

Alefacept—Alefacept (Amevive; ASP0485; CD2-binding fusion protein; Astellas Pharma, Deerfield, Ill) is a fusion protein composed of the first extracellular domain of lymphocyte

function–associated antigen 3 (CD58) and the human IgG_1 Fc domain.[50] Binding of the lymphocyte function–associated antigen 3 fragment to CD2 blocks costimulation and activation of T cells.[50] Furthermore, by binding to CD2 and the FcγR receptors, particularly FcγRIII (CD16), alefacept mediates cognate interaction between T cells and NK cells, resulting in T-cell apoptosis.[51,52] In patients with psoriasis, alefacept decreased the number of memory $CD4^+$ and $CD8^+$ cells, as well as activated ($CD25^+$) T cells, in lesional skin and synovial tissue.[53,54]

Although there are no reports of the use of alefacept for the treatment of systemic eosinophilic disease, reduction of eosinophilia in the blood and skin of 10 patients with AD treated with alefacept has beendescribed.[55] Alefaceptdecreasedthe numberand activation of peripheral blood T cells in patients with AD. Skin biopsy specimens revealed a significant reduction in dermal infiltrating cell counts and cytokine expression, particularly IL-5 and IL-13. The reduction of B cells and eosinophils in blood and skin was probably a secondary effect because of decreased numbers, activation, and cytokine expression of T cells. More importantly, clinical improvement was observed in all 10 patients.[55] In a second pilot study of alefacept in patients with AD, symptoms were reduced in 6 of 9 patients.[56] Unfortunately, manufacture of alefacept was discontinued in December 2011 (www.amevive.com).

T_H2 targets

Omalizumab—Omalizumab (anti-IgE; Novartis/Genentech, South San Francisco, Calif) is a recombinant therapeutic mAb against IgE approved for use in the treatment of allergic asthma. Omalizumab bindsto IgE and prevents its binding to FcεRI, leading to inhibition of mast cell and basophil activation.[57] Omalizumab can also affect dendritic cell function by downregulating FcεRI. Although the action of anti-IgE on immediate hypersensitivity is well established, less is known about the effects of anti-IgE therapy on other important inflammatory cells, such as the eosinophil.

Both bronchial and sputum eosinophilia were reduced in asthmatic patients after 16 weeks of omalizumab treatment, despite a lack of improvement in methacholine PC_{20}.[58] In a second study patients with mild asthma treated with 12 weeks of omalizumab demonstrated a significant reduction in $EG2^+$ cell staining in the lung submucosa coincident with a reduction in sputum eosinophil counts.[59] In contrast to the earlier study, there was a significant improvement in FEV_1 and peak flows. In a meta-analysis omalizumab reduced circulating levels of blood eosinophils in asthmatic patients receiving concomitant corticosteroid therapy.[60] Overall, these clinical data suggest that omalizumab is able to modulate eosinophil counts in blood and sputum and within the lung.

The mechanism by which anti-IgE modulates eosinophil recruitment, the extent of the reduction and its relationship to efficacy are areas of active investigation. The demonstration of this antieosinophil activity has focused on atopic diseases. Although approved for asthma, given the modest clinical trials experience with anti-IgE therapy of eosinophilic diseases, the use of omalizumab as an antieosinophil drug should be considered investigational at present.

Pitrakinra—Pitrakinra (AER001; BAY 16-9996; IL-4/IL-13 receptor antagonist; Aerovance, Berkeley, Calif) is a recombinant variant of IL-4 with 2 point mutations (position 121 mutated from arginine to aspartic acid and position 124 mutated from tyrosine to aspartic acid). Although pitrakinra binds to IL-4 receptor (IL-4R) α, signal transduction does not occur, and the molecule acts as a competitive antagonist of both IL-4 and IL-13 because IL-13 binds to a receptor composed of a dimer of IL-4Rα and the IL-13 receptor.

Two double-blind, placebo-controlled allergen challenge studies have been performed to examine the efficacy of pitrakinra in asthmatic patients.[61] One used subcutaneous delivery

(once-daily treatment with 12 subjects in each group), and the other used nebulized delivery (twice-daily treatment with 15 subjects in each group). After baseline allergen challenge, subjects were treated for 1 month before a second allergen challenge. The primary outcome measure for both studies was the change in FEV_1 over the 4- to 10-hour period (late response). In both studies the pitrakinra-treated group had a reduced late-phase response, with a 3.0- to 3.7-fold reduction in the decrease in FEV_1 (although this achieved a significance of <.05 only in the inhalation group). There was no effect on the early response or airway hyperresponsiveness. There was a significant reduction in baseline exhaled nitric oxide levels after treatment with pitrakinra but no effect on postchallenge exhaled nitric oxide levels, sputum or blood eosinophilia, or total IgE levels.

