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Novel Targeted Therapies for Eosinophil-Associated Diseases and Allergy

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Abstract

Eosinophil-associated diseases often present with life-threatening manifestations and/or chronic organ damage. Currently available therapeutic options are limited to a few drugs that often have to be prescribed on a life-long basis to keep eosinophil counts under control. In the last 10 years, treatment options and outcomes in patients with clonal eosinophilic and other eosinophilic disorders have improved substantially. Several new targeted therapies have emerged, addressing different aspects of eosinophil expansion and inflammation. In this review, we discuss available and currently tested agents, as well as new strategies and drug targets relevant to both primary and secondary eosinophilic diseases, including allergic disorders.

Keywords

tyrosine kinase inhibitor; biologicals; asthma; chronic eosinophilic leukemia; hypereosinophilic syndrome

Introduction

Eosinophil-associated diseases include a variety of neoplastic and reactive conditions of different etiologies, including allergic and autoimmune disorders (1), where an elevation of eosinophil levels is the common denominator. The eosinophil is a granulocytic cell that stores highly cytotoxic proteins within its granules. Although the cytotoxic effects of eosinophils and their toxins were first described in the context of parasitic helminth infections (2), they can also be key mediators of tissue damage in inflammation. Normally,

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eosinophils reside in diverse tissues, such as the gastrointestinal tract (GIT), female genital tract, mammary glands, and thymus (3). Eosinophil infiltration in mucosal tissues is believed to contribute to the maintenance of a barrier against invasive microbes (4). More recently, immunomodulatory functions of eosinophils have increasingly come into focus (5), with murine models demonstrating a role for eosinophils in plasma cell homeostasis in bone marrow (6) and regulation of glucose metabolism in adipose tissue (7).

Eosinophils are generated in the bone marrow from multipotent and lineage-restricted progenitor cells which are themselves constantly replenished from a small pool of self-renewing hematopoietic stem cells (HSC) (8). Essential growth and differentiation factors for eosinophils include interleukin (IL)-5 (9), granulocyte/macrophage colony-stimulating factor (GM-CSF) (10), and IL-3. These cytokines are collectively called eosinopoietins. Whereas GM-CSF and IL-3 are well-described growth factors for other leukocytes, the effects of IL-5 are largely restricted to the eosinophil lineage. IL-5 regulates the proliferation of early progenitor cells, the mobilization of eosinophils from the bone marrow, as well as eosinophil maturation, activation, tissue recruitment, priming, and survival. The receptor for IL-5 shares a common β-chain with the receptors for GM-CSF and IL-3, but contains a specific α-chain (IL-5Rα) (11), expressed almost exclusively on eosinophils, basophils, mast cells, and their progenitors (12).

Eosinophilia is the result of an imbalance between eosinopoiesis, eosinophil clearance, and eosinophil death (13, 14). This may be due to intrinsic, eosinophil-related causes (e.g. by mutations in eosinophil precursor cells) or extrinsic causes (e.g. by enhanced production of eosinopoietins) (1). If blood eosinophil levels exceed 1.5×10^6/l, the condition is referred to as hypereosinophilia (HE) (15–17). In the absence of any of the common triggers of eosinophilia (allergens, parasites, drugs), the term *hypereosinophilic syndrome* (HES) is appropriate if HE is documented over a period of at least 4 weeks and is accompanied by eosinophil-mediated organ damage (16).

A number of different HES classifications have been proposed, attempting to identify subgroups of patients who may respond similarly to particular therapies (1, 15–17). It is useful to distinguish between patients with an underlying primary or clonal process and those with secondary eosinophilia. Patients with primary or clonal HE suffer from a myeloid or stem cell-derived neoplasm, i.e. eosinophils belong to the malignant clone. The FIP1-like 1 (FIP1L1) - platelet-derived growth factor receptor alpha (PDGFRA) fusion gene is the most frequent recurrent aberration in clonal HE and is detected in 30-50% of all cases (18). However, HES may also occur in the setting of other myeloid neoplasms accompanied by clonal HE (1, 15–17).

Secondary HES variants are mediated by production of one or several eosinopoietins, e.g. by normal/reactive (activated) T cells, clonal T cells, or other tumor cells (15–17). Both CD4+ and CD8+ T cells have been identified as eosinopoietin-producers (19). When eosinopoietin-producing T cells drive HE, the term lymphocytic HES (LHES) is appropriate (1, 15–17). In many patients with LHES, expansion of a T cell clone can be identified (1, 15–17, 20). In a subset of these patients, overt Non-Hodgkin's lymphoma (NHL) may eventually develop (21).

The eosinophilia or HE observed in the setting of eosinophilic allergic disorders is typically mediated by eosinopoietin-producing T cells (1). Furthermore, the clinical manifestations of these disorders overlap with those of HESs. Although therapeutic approaches to HESs and eosinophilic allergic disorders have historically differed, the availability of novel targeted therapies and a better understanding of the pathogenesis of HE and HES variants now allow a more structured approach (1, 15–17). In this review, we discuss targeted therapeutic options currently being investigated for primary and secondary eosinophilic diseases, including allergic disorders.

Clonal Eosinophilic Disorders

Somatic mutations of certain genes involved in proliferation and survival of eosinophil progenitor cells can result in clonal HE and/or a primary (clonal) HES. In recent years, a number of molecular defects have been identified in patients with clonal eosinophilic disorders, the most common being the FIP1L1-PDGFRA gene fusion (22). The FIP1L1-PDGFRA fusion results in constitutive, ligand-independent PDGFRA tyrosine kinase activity (22). Interestingly, the oncogenic potential of the FIP1L1-PDGFRA mutant can be enhanced by escape of the fusion product from normal protein degradation processes, leading to its accumulation (23).

Other, fusion genes involving PDGFRα or PDGFRβ can also cause clonal HE or HES (22). Most result in a constitutively active tyrosine kinase receptor that acts as oncogenic driver. Rarely, clonal HE or HES is caused by a chromosomal translocation involving the fibroblast growth factor receptor 1 (FGFR1) gene on chromosome 8p11-12, the so-called “8p11 syndrome” (24). This syndrome typically has an aggressive course with primary multilineage involvement and acute leukemia of mostly myeloid or mixed lineage in the terminal phase. As these patients are usually treatment-resistant, their prognosis is poor (24). Finally, clonal eosinophilia has been described in D816V KIT positive systemic mastocytosis (25) and in association with cytogenetic abnormalities, including PCM1-JAK2 (26). From a therapeutic standpoint, this is important to recognize since these genetic abnormalities do not respond to imatinib and require alternative approaches.

