Biotherapeutics for human eosinophil associated disorders

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ABSTRACT

Eosinophils which play an important role in pathophysiology of eosinophil associated diseases (EADs) contain plethora of mediators of inflammation and immune responses, including lipid mediators and eosinophil granules containing secretory cationic proteins. When secreted, they cause significant tissue damage. To circumvent the effects, numerous strategies have been employed for development of biologic medicines for EADs. Strategies used for development of biotherapeutics for EADs include eosinophil modulating therapies, destruction of eosinophils by means of ADCC or opsonization with removal by reticuloendothelial system, targeting key eosinophil survival cytokines and blocking eosinophil migration into inflamed tissues is another promising strategy for the reduction of end-organ manifestation of eosinophilia. EADs are categorized as "orphan" diseases and most of the current treatment options for patients with these conditions are used off-label. Better therapeutic targets that offer enhanced treatment selectivity and benefit for these conditions need to be identified and developed. In this review we provided an overview of potential molecular targets of pharmacotherapy and discussed the potential eosinophil targeting agents, which includes those in preclinical and clinical development for treatment of EADs.

Keywords: Biotherapeutics, Eosinophil, Monoclonal antibody, Eosinophil associated diseases

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HUMAN EOSINOPHIL ASSOCIATED DISORDERS

Eosinophils are sources of many mediators of inflammation and immune responses, including lipid mediators (e.g. leukotriene C4, platelet-activating factor and eoxins). Eosinophil granules contain four principal cationic proteins - major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN), and eosinophil peroxidase (EPO). Eosinophils remain as one of the most enigmatic cells in the immune system, as our understanding of their detrimental activities such as in asthma and allergic disease far outweighs our understanding of their beneficial effects as antibacterial, antiviral or antiparasitic activities. The ribonucleases eosinophil-derived neurotoxin (EDN) and

eosinophil cationic protein (ECP) are among the major secretory effector proteins of eosinophils in primates. ECP and EDN are members of the RNase A superfamily, which play a crucial role in host defense against various pathogens as they are endowed with several biological activities. ECP has cytotoxic activity against Gram positive and Gram negative bacteria [1], viruses such as respiratory syncytial virus (RSV) [2], parasites such as *Trichinella spiralis, Schistosoma mansoni, Trypanosoma cruzi, Leishmania donovani etc [3–6]*. These cationic proteins are secreted from the granules and can cause significant tissue damage [7]. In addition to the previous mentioned beneficial effects, eosinophils are involved in many life threatening disorders, referred to as eosinophil associated diseases (EADs). These refer to a group of disorders in which eosinophils are believed to play a central role in the pathophysiology [8]. They often present with life-threatening manifestations and/or chronic organ damage. Some EADs includes hypereosinophilic syndrome (HES), eosinophil myalgia syndrome, eosinophilic gastrointestinal disorders, and other clinical subtypes which includes allergic disorders. Eosinophilic asthma is a subtype of asthma characterized by peripheral eosinophilia and sputum eosinophilia of 2% or more [9].

The earlier therapeutics options for EADs were topical or systemic corticosterioids which are initially effective in controlling eosinophilia and symptoms in many patients but with time due to development of resistance and toxicity, there is a need for second-line agents [10–11] The recent interest in developing targeted therapies - for their selectivity and low systemic side effects in human disorders - has resulted in efforts to develop such therapies for EADs.

BIOTHERAPEUTICS

Bioptherapeutics are large complex molecules derived from natural or genetically engineered systems. They are used in the treatment, diagnosis or prevention of human diseases [12]. Bioptherapeutics which are also known as biologic medicines, include hormones, recombinant proteins, cytokines, nucleic acids and monoclonal antibodies (mAbs). The interest in biotherapeutics developed due to their advantage of targeting specific cells or biochemical pathways in human body, theoretically increasing efficacy and limiting complications related to nonspecific effects of more traditional therapies [13]. Bioptherapeutics due to their sensitivity and large size, are always administered by injecting into a patient's body and individual patient's response is evaluated to titrate the therapy. Biotherapeutics are expanding the arsenal of agents available for preventing and treating many diseases. Monoclonal antibodies due to their speicifity of binding are exquisitely selective for the molecular target of interest. One disadvantage of targeted therapies lies in the fact that that the molecular targets of interest is also expressed on normal cells, thereby disrupting the normal cellular function. Therefore, inspite of highly selective nature of targeted therapies, a range of previously unknown and often unpredictable adverse event occurs [14]. Even though biotherapeutics were shown to have limited potential for side effects due to the specificity of their binding, few of them have been shown to cause unexpected adverse drug reactions (ADRs). However, the benefit associated with targeted therapy outweighs the adverse events associated with them and make them a favoured choice.