As a result of the 2 initial studies, a large, multicenter, placebo-controlled trial was conducted to assess the efficacy of pitrakinra in preventing asthma exacerbations in patients with moderate-to-severe asthma.[62] There was a significant reduction in exacerbations in a prespecified subgroup of patients with a high peripheral blood eosinophil count. Pitrakinra also demonstrated a significant interaction between anti–IL-4Rα therapy and *IL4RA* gene variation, identifying pharmacogenetically a subgroup that was more responsive to therapy with this antagonist.

TPI ASM8—TPI ASM8 (antisense CCR3 and common β chain; Pharmaxis, Sydney, Australia) contains 2 phosphorothioate antisense oligonucleotides directed against the mRNA for human CCR3 and of the common β chain (βc) of IL-3, IL-5, and GM-CSF receptors, thereby downregulating expression on the cell surface of CCR3 and βc. In animal models TPI ASM8's equivalent has been shown to downregulate its targets and the resultant airway hyperresponsiveness and inflammation after allergen challenge. Systemic distribution of the inhaled product in animals and human subjects is less than 1%, and it has been shown to be safe. When administered to healthy subjects in 2 single-dose phase 1 studies at doses of up to 6 mg, there was a trend toward less adverse events than in placebo-treated patients.

Seventeen patients with mild atopic asthma were randomized in a crossover study to inhale 1.5 mg/day TPI ASM8 (estimated lung deposition, 90–220 μg) or placebo for 4 days to examine the effects of inhaled TPI ASM8 on allergen-induced sputum eosinophil counts, CCR3 and βc mRNA levels in sputum cells, and the early and late asthmatic responses in patients with mild asthma after allergen challenge.[63] TPI ASM8 reduced allergen-induced sputum eosinophil counts by 46% ($P = .02$) on day 3. The allergen-induced (day 2 to day 3) levels of βc mRNA in sputum cells were also significantly inhibited by TPI ASM8 compared with placebo (1.1-fold increase compared with 11.9-fold increase respectively; $P = .039$). The allergen-induced levels of CCR3 mRNA increased 1.4-fold (SD, 6.5) with TPI ASM8 and 6.4-fold (SD, 6.9) with placebo ($P = .055$). TPI ASM8 significantly reduced the early asthmatic response ($P = .03$), with a trend toward the late asthmatic response ($P = .08$). TPI ASM8 was well tolerated. There were no serious adverse events from TPI ASM8 inhalation, and adverse events were similar in number to those reported with placebo.

Thus TPI ASM8 has been shown to reduce allergen-induced airway eosinophilia and attenuate the physiologic response in subjects with mild asthma through downregulation of the target genes encoding CCR3 and βc.[63] Further studies to test this approach in allergic and eosinophilic inflammation are ongoing.

PROMISING AGENTS IN PRECLINICAL DEVELOPMENT

Eosinophil survival

There are numerous preclinical pathways that are currently being pursued for the treatment of eosinophil-associated diseases. Interfering with eosinophil-inhibitory receptors involves direct engagement of inhibitory receptors with activating ligands (including antibodies) that result in impairment of eosinophil functional responses or survival signals or directly induce apoptosis. For example, anti–Siglec-8 (or anti–Siglec-F in the mouse) has the capacity to induce direct eosinophil apoptosis in both human subjects[64,65] and murine models.[66] Inhibitory receptor signaling might be particularly important for eosinophil survival in tissues, as evidenced by the effects of interference with signal-regulatory protein α (CD172a) signaling on eosinophil survival and accumulation in a number of tissues, including the small intestine.[67] Furthermore, ligands that activate inhibitory receptors on eosinophils (eg, CD300a) could be directed to eosinophils in a specific manner by cotargeting eosinophil-specific receptors, such as CCR3.[68] Lastly, new classes of inhibitory receptors on eosinophils (eg, paired immunoglobulin-like receptor B) have been identified and shown to regulate baseline, allergen-induced, and IL-13–induced eosinophilia.[69]

Eosinophil migration

Blocking eosinophil migration into inflamed tissues is another promising strategy for the reduction of end-organ manifestations of eosinophilia. Eosinophil-selective chemokines, such as the eotaxin subfamily of chemokines and their receptor CCR3, are critical for the recruitment of eosinophils into the lung and intestine.[70–74] Low-molecular-weight antagonists for CCR3 have been shown to attenuate airway eosinophil accumulation and lung pathology in experimental asthma models.[75,76] As such, competitive antagonists and neutralizing antibodies targeted to the CCR3/CCL11 axis are currently under investigation in human subjects (NCT01160224 and NCT01551771).