Tyrosine Kinase-Targeting Drugs

Imatinib—Patients with clonal eosinophilia typically do not have a sustained response to glucocorticosteroid therapies. Imatinib was originally designed to target the fusion oncogene, BCR/ABL, in chronic myeloid leukemia (CML) (27). The FIP1L1-PDGFRA kinase is 200-fold more sensitive to imatinib than BCR/ABL (28) and imatinib (100-400 mg/d) is first-line therapy for patients with PDGFR-associated disease (17). Clinical and hematological responses are rapid and dramatic (29) with molecular remission (no detectable FIP1L1-PDGFRA) typically observed within 2-3 months (30). Although imatinib is generally well-tolerated, myocardial necrosis has been reported in patients with eosinophilic cardiac involvement. Thus, in patients with elevated serum troponin levels or echocardiographic evidence of endomyocardial fibrosis, concomitant glucocorticosteroid therapy is recommended with imatinib initially to reduce this risk. Imatinib is not curative in the majority of cases (30, 31) and life-long therapy is recommended.

Though rare, if no hematological response is observed within 4 weeks, primary resistance should be considered (32). On a molecular basis, primary resistance to imatinib has been linked to the occurrence of a S601P mutation in PDGFRA, which leads to destabilization of the inactive conformation of the kinase domain binding imatinib (33). Acquired resistance to imatinib also appears to be uncommon. Most cases have been in association with the T674I mutation of FIP1L1-PDGFRA, a single base substitution in the imatinib-binding sequence analogous to the T315I mutation in BCR/ABL, which also promotes resistance to imatinib and related TKIs (28).

Second- and third-generation TKIs—Second-generation TKIs were developed to address the unfavorable outcome due to imatinib intolerance or resistance observed in approximately 30% of CML patients (34, 35). Indeed, higher response rates and less resistance were found in CML patients receiving nilotinib, dasatinib, or bosutinib in clinical trials (36–38). However, although sorafenib and nilotinib show in vitro efficacy against T315I BCR/ABL and T674I FIP1L1-PDGFRA, their clinical success with the T674I FIP1L1-PDGFRA resistance mutation has been disappointing (39, 40). Bosutinib is largely ineffective as this BCR/ABL blocker does not recognize PDGFRs (41). Therefore, the treatment of choice for such patients remains allogeneic stem cell transplantation (40).

Newer TKIs, such as ponatinib (42) and DCC-2036 (43), third-generation BCR/ABL blockers that are effective in vitro against the T674I-mutation in FIP1L1-PDGFRA are in development. Third-generation TKIs, including gefitinib (44), midostaurin (45), and ponatinib (46) have also shown in vitro efficacy against FGFR1 fusion genes, and may prove effective in PDGFR negative clonal eosinophilic disorders, such as 8p11 syndrome. In one patient with a ZNF198-FGFR1 gene fusion, a stable 6-month remission was obtained upon administration of midostaurin; increased maturation of myeloid cells and normalization of the myeloid-to-erythroid ratio in the bone marrow was achieved within 12 weeks (45).

It is important to recognize that, unlike imatinib, which is extremely well-tolerated, these novel TKIs can induce clinically relevant side effects. For instance, in CML patients treated with nilotinib and ponatinib, an increased rate of severe peripheral and central arterial occlusive diseases, leading to infarction, amputations, and disability, have been described (47, 48). In 2013, ponatinib was even taken off the market because of these side effects.

Interestingly, most of the currently available TKI, including imatinib, midostaurin, nilotinib, and ponatinib, but not bosutinib, recognize and block wild-type KIT tyrosine kinase activity (49). This finding is important, because leukemic stem cells (LSC) utilize this stem cell kinase as a critical mediator of survival and proliferation, suggesting that combination therapy using more toxic TKIs with enhanced activity against LSC to attack the vascular stem cell niche in an early (induction) phase, followed by maintenance therapy with imatinib, might be used in the future for patients with aggressive disease. It should be noted that midostaurin and ponatinib (50), but not imatinib and nilotinib (51), are effective against D816V, the most common KIT mutation in patients with systemic mastocytosis.

Clearly, the development of resistance, the requirement for life-long administration of imatinib, and the toxicity of the second- and third-generation TKIs remain clinical problems.

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In the following sections, we discuss new strategies that may help overcome these limitations in treatment of clonal eosinophilic disorders (Figure 1).

New Targeted Treatment Strategies

Targeting eosinophil-specific surface receptors—Although neutralization of IL-5 by antibody treatment can be beneficial for patients with FIP1L1-PDGFRA positive clonal eosinophilic disorders (52), this strategy has limited potential in this group of patients since it fails to address the underlying LSC. Another approach is to target eosinophils directly using afucosylated antibodies that bind to eosinophil-specific surface receptors, thus marking the cell for enhanced natural killer (NK) cell – mediated killing (Figure 1A). Of the antibodies currently in clinical development, benralizumab, an afucosylated antibody that binds to the surface receptor for IL-5 (IL-5Rα) (53), is likely the most promising since IL-5Rα is expressed on both eosinophils and eosinophil precursors in the bone marrow, peripheral blood, and tissues (12). Benralizumab has been tested in phase I and phase II trials in normal subjects and patients with asthma. In a recent study, bone marrow biopsies performed following a single dose of benralizumab demonstrated depletion of eosinophils and precursors in all 4 subjects (54). The fact that IL-5Rα is also expressed on mast cells and basophils (12) is an additional potential advantage in treatment of clonal HESs, since these lineages are often involved.

Several additional antibodies targeting eosinophil surface receptors are in preclinical development. The epidermal growth factor-like modul-containing, mucin-like hormone receptor 1 (EMR1) is an eosinophil-specific receptor (55) expressed on mature eosinophils. A novel, humanized, afucosylated anti-EMR1 IgG_1 antibody was recently shown to enhance NK cell-mediated killing of eosinophils in vitro and in non-human primates (55).

Siglec-8 is an inhibitory receptor expressed on eosinophils, basophils, and mast cells (56). Crosslinking of Siglec-8 induces eosinophil cell death and inhibition of mast cell degranulation in vitro (57). More importantly, administration of anti-Siglec-F antibody in a mouse model (mouse Siglec-F is the closest functional paralog of human Siglec-8) reduced eosinophil levels in the blood and tissues (58). Of note, human intravenous immunoglobulin preparations contain natural anti-Siglec-8 autoantibodies (59). Unfortunately, since neither EMR1 nor Siglec-8 is expressed in early eosinophil differentiation stages, antibodies targeting these receptors are unlikely to deplete the causative cells in clonal eosinophilic disorders.

Enhancing protein degradation—The FIP1L1-PDGFRA gene fusion product is able to escape protein degradation, resulting in enhanced oncogenic potential (23). In non-leukemic states, receptor tyrosine kinase activation leads to Cbl-mediated mono-ubiquitination of lysines, and subsequent degradation. Since chimeric and patient-derived FIP1L1-PDGFRA have more stable expression compared with wild-type PDGFRA (23), specifically targeting the mutant proteins for degradation could be a promising therapeutic strategy (Figure 1B).