BIOTHERAPEUTICS IN HUMAN EOSIN-OPHIL ASSOCIATED DISORDERS

The search for specific targets or pathways while increasing efficacy and limiting adverse events, led to research of biotherpaeutic agents for treating EADs. Eosinophils are ideal target for treatment of EADs. Eosinophils express specific lineage-restricted surface receptors which can be exploited in developing biologic therapies for EADs [15]. Treatment for EADs which is currently avalibale is limited to few drugs that have to be prescribed on a lifelong basis to keep eosinophil counts under control. In the past few years, biotherapeutics have substantially improved the treatment options and outcome in patients with EADs.

The earlier biologic therapies focused on targeting the blocking of IL-5 expressed on eosinophils. Anti-IL-5 antibodies bind to IL-5R, therefore blocking the IL-5 to bind its receptor IL-5R, which interfere with the activation of eosinophils, eosinophil actiation is central to their role in causing tissue damage. Humanized antibodies to IL-5 have been developed in recent years, which are currently in clinical trials for EADs.

Though the early efforts focused primarily on blocking the action of IL-5, the number of biologic agents with varied targets, in clinical development for the treatment of EAD has expanded dramatically in the last five years. In addition to targeting IL-5 and IL-5Rα, many novel targets for the treatment for eosinophil associated diseases have been intensively examined for future therapeutic uses. Such novel targerts include IgE blockers, IL-4 and IL-13 blockers, IL-4 and IL-13 receptor inhibitors, tyrosine kinase inhibitors; IFN-α, ICAM-1, VCAM-1, integrin, eotaxin/CCR3 pathways; blocking eosinophil migration (targeting VLA-4); immune inhibitory receptors on eosinophils; sialic acid binding immunoglobulin-like lectin (Siglec)-3, Siglec-7, Siglec-8, Siglec-10, FcgRII, CD85a, and CD300a [16–20] As these agents become commercially available, careful assessment of the potential advantages and limitations will be required to select the most appropriate agent for a given patient.

This review discusses the various therapeutic targets for treatment of esosinophil associated diseases (EADs). Further, it discusses the clinical trials using the first generation of eosinophil-targeted biologics anti–IL-5

antibodies and the advantages and potential limitations of currently available and novel targeted therapies to treat eosinophilic disorders.

THERAPEUTIC TARGETS FOR TREATMENT OF EADS

Activated T cells and their mediators cause eosinophilia, as can be observed in patients with allergic diseases, autoimmune diseases, lymphoproliferative forms of HESs, and T-cell lymphomas [21].

There are various strategies employed for development of biotherapeutics for eosinophil-associated diseases. The ideal target for the treatment of EADs is IL-5 and IL-5R. IL-5 has a role in differentiation, activation and survival of eosinophils. Targeting IL-5 and IL-5R in preclinical and clinical settings gave promising results.

Other modalities used for development of biologic medicines for eosinophil-associated diseases include eosinophil modulating therapies targeting IGE, IL-4, IL-13, TSLP, IL-25, IL-33, destruction of eosinophils by means of ADCC or opsonization with removal by reticuloendothelial system, targeting key eosinophil survival cytokines, IL-5, IL-3 and GM-CSF, and blocking eosinophil migration into inflamed tissues is another promising strategy for the reduction of end-organ manifestation of eosinophilia.

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.