Adhesion molecules also have an important role in directing eosinophil recruitment into tissues. In particular, VLA-4 (α4β1 integrin) has been shown to be critical for eosinophil recruitment into the lung after allergen challenge in mice.[77] In preclinical studies blockade of VLA-4 resulted in significantly reduced tissue eosinophil counts.[78,79] Although natalizumab (Tysabri; Biogen Idec, Cambridge, Mass, and Elan, Dublin, Ireland), a humanized mAb to VLA-4, is commercially available under a special prescription program to treat multiple sclerosis, clinical trials in patients with eosinophilic disorders have not been initiated because of an increased risk of progressive multifocal leukoencephalopathy reported in patients with multiple sclerosis.[80]

Prostaglandin D_2 and its receptor CRTH2 provide another attractive target for blocking eosinophil recruitment. Low-molecular-weight CRTH2 antagonists have been shown to partially attenuate pulmonary eosinophilia in a number of different experimental models,[81,82] and prostaglandin D_2 signal transduction has been shown to play a role in eosinophil mobilization from the bone marrow and eosinophil activation and chemoattraction. A number of inhibitors of this pathway are in development and currently being tested for clinical utility for eosinophil-associated disorders.[82]

SUMMARY AND FUTURE DIRECTIONS

Eosinophilic disorders are chronic conditions that require long-term treatment for the prevention of clinical manifestations. Morbidity and mortality for many eosinophilic disorders remain high, and current treatment options are limited by lack of efficacy, significant toxicity, or both. Recent advances in our understanding of eosinophil biology have paved the way for the development of several promising novel therapies. Although

clinical trial development in these therapeutic areas has been enhanced by the recent creation of patient registries (eg, www.regid.org), research remains a challenge because of the paucity of subjects available for study at a given site and the lack of *bona fide* clinical biomarkers for use as end points in clinical trials. Larger studies assessing different dosing strategies might also identify subgroups of responders to different therapies. In this regard multicenter collaborations, translational research, and support from granting agencies and the pharmaceutical industry remain a priority. With further investigation, we hope to gain a better understanding of the biology of these eosinophilic disorders, to identify newer targets, and ultimately to apply this knowledge to the treatment of patients with HESs, as well as other more common eosinophilic disorders, such as asthma.

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Abbreviations used

AD	Atopic dermatitis
ADCC	Antibody-dependent cell-mediated cytotoxicity
βc	Common β chain
CSS	Churg-Strauss syndrome
ECP	Eosinophil cationic protein
EDN	Eosinophil-derived neurotoxin
EGID	Eosinophil-associated gastrointestinal disorder
EoE	Eosinophilic esophagitis
HES	Hypereosinophilic syndrome
IL-4R	IL-4 receptor
IL-5R	IL-5 receptor
MPN	Myeloproliferative neoplasm
NK	Natural killer
VLA-4	Very late antigen 4

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What do we know?

- HESs are a heterogeneous group of eosinophilic disorders that include idiopathic HES, CSS–related vasculitis, and EGIDs and that are characterized by marked eosinophilia in the peripheral blood, tissues, or both without a secondary cause.
- Eosinophils are sources of many mediators of inflammation and immune responses, including lipid mediators (eg, leukotriene C_4, platelet-activating factor, and eoxins) and eosinophil granules containing 4 principal cationic proteins (ie, major basic protein, ECP, EDN, and eosinophil peroxidase), which can be secreted, causing significant tissue damage.
- Promising targets currently being investigated include IL-5 and IL-5R, CD2 binding protein, IgE, and IL-4/IL-13 receptor.

What is still unknown?

- The specific mechanisms of action of the different anti-eosinophil–targeted therapies and why a specific therapy works in some but not in all patients with eosinophilia remain unclear.
- Dosing strategies and treatment options for patients with hypereosinophilia remain ill defined but are actively being investigated.
- Biomarkers, if any, that accurately predict responsiveness to therapy need to be identified.