Targeting regulators of autophagy—Pharmacological targeting of different steps in the autophagy process in imatinib-treated CML cells significantly increased cell death (60). Furthermore, siRNA-mediated down-regulation of the autophagy-related protein 5 (ATG5)

or ATG7 resulted in significant reductions in colony formation by imatinib-treated CML cells (60). Autophagic activity has not yet been investigated in clonal HESs. However, since autophagy regulates both self-renewal and differentiation of adult stem cells (61), it is possible that inhibitors of autophagy could both overcome TKI resistance and induce death in quiescent LSC in the bone marrow (Figure 1C).

Targeting regulators of apoptosis—Cell death is largely regulated by members of the BCL-2 family. Therefore, targeting anti-apoptotic members of this family, such as BCL-2 or myeloid cell leukemia-1 (MCL-1), is an emerging strategy in cancer therapy (Figure 1D). Targeting aberrantly activated BCL-2 family proteins is not only a promising approach to overcome TKI resistance, but it may also sensitize LSC for tyrosine kinase inhibition (62).

Omacetaxine is an inhibitor of protein synthesis (63). Inhibition leads to the down-regulation of short-lived anti-apoptotic proteins, such as MCL-1, with subsequent induction of apoptosis. The lack of target specificity (i.e. independence from BCR/ABL binding) makes it also independent of the BCR/ABL mutation status. The clinical effectiveness of omacetaxine in imatinib-resistant CML is as high as 77% (64), but remains unknown in FIP1L1-PDGFRA positive HES.

Sabutoclax is a novel inhibitor of anti-apoptotic members of the BCL-2 family (including MCL-1), currently being tested in various in vitro leukemia models. For instance, sabutoclax rendered CML blast crisis bone marrow stem cells sensitive to the tyrosine kinase inhibitor dasatinib, but had no effect on normal progenitors (62). Obatoclax, another small molecule targeting MCL-1 and other anti-apoptotic members of the BCL-2 family (65), is currently being tested in phase II clinical trials for treatment of leukemia, lymphoma, myelofibrosis, and mastocytosis.

Finally, antisense oligonucleotides, such as PNT2258, can be used to target anti-apoptotic BCL-2 family members. In a phase I study in patients with solid tumors, PNT2258 was both safe and well tolerated (66). A phase II study in patients with Non-Hodgkin's lymphoma is currently underway.

Targeting signaling pathways and transcription—Investigation of the role of self-renewal signaling pathways in CML-LSC revealed an important role of β-catenin, a key effector in the Wnt pathway (67). Targeting β-catenin with the COX2 inhibitor indomethacin increased the therapeutic effect of imatinib (Figure 1E) (67, 68). Similarly, pan-histone deacetylase inhibitors sensitized CML-LSC to imatinib treatment (69), resulting, however, in parallel toxicity to normal HSC (70). Sirtulin 1 (SIRT1), which belongs to a subgroup of histone-deacetylases not targeted by the pan-histone-deacetylase inhibitor, has been linked to self-renewal maintenance as well as to the regulation of tumor suppressors like p53 and FoxOs (71). In CML-LSC, SIRT1 overexpression has been documented and pharmacological inhibition or knock-down of its expression resulted in increased activation of p53 and increased apoptosis (70). Additionally, SIRT1 inhibition was able to potentiate the effect of imatinib (70).

Targeting surface receptors on LSC—It is generally appreciated that LSC represent an important target cell population, especially for any curative treatment approaches (72, 73). Although the exact immunophenotype of LSC in clonal eosinophilic disorders is unknown, based on similar disease models, it is unlikely that the LSC in clonal eosinophilic disorders reside within a CD34+ fraction. In CML, these cells have been defined and their target expression profiles have been characterized in some detail (73). Major targets expressed on LSC in CML include CD25 (IL-2RA), CD33 (Siglec-3), CD44 (Pgp-1), CD52 (Campath-1), CD123 (IL-3RA), and CD184 (CXCR4). Targeted antibodies against these surface structures have the advantage that slowly cycling, or even quiescent cells, could be depleted, which might be an achievable goal in both CML and CEL (Figure 1A).

Enhancing LSC release from the stem cell niche—A pivotal factor in promoting bone marrow homing of both hematological and leukemic progenitor cells is CXCR4 (CD184) (74, 75). In diverse leukemias, CXCR4 is expressed on LSC and progenitor cells. Plerixafor is a CXCR4 antagonist, currently FDA-approved for use in mobilization of HSC from the bone marrow for autologous stem cell transplantation (75). Recent data suggest that plerixafor can also mobilize neoplastic stem cells in various types of leukemias and may thereby enhance the responsiveness of these cells to various anti-leukemic drugs (Figure 1F) (74). As CXCR4 is an important homing factor for diverse types of HSCs, this stem cell receptor is also likely to play an important role in clonal eosinophil progenitors homing to shelter sites, and, thus, might be a target for enhancing TKI efficacy in patients with clonal eosinophilic disorders in the same way as in other leukemia models. The limitation of this strategy is certainly the lack of distinction between leukemic and normal hematopoietic progenitors in the mobilization from the bone marrow. On the other hand, the effects of concomitant TKI administration might lead to a more specific destruction of LSC.

Targeting the stem cell niche—Similar to HSC, LSC are believed to depend critically on their niches in the bone marrow (72). In the currently emerging scenario, LSC are thought to remodel the bone marrow niche to achieve a survival advantage over non-leukemic stem cells (76). One hypothesis is that hypoxic areas are enhanced in the leukemic bone marrow. Consequent stabilization of hypoxia-inducible factor α (HIF-α) in LSC alters their metabolism to glycolysis enabling survival in a quiescent state (Figure 1G) (77). In addition, hypoxia promotes VEGF production and, thus, bone marrow angiogenesis together with the related vascular niche. Based on these assumptions, targeting of hypoxic niches may be a promising new approach for the destruction of LSC. Experimental therapy with PR-104, a dinitrobenzamide mustard, activated by reduction to the cytotoxic hydroxylamine metabolite, caused a reduction of LSC and increased host survival in immune-deficient mice injected with primary, acute lymphoblastic leukemia cells (78), providing a proof of principle.

Recent data suggest that LSC evolution is regulated by the endosteal and vascular stem cell niches (72, 79). Therefore, several strategies target LSC-related angiogenesis with the final goal of destroying both the niche and niche-dependent LSC. Interestingly, some of the new TKIs, such as nilotinib, act on endothelial cells to inhibit their growth and, hence, angiogenesis. These TKIs and other anti-angiogenic drugs, such as lenalidomide (80), may

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therefore be interesting drugs to target the vascular LSC niche in patients with drug-resistant leukemia.