BLOCKING IL-5 AND INHIBITION OF IL-5 BINDING TO IL-5R FOR TREATMENT OF EADS

Among a plethora of therapeutic targets for treatment of EADs, the earliest efforts were made to focus on blocking the action of IL-5. It is considered as an ideal target for treatment of EADs, beacause they are expressed as lineage-restricted surface receptors on eosinophils and central to the differentiation, activation and survival of eosinophils, they have insignificant effect on other lineages [22]. The earlier pre-clinical studies on mice and monkey models of allergic asthma demonstrated a sustained decrease in eosinophils in bronchoalveolar lavage after treatment with anti–IL-5 antibody. These data led to the clinical developmentof 2 different humanized mAbs that specifically bind IL-5 with high affinity: mepolizumab (GlaxoSmithKline) and reslizumab (Teva Pharmaceuticals).

IL-5Rα is a high-affinity receptor specific for the eosinophil lineage [23]. It is also expressed on basophils, mast cells, and their precursors in the bone marrow. IL-5Rα occurs as a heterodimer with the beta subunit common to IL- 5, IL-3, and GM-CSF receptors. Clinical development of a humanized monoclonal antibody, Benralizumab to IL5Rα showed both inhibition of IL-5 mediated cell proliferation by reducing IL-5 binding to its receptor and depletion of IL-5Rα bearing cells through enhanced antibody-dependent cell-mediated cytotoxicity.

Mepolizumab

Target: Cytokine IL-5 is specific for the esonophil lineage and is related to tissue damage due to eosinophil activation. It is an ideal target for biotherapeutic agents for treatment of EADs. Anti-IL-5 antibodies directly bind to IL-5 and inhibit the interaction of IL-5 to IL-5Rα expressed on the eosinophil surface. With this approach humanized anti–IL-5 antibodies, mepolizumab have been developed which specifically binds to IL-5 and have shown efficacy in clinical trials for asthma and HESs.

Conditions: Mepolizumab has been investigated in clinical trials for the treatment of asthma, atopic dermatitis (AD), FIP1L1/PDGFRA-negative HESs, eosinophilic esophagitis (EoE) and nasal polyposis.

Clinical trial studies: Intial studies on mepolizumab were published in 2009 in patients with treatment-refractory eosinophilic asthma.The study demonstrated that mepolizumab therapy reduced asthma exacerbations with improvement in asthma symptoms as compared to placebo [24]. Another study was carried out on patients on oral corticosteroid therapy for controlling asthma. The results demonstrated a reduction in maintenance corticosteroid therapy in such patientsl. No safety concerns for mepolizumab were identified. In study 1 severe asthma exacerbations were transiently increased in the 3 to 6 months after cessation of mepolizumab therapy.

In a study on 621 patients with recurrent exacerbations in eosinophilic asthma, three different dosing regimens on intravenous mepolizumab were given. The Dose Ranging Efficacy And safety with Mepolizumab (DREAM) study data showed asthma exacerbations were decreased by 39% to 52% in patients receiving mepolizumab [25].

Cluster analysis identified 3 predictors of response to mepolizumab: absolute eosinophil count, airway reversibility, and body mass index. In contrast, oral corticosteroid use was not associated with exacerbation rate.

Mepolizumab was further assessed on with recurrent asthma exacerbations and eosinophilic inflammation despite corticosteroid therapy. The trial MENSA was a 32-week double-blind, double-dummy, placebo-controlled, parallel group multicentre study performed with 576 patients with severe asthma, who had a history of frequent exacerbations despite treatment with high-dose inhaled corticosteroids (ICS), with at least one other controller medication.The patients in the clinical trials received monthly 100 mg subcutaneously or 75 mg intravenously mepolizumab orplacebo [26]. An effective reduction of asthma exacer bations, FEV1, and clinical symptoms was demonstrated by both the routes in the mepolizumab group as compared to placebo group.

Another trial SIRIUS was a 24-week double-blind, double-dummy, placebo-controlled, parallel group multicentre study performed with 135 patients with severe asthma, who were being treated with oral corticosteroid (OCS), high-dose inhaled corticosteroid (ICS) plus an additional controller medication. The aim of the study was to evaluate the reduction in daily OCS use, while maintaining asthma control with mepolizumab 100mg SC, every 4 weeks in comparison to placebo. In SIRIUS, the daily dose of oral corticosteroid (OCS) was significantly reduced during weeks 20-24 when compared with the pre-determined dose during the optimization phase.

In both MENSA and SIRIUS trials, patients experienced an improved control of asthma thereby having a higher quality of life.