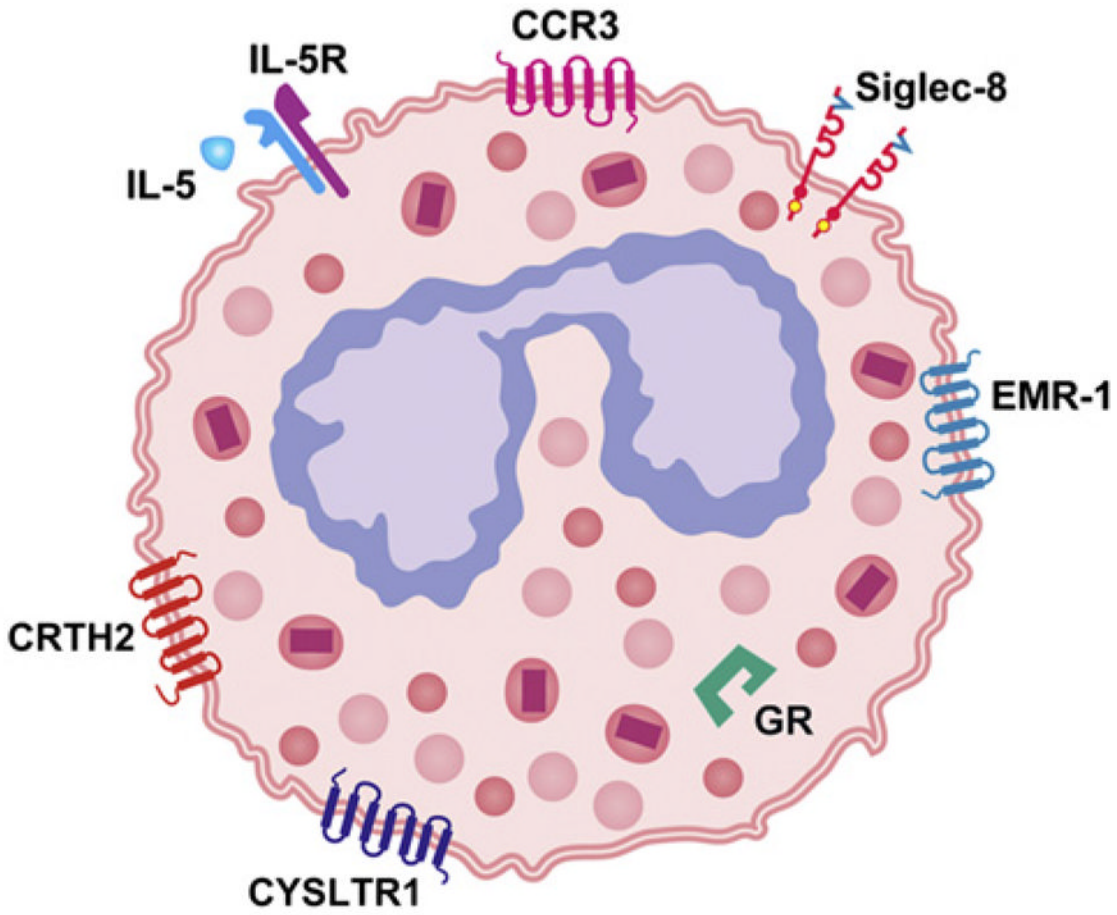

FIG. 1.

Active and theoretic eosinophil-selective therapeutic targets. The eosinophil possesses multiple targets that are the focus of active research in patients with hypereosinophilic diseases. These include IL-5, CCR3, Siglec-8, EMR1, CRTH2, cysteinyl leukotriene 1 *(CYSLTR1)*, and the gluco-corticoid receptor *(GR)*. Multiple other targets on the eosinophil and in pathways indirectly related to the eosinophil exist and are not depicted in this image. Medical Illustrator Jacqueline Schaffer provided this work.

TABLE I

HESs

Variant	Definition
HESs	Blood eosinophilia >1500/mm^3 (HE) on ≥2 occasions or evidence of prominent tissue eosinophilia associated with symptoms and marked blood eosinophilia AND exclusion of secondary causes of eosinophilia, such as parasitic or viral infections, allergic diseases, drug- or chemical-induced eosinophilia, hypoadrenalism, and neoplasms
Myeloproliferative HESs	HESs with features of myeloproliferative disease with or without proof of clonality (eg, *FIP1L1/PDGFRA*$^+$ myeloproliferative neoplasms)
Lymphoproliferative HESs	HESs with populations of T cells secreting eosinophil hemopoietins (eg, clonal T cells exhibiting an abnormal immunophenotype)
Undefined HESs	Symptomatic HESs without features of myeloproliferative or lymphocytic HESs
Organ-restricted HE	Tissue hypereosinophilia with or without blood eosinophilia; examples include eosinophilic gastrointestinal disorders and eosinophilic pneumonias
Associated HESs	HESs in association with a defined diagnosis, such as CSS
Benign HESs	HESs without signs, symptoms, or evidence of eosinophil-related organ damage
Familial HE/HES	HE or HESs with familial clustering, typically autosomal dominant