Lymphocytic Hypereosinophilic Syndromes

The hallmark of LHESs is increased production of eosinopoietins, especially IL-5, by activated polyclonal T cells or T cell clones (1, 15–17, 20, 81). Several different T cell immune-phenotypic abnormalities have been described, including double negative T cells (CD3+CD4-CD8-) (81) and T cells lacking CD3 expression (CD3-CD4+) (82), although the surface phenotype may be normal even in the setting of a T cell clone. Abnormal expression of other lineage-associated markers, such as CD2, CD5, CD6, and CD7, are often present (20).

Although glucocorticosteroid therapy is effective in reducing eosinophilia and symptoms in the majority of patients with LHES, moderate to high doses are often necessary (20, 29, 30). Furthermore, glucocorticosteroid treatment does not eliminate cytokine-producing T cells. In this regard, IFN-α, the most common second-line agent for LHESs, has the theoretical advantage of potentially depleting the cytokine-producing T cell clone (21). In severe asthma, IFN-α has been shown to support Th1 differentiation and IL-10 production (83), suggesting additional mechanisms of anti-eosinophil activity. It should be noted that IFN-α has also been successfully used in the treatment of HESs (29, 30). In the following section, we will address a few selected, novel therapeutic approaches for the treatment of LHESs (Supplemental Table 1 and Figure 2).

Eosinophil Targets

IL-5—Both mepolizumab and reslizumab, humanized antibodies to IL-5 in development for treatment of asthma, have demonstrated efficacy in patients with non-clonal HES (Supplemental Table 1). Based on the results of two small studies, which demonstrated that mepolizumab could safely reduce eosinophilia and improve symptoms in patients with non-clonal HES (84, 85), 85 FIP1L1-PDGFRA negative HES patients were enrolled in a double-blind, placebo-controlled study of monthly mepolizumab as a steroid-sparing agent (86). Overall, 84% of the subjects who received mepolizumab met the primary endpoint of a glucocorticosteroid dose of ≤ 10 mg/d for ≥ 8 weeks (86). Subgroup analysis revealed a comparable steroid-sparing effect in the 13 patients with LHES caused by an IL-5-producing T cell clone compared to the group as a whole, although eosinophil counts were suppressed to a lesser degree (87). Among 78 patients participating in a long-term open-label extension of the placebo-controlled study, 62% were prednisone-free for ≥12 consecutive weeks, confirming the long-term efficacy of mepolizumab in suppressing eosinophilia and symptoms in patients with HES (88). Ex vivo experiments suggested that the high efficacy resulted both from a decrease in total eosinophil numbers and from reduced activation of the remaining eosinophils (89). Although data is limited to a single open-label study in 4 patients with treatment-refractory disease, reslizumab is likely to show similar efficacy to mepolizumab in reducing eosinophilia in HESs, including LHESs (52).

Both mepolizumab and reslizumab have been well-tolerated in HES patients in clinical trials to date, and no safety concerns were identified in the long-term mepolizumab study (88).

Furthermore, the physiological eosinophil infiltration of the gastrointestinal tract was unaltered following anti-IL-5 antibody treatment in patients with eosinophilic esophagitis (EoE) (90). Nevertheless, maturational arrest in the bone marrow, increased production of intracellular IL-5 in CD4+ and CD8+ T cells and increased serum levels of IL-5 after mepolizumab treatment (89) have raised concern for potentially uncontrolled IL-5 - mediated inflammation upon interruption of therapy (91). In fact, an increase in blood eosinophilia above baseline, accompanied by an exacerbation of symptoms, has been observed after a single dose of reslizumab as drug levels declined several weeks after drug discontinuation (52). It is possible that increased eotaxin levels following IL-5 neutralization (92) contribute, at least partially, to such unwanted events following drug withdrawal.

IL-5Rα—Targeting IL-5Rα by benralizumab is thought to exert an anti-eosinophil effect both by interfering with IL-5 binding and by promoting antibody-dependent cell-mediated cytotoxicity (ADCC) with consequent enhanced apoptosis (53). When cultured in the presence of benralizumab, eosinophils and basophils undergo NK cell-mediated apoptosis. Importantly, such apoptosis was not associated with eosinophil degranulation under these conditions (53). In a phase I dose-escalation trial in patients with mild atopic asthma, benralizumab treatment caused a dose-dependent decrease in peripheral blood eosinophil counts within 24 h that persisted for up to 12 weeks after a single 3 mg/kg dose (93). A subsequent phase II trial in 27 subjects with eosinophilic asthma confirmed safety and efficacy of benralizumab in reducing blood, bone marrow, and tissue eosinophilia (54). A phase III trial in patients with asthma and a phase II trial in patients with HES are ongoing.

Siglec-8 and EMR1—As discussed above, monoclonal antibodies to the eosinophil surface receptors, Siglec-8 and EMR1, have been developed (55, 56) and are in pre-clinical development for the treatment of eosinophilic disorders. Both receptors are highly expressed on eosinophils from normal and eosinophilic individuals (55, 94) and are promising therapeutic targets in HESs, including LHESs.

T Cell Targets

CD52—CD52, also known as Campath-1 antigen, is a surface glycoprotein expressed on T cells, B cells, NK cells, most monocytes, macrophages, and eosinophils (91). Alemtuzumab is a humanized anti-CD52 monoclonal antibody that was initially approved for the treatment of B-cell chronic lymphocytic leukemia (CLL). Two small case series and several additional case reports demonstrated the potential of alemtuzumab to induce hematological remission in HES patients (95, 96). Since both eosinophils and T cells express CD52, potential mechanisms of action include a direct effect on eosinophils and/or destruction of eosinopoietin-producing T cells.

Despite initial benefit in a majority of patients in the two published case series, 70% and 83% of patients relapsed upon cessation of therapy (95, 96). In addition, alemtuzumab can lead to severe side effects, including severe hematological toxicity with resulting opportunistic infections (96). In one case series in HES, almost 50% of the patients died during the follow-up period and in the majority, the cause of death was attributed to the treatment rather than to the underlying eosinophilia (95).

CD2—Alefacept is a fusion protein of the human IgG_1 Fc domain and lymphocyte function-associated antigen (LFA)-3 (CD58) (97). LFA-3 binding to CD2 prevents co-stimulation, which is required for T cell activation. Furthermore, binding of IgG_1 to Fcγ receptors, particularly CD16, is thought to mediate interaction between T and NK cells, with consequent memory T cell apoptosis (91). Alefacept has been shown to be effective in the treatment of patients with atopic dermatitis (98) and psoriasis (99) and has demonstrated anti-eosinophil activity in patients with atopic dermatitis (98), perhaps, due to the depletion of cytokine-producing T cells (91). However, the manufacturer discontinued the development of alefacept in 2011 (www.amevive.com).

Allergic Diseases

Allergic diseases are characterized by Th2-driven inflammatory responses, often resulting in increased IgE levels and eosinophilia (Figure 2A). They comprise a wide spectrum of conditions affecting many different organ systems, each of which may require treatment with different drugs. Glucocorticosteroids are widely used to suppress the underlying chronic inflammation in allergic diseases, including those of the nose, eye, lung, skin, and gastrointestinal tract. However, glucocorticosteroids have potentially debilitating, unwanted effects when used incorrectly or inappropriately. Moreover, these drugs are not effective initially in all patients and many patients develop glucocorticosteroid resistance over time. Clearly, the development of novel, more specific and targeted therapies is needed. Below, we discuss new developments in therapies that target different components of the immune system (Supplemental Table 1, Figure 2B-F), with the exception of so-called immunotherapy, which has been reviewed elsewhere (100). It should be noted that, since allergy-associated eosinophilia is driven by eosinopoietin-producing T cells, all anti-eosinophil strategies described for LHESs also apply to the treatment of allergic diseases.

Targeting IgE

Recombinant humanized anti-IgE monoclonal antibody treatment with omalizumab was shown to be beneficial as a steroid-sparing agent in asthma patients, reducing asthma exacerbations, improving symptom severity, and enhancing the quality of life in these patients (101). Of note, the effectiveness of omalizumab for reduction of asthma exacerbations was shown to be best in allergic asthmatics with high blood eosinophilia, high periostin levels (a marker for IL-4 and IL-13 activity and a potential predictor of airway eosinophilia) and increased fractional exhaled nitric oxide (a marker of airway inflammation) (102). Serum levels of IgE, on the other hand, were not predictive of responsiveness to omalizumab (103). In a cohort of patients with nasal polyposis and comorbid asthma, treatment with omalizumab led to a significant reduction in airway symptoms and endoscopic polyp scores, whether or not associated with allergy (104). Recently, treatment of standard, therapy-resistant urticaria has evolved as a novel, currently still off-label, indication for omalizumab (105). Omalizumab is generally well tolerated (106).

Omalizumab binds free IgE precluding its binding to IgE receptors on mast cells and basophils (Figure 2B) (107). This leads to decreased expression of IgE receptors on mast

cells, basophils, and dendritic cells (108), and a decrease in the functional responses of these cells to IgE-dependent stimuli. Downregulation of IL-2 and IL-13 production by T cells (109), as well as reduced bronchial eosinophil infiltration and sputum eosinophil counts, were also observed in patients treated with omalizumab (110). Moreover, IgE synthesis in B cells was decreased, potentially due to induction of a state of anergy in IgE-bearing B cells (111). Omalizumab treatment also reduced expression of TSLP and increased expression of IL-10 in the skin of children with severe atopic dermatitis (112).

Recently, an IgE-specific DARPin (designed ankyrin repeat protein) has been reported to be able to bind IgE with very high affinity causing IgE molecules to be easily dissociated from high-affinity IgE receptors (113). Clearly, such compounds may pave the way for the next generation of IgE-blocking molecules with the goal of blocking allergic reactions.

Eosinophil Targets

IL-5 and IL-5Rα—Despite the failure of early studies to show efficacy of anti-IL-5 antibody therapy in the treatment of asthma (114, 115), recently multiple studies have demonstrated improvement of peripheral eosinophilia, a significant reduction of asthma exacerbations, and/or improvements in airway function among asthmatic patients with blood and/or sputum eosinophilia receiving mepolizumab or reslizumab (116–119).

Eosinophilic granulomatosis with polyangiitis (EGPA), previously known as Churg-Strauss syndrome, is characterized by asthma, sinusitis with nasal polyposis, eosinophilic vasculitis with extravascular granulomas, and peripheral blood eosinophilia (120). Although the pathogenesis of EGPA is believed to be multifunctional, elevated IL-5 levels and the expression of eosinophil activation markers are common, especially in ANCA-negative patients. The safety and efficacy of mepolizumab as a steroid-sparing agent in EGPA has been demonstrated in a small placebo-controlled trial (121). In a second small open-label trial, mepolizumab treatment decreased the relapse rate in patients with relapsing or refractory EGPA, although the effect was not sustained when the drug was discontinued (122, 123). A multicenter, phase III trial of mepolizumab as a steroid-sparing agent in patients with relapsing or refractory EGPA is currently underway. Reslizumab has not been studied in EGPA, but has been reported to have an impact on nasal polyposis in patients with elevated IL-5 in nasal secretions (124).

Results of clinical trials of anti-IL-5 therapy for other allergic eosinophilic disorders have been disappointing. In pediatric and adult patients with EoE, significant clinical improvement was not observed despite reductions in the numbers of blood and tissue eosinophils (92, 125, 126). A similar lack of clinical efficacy was seen in a placebo-controlled trial of mepolizumab in patients with atopic dermatitis (127).

In a multicenter, double-blind, placebo-controlled phase II study in asthmatic patients with sputum eosinophilia, benralizumab reduced airway eosinophil counts by 95.8%, sputum eosinophil counts by 89.9% and blood eosinophilia by 100% after 4 weeks of subcutaneous administration (54). Moreover, benralizumab suppressed eosinophil counts in the airway mucosa by 61.9% following intravenous application. No serious adverse events related to

benralizumab were reported (54). A phase III study of subcutaneous benralizumab in asthmatic patients is currently underway (Supplemental Table 1).

Eotaxin – CCR3—Eotaxins are a family of three chemokines, CCL11 (eotaxin-1), CCL24 (eotaxin-2) and CCL26 (eotaxin-3), which evoke chemotactic activity in cells expressing the eotaxin receptor CCR3, primarily eosinophils and basophils (Figure 2F) (128). Eotaxin-1/2 knockout mice fail to recruit eosinophils to the lung upon allergen challenge. IL-4 and IL-13 production were significantly reduced in these mice, as well as in eosinophil-depleted mice (128). These data suggest that targeting the eotaxin-CCR3 axis would strongly reduce eosinophil recruitment to allergic sites.

Bertilimumab, a human anti-eotaxin-1 antibody, is currently in a phase II clinical trial for ulcerative colitis, an autoimmune inflammatory disease known to be associated with elevated CCL11 levels (129). Ongoing studies with GW766944 and other investigational CCR3 antagonists have been reviewed recently (130).

TPI ASM8 is a novel agent consisting of antisense oligonucleotides to human CCR3 and common β-chain transcripts. Its application leads to decreased expression of CCR3 and the common β-chain of the IL-5R, IL-3R, and GM-CSFR. In mild atopic asthmatics, inhaled TPI ASM8 significantly reduced allergen-induced sputum eosinophilia (131). In a second study, early and late asthmatic responses were significantly attenuated (132).

T Cell Targets

TSLP—Thymic stromal lymphopoietin (TSLP) has been linked to the initial phases of allergic disorders and is a critical driver of Th2 differentiation through its effect on dendritic cells (133). Overexpression of TSLP in the skin of mice causes a disease that resembles atopic dermatitis (134) and lung-specific TSLP-expressing transgenic mice develop a chronic disease with characteristics of bronchial asthma (135). Furthermore, increased TSLP expression has been observed in skin biopsies of patients with atopic dermatitis and genetic alterations in the TSLP gene have been associated with EoE (136), consistent with the hypothesis that TSLP is critically involved in the evolution of various allergic diseases. Recent data demonstrating that the TSLP receptor is expressed on eosinophils and that eosinophil extracellular DNA traps are formed upon TSLP stimulation (137), suggest that eosinophils play a key role in TSLP-mediated pro-inflammatory effects. Basophils may also be important as evidenced by studies linking TSLP gain-of-function polymorphisms in EoE patients to higher basophil activity and a dependency on TSLP and basophils in a mouse model of EoE-like disease (138). Thus, therapy targeting TSLP could result in both anti-basophil and anti-eosinophil effects (Figure 2D). Anti-TSLP antibody is currently being investigated in several phase I clinical trials (Supplemental Table 1).

IL-4/IL-13 and their receptors—Targeting both arms of the IL-4 and IL-13 axis is a promising strategy for the treatment of asthma (139). Such an approach has been taken with pitrakinra, a recombinant protein derived from human IL-4 that acts as a competitive antagonist of both IL-4 and IL-13 (Figure 2C) (139). Pitrakinra significantly reduced allergen-induced airway hyperreactivity in an experimental animal model of asthma (140) and prevented asthma exacerbations in a subgroup of asthma patients (139), although

clinical efficacy was dependent on the presence of a specific IL-4 receptor polymorphism (141). Dupilumab, a fully human antibody directed against the IL-4Rα shared by IL-4 and IL-13 receptors (Figure 2C), has been evaluated in persistent, moderate-to-severe asthma with elevated blood eosinophil counts (142). Dupilumab treatment, as compared with placebo, brought fewer asthma exacerbations and improved lung function following the withdrawal of inhaled glucocorticosteroid and long-acting beta-agonist therapies (142). Levels of TARC, eotaxin-3, and IgE were significantly reduced following dupilumab treatment, confirming that the IL-4-driven pathway was blocked (142). Of note, neither blood eosinophil counts or sputum eosinophils were significantly decreased by pitrakinra or dupilumab treatment (139, 142). A human IgG4 bispecific antibody able to neutralize both cytokines is currently in clinical development (143).

Neutralization of IL-13 alone has also been investigated (Figure 2C). Two anti-IL-13 antibodies have been developed and used in clinical trials (Supplemental Table 1). Lebrikizumab is a humanized monoclonal anti-IL-13 antibody and was evaluated in a randomized, double-blind, placebo-controlled study in 219 adults with inadequately controlled asthma despite inhaled glucocorticosteroid therapy (144). Interestingly, the improvement in lung function correlated with serum periostin levels, an indicator of IL-13 activity (144). These data provided further evidence of the heterogeneous nature of asthma and confirmed the utility of careful patient selection before applying targeted treatment approaches. More recently, lebrikizumab was also shown to reduce the late asthmatic response in patients with mild asthma (145). Tralokinumab is a fully human anti-IL-13 monoclonal IgG_4 antibody. In a large randomized, placebo-controlled study enrolling 194 patients with moderate-to-severe uncontrolled asthma, tralokinumab safely improved lung function (146).

CRTh2—In recent years, evidence has accumulated for the critical role of prostaglandin D2 (PGD2), a prostanoid produced mainly by mast cells, in allergic inflammation. PGD2 has been shown to mediate activation and recruitment of eosinophils, basophils, and Th2 cells, as well as promote enhanced Th2 cytokine production through the PGD2 receptor chemoattractant receptor-homologous molecule on Th2 cells (CRTh2) (147). Moreover, CRTh2 agonists increased allergic inflammation in murine models of atopic dermatitis and allergic asthma (148). In agreement with these findings, CRTh2 deficient mice mounted a less pronounced inflammatory response to hapten-specific IgE injections, and this was associated with decreased tissue infiltration of eosinophils, basophils, and lymphocytes (149). CRTh2 has thus evolved as a promising new therapeutic target for treatment of allergic disease (Figure 2D; Supplemental Table 1). The CRTh2 antagonist OC000459 significantly reduced eosinophil-inflammation in asthma (150) and in EoE (151), leading to clinical improvement in both patient groups as compared to patients receiving placebo. OC000459 has also shown efficacy in reduction of grass pollen-induced conjunctivitis and rhinitis symptoms (152).

GATA-3 specific DNAzyme—The importance of the transcription factor GATA-3 in Th2 cell activation and regulation of mast cells, eosinophils, and epithelial cells has led to investigationof GATA-3 as an emerging new therapeutic target. Topical application of so-

called DNAzyme, single-stranded DNA molecules targeting and cleaving specific sequences in target mRNA molecules (i.e., gd21 and hgd40), was shown to be highly effective in the prevention of allergic inflammation and airway hyperresponsiveness in murine models of acute allergy airway inflammation (153, 154). No off-target effects were observed with gd21 (153) and aerosol inhalation of hgd40 in rats and dogs was well-tolerated with only mild systemic effects (154). A dose-dependent increase in IL-10 and IFN-γ was seen in the hgd40 study, but at very low levels (154).

Targeting B Cells

Rituximab is a chimeric monoclonal anti-CD20 antibody, originally developed for the therapy of B cell malignancies. CD20 is expressed on pre-B cells and mature B cells, but not on plasma cells. In an investigator-driven study, six patients with severe atopic dermatitis were treated with rituximab (155). All patients showed improvement in skin symptoms within 4 to 8 weeks. Of note, expression of IL-5 and IL-13 was reduced after therapy, whereas allergen-specific IgE levels were not altered (155). These data suggest that depletion of B cells may decrease T cell activation, resulting in reduced Th2 cytokine expression.

Concluding Remarks

During the past few years, our understanding of HE and HES has increased substantially, and several new treatment approaches have been translated from preclinical models into clinical practice. Today, further development of new targeted therapies for eosinophilic disorders, including allergic diseases, is closely linked to an understanding of mechanisms for evolution of disease progression and drug resistance. In line with this notion, any targeted therapy requires optimal patient selection confirmed by adequate biomarkers. Ongoing research aimed at defining the physiological and pathological roles of eosinophils will provide additional targets and drugs that can be applied to eosinophilic diseases and allergic disorders. In eosinophil neoplasms, the ultimate goal will be to eradicate LSC in order to achieve long-term cures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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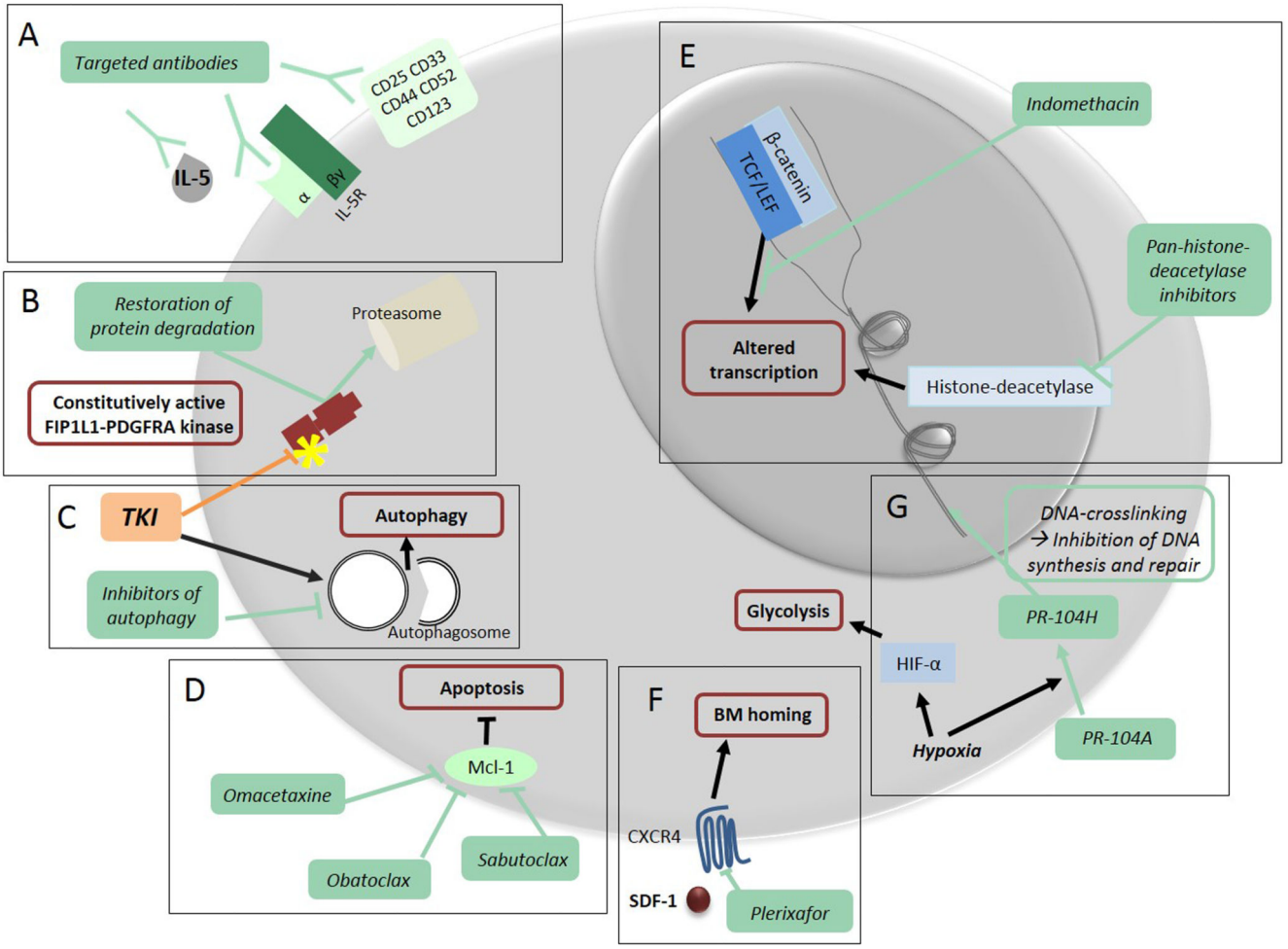

Figure 1. Potential novel targeted-treatment strategies for clonal eosinophilic disorders.

The development of clonal or primary HES can often be linked to the presence of a constitutively active stem cell kinase, such as the FIP1L1-PDGFRA oncoprotein. Imatinib is a highly effective inhibitor of FIP1L1-PDGFRA and remains first-line treatment in patients with this fusion gene. However, resistance may occur, often in association with secondary mutations in FIP1L1-PDGFRA, and discontinuation of imatinib in remission leads to reoccurrence of the disease, probably because of persisting LSC. Besides new, more effective TKIs, other promising novel therapeutic strategies for patients with clonal or primary HES are presented below:

A. **Antibodies targeting eosinophil-specific and LSC surface receptors**. Although IL-5 is the most important eosinopoietin, clonal eosinophils are known to grow independently of IL-5. Consequently, anti-IL-5 antibodies are not likely to be effective in most cases. Whether targeting the IL-5Rα will elicit better responses is unknown. The immunophenotype of the clonal eosinophil LSC is currently unknown, but based on other leukemic models, it seems unlikely that these cells express IL-5 receptors. Specific antibodies to LSC surface molecules, alone or in combination with

imatinib, may be a promising strategy for eliminating LSC and thus achieving cures in patients with clonal eosinophilic disorders. Please note that imatinib as well as the second-generation TKIs are also potent inhibitors of the wild-type stem cell kinase KIT.

B. **Restoration of protein degradation**. Oncogenic enhancement is further supported by an escape of the fusion protein from protein degradation. Therefore, promoting ubiquitination, thus targeting the fusion protein for proteasomal degradation, might augment TKI effects.

C. **Inhibition of autophagy**. Autophagy is a survival mechanism and is known to be highly active in adult stem cells. Targeting autophagy in cell lines and primary CML cells significantly increased cell death. In addition, TKI have been identified as activators of autophagy, a mechanism, which can contribute to drug resistance.

D. **Induction of apoptosis**. Aberrantly activated anti-apoptotic BCL-2 family members can contribute to inappropriate survival and accumulation of eosinophils in clonal eosinophilia. Different strategies to inhibit anti-apoptotic members of the BCL-2 family are currently being tested in vitro and in clinical trials with the goal of tipping the balance in favor of cell death. Both sabutoclax and obatoclax target MCL-1 and other BCL-2 family members. Omacetaxine, on the other hand, causes a decrease of the short-lived MCL-1 through inhibition of protein synthesis.

E. **Inhibition of altered transcription**. Altered transcription pathways have been linked to self-renewal maintenance and blocking of expression of tumor suppressors. As examples, drugs which inhibit histone deacetylation or β-catenin expression are indicated. Increased levels of β-catenin enhance its interaction with the transcription factors of the TCF-LEF family, its translocation to the nucleus, and modulation of transcription of downstream genes. Indomethacin was shown to attenuate transcription of β-catenin-TCF responsive genes.

F. **Mobilization of clonal eosinophil LSC.** Plerixafor is an antagonist of the stem cell homing receptor CXCR4 that is also expressed on eosinophils. Correspondingly, the CXCR4 ligand SDF-1 is a strong chemoattractant for both stem cells and eosinophils, pointing to a major role of SDF-1/CXCR4 interactions in clonal eosinophilic disorders. Plerixafor disrupts this interaction and thus leads to rapid mobilization of normal and neoplastic stem- and progenitor cells. Following mobilization from the bone marrow niche, these cells are more likely to respond to anti-leukemic therapies, e.g. imatinib.

G. **Targeting the LSC niche.** It is hypothesized that hypoxic areas are increased in the leukemic bone marrow. PR-104 is a small-molecule pre-drug activated by reduction to a DNA cross-linker. It inhibits DNA repair and synthesis, causing cell-cycle arrest, and apoptosis in LSC and

progenitor cells. There are many other drugs that can attack the vascular bone marrow niche. Some of these drugs can directly inhibit the growth of endothelial cells and angiogenesis. Among these drugs are lenalidomide and some of the second- and third-generation BCR/ABL TKIs (nilotinib, ponatinib).

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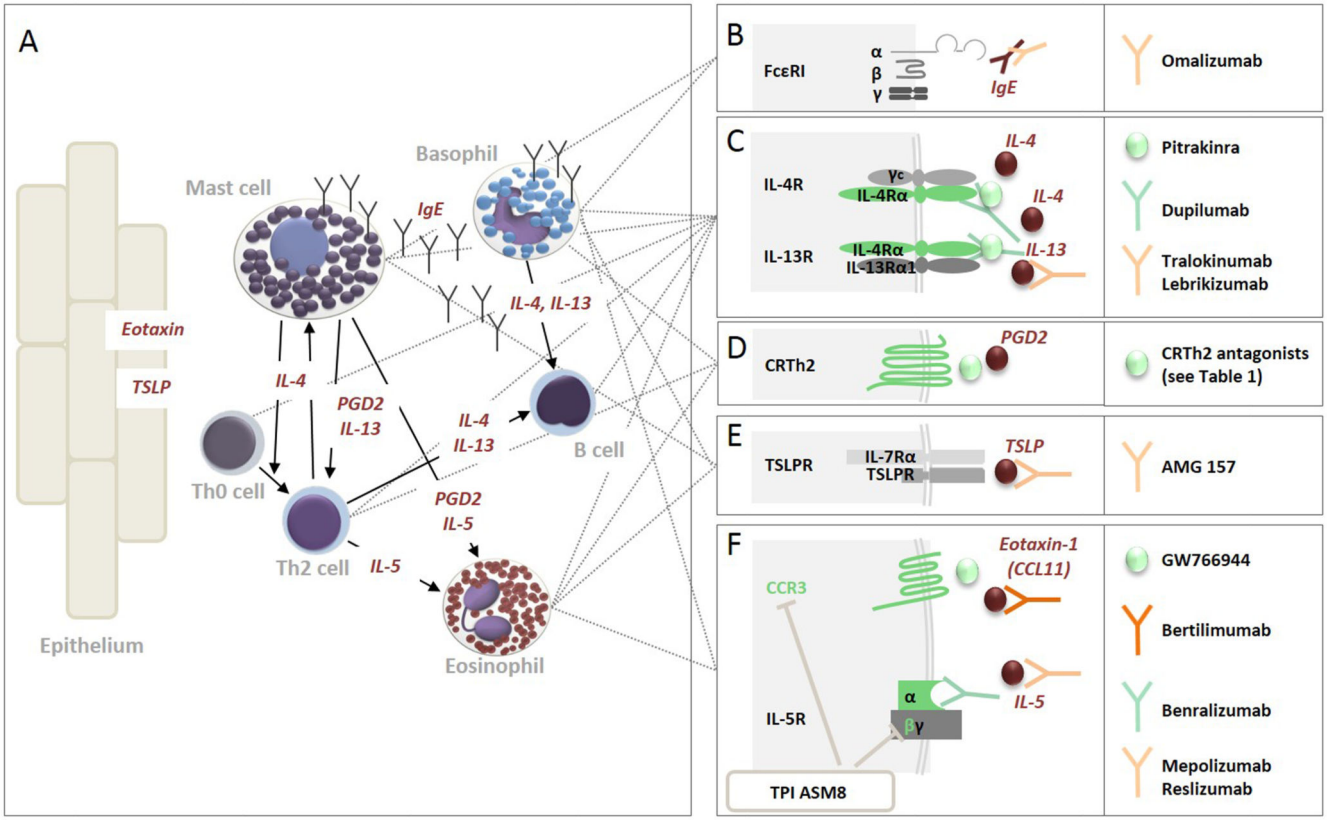

Figure 2. Current targeted therapeutic approaches for allergy are presented below.
Compounds not yet in clinical trials are not included.

A. Schematic overview of the targeted cells and effector molecules involved in the pathogenesis of allergy. Briefly, mast cell and basophil activation is initiated upon crosslinking of surface-bound IgE. The mediators released following activation include chemotactic factors for T cells and eosinophils (PGD2), as well as IL-4 and IL-13. IL-4 and TSLP, largely produced by epithelial cells, which promote the differentiation of Th0 to Th2 cells. IL-4 and IL-13 enhance IgE production in B cells. IL-5 and eotaxin, released by epithelial cells, enhance eosinophil infiltration. Targeted therapeutic strategies are directed against both effector molecules and surface receptors. The expression of critical receptors that can be targeted by currently available drugs is indicated for each cell with a dotted line.

B. Omalizumab, a humanized antibody, binds to free IgE and, as a consequence, decreases FcεR-bound IgE. This also leads to a down-regulation of the high-affinity FcεR on mast cells and basophils.

C. Different strategies for blocking the IL-4/IL-13 axis are currently under investigation. Both receptors contain an IL-4Rα chain. Antagonism of both IL-4 and IL-13 signaling pathways may thus be achieved by blocking IL-4Rα either through targeted antibodies (dupilumab) or receptor

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antagonism (pitrakinra). Furthermore, IL-13 blocking antibodies have shown promising results.

D. Blocking the effects of PGD2 is currently being addressed by several receptor antagonists.

E. The human monoclonal antibody AMG 157 binds to TSLP, thereby interfering with the interaction of TSLP with its receptor on mast cells, basophils, and eosinophils, as well as dendritic cells (not shown).

F. Eotaxins are critical chemotactic factors for eosinophils. The currently investigated antibody targeting these chemokines is bertilimumab, directed against eotaxin-1 (CCL11). IL-5 is a critical pro-survival and activating factor of eosinophils. Its effect can be blocked by either targeting the cytokine with mepolizumab or reslizumab, or by targeting IL-5Rα. Receptor antagonism of CCR3 (CD193) is an attempt to directly block eosinophil chemotaxis. Decreased expression of both CCR3 and the common β-chain of the IL-5R is the aim of treatment with TP ASM8, an antisense oligonucleotide.