Mepolizumab was studied in hypereosinophilia syndrome, which is a heterogeneous group of disorders defined by peripheral eosinophilia and eosinophil-related end-organ manifestations. A double blind, placebo-controlled clinical trial evaluated the efficacy of mepolizumab as a corticosteroid-sparing agent in patients with FIP1L1/PDGFRA-negative HESs. The primary end point included maintenance of disease control with <10 mg/d prednisone for a period of >8 consecutive weeks. The primary end point was achieved in a significantly higher proportion of subjects who received mepolizumab compared with those receiving placebo (84% vs 43%, P <.001). There was a significant reduction in the mean dose of prednisone required 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). More subjects were able to discontinue prednisone until the end of study (47% on mepolizumab vs 5% in the placebo group, P < .001). Mepolizumab was well tolerated and effective with repeated dosing over nine months. Despite these results, mepolizumab was not approved by the Food and Drug Administration for the treatment of HES [27] and is available only for compassionate use in patients with life-threatening, treatment-refractory disease.

Mepolizumab was also studied in patients with eosinophilic granulomatosis with polyangiitis (EGPA). This is a multisystem disorder characterized by asthma, peripheral eosinophilia, and small to medium vessel eosinophilic vasculitis. Mepolizumab has been found to be successful in the treatment of a patient with treatment-refractory EGPA [28]. Two small open-label pilot studies of monthly mepolizumab infusions were conducted in patients with corticosteroid-dependent EGPA. It was shown that treatment with mepolizumab suppressed the clinical symptoms and eosinophilia, despite tapering of maintenance corticosteroid therapy. Once mepolizumab was discontinued, eosinophilia recurrence take place. These studies demonstrated that eosinophils play an important role in disease pathogenesis [29].

Mepolizumab has been studied in eosinophilic esophagitis. This is a chronic disorder characterizedby the presence of esophageal eosinophilia. Initial safety trials in four patients, with monthly mepolizumab thearapy showed a significant decrease in esophageal eosinophilia and peripheral eosinophilia with a marked improvement in clinical symptoms. Further double-blind and placebo-controlled studies failed to demonstrate the clinical efficacy of mepolizumab therapy in patients with eosinophil esophagitis despite reduction in blood and esophageal eosinophilia [30].

Mepolizumab treatment studies in atopic dermatitis patients did not show significantly promising results. Eosinophils are typically prominent in skin biopsies from patients with atopic dermatitis and have been implicated in disease pathogenesis. In patients with atopic dermatitis, following allergen challenge by intravenous administration, a reduction was present in eosinophil infiltration in skin biopsies; however the size of the reaction was unaffected. In18 patients with severe atopic dermatitis, mepolizumab given as two weekly doses showed a marked reduction in peripheral eosinophil counts, however, mepolizumab was ineffective in completely alleviating clinical symptoms or reducing the size of the reaction to allergy patch testing.

In placebo-controlled clinical trials, mepolizumab was further assessed in 30 patients with treatment-refractory severe nasal polyposis. The study demonstrated a statistically significant reduction in polyp size in patients treated with mepolizumab as compared to placebo.

Reslizumab

Target: Reslizumab is another monoclonal antibody designed to target IL-5. It is a humanized anti-IL-5 mAb which binds to IL-5 and interfere in its binding to IL-5Rα on the eosinophil surface.

Conditions: Reslizumab is in clinical development for the treatment of eosinophilic inflammatory disorders, such as eosinophil esophagitis and asthma. Reslizumab has high affinity for human IL-5 and inhibits the IL-5–dependent proliferation of the human erythroleukemic cell line TF-1.

Clinical Trial studies: Pre-clinical animal experiments with reslizumab demonstrated a marked inhibition in the development of pulmonary eosinophilia, bronchoconstriction, cutaneous eosinophilia, and esophageal eosinophilia. Based on these results further clinical trials were conducted to study the effectiveness of reslizumab in in patients with poorly controlled eosinophilic asthma despite inhaled corticosteroids [31]. Reslizumab therapy was found to be effective in theses patients.

Data from two 52-week Phase III global trials of reslizumab showed that compared to placebo, treatment with reslizumab significantly reduced the annual rate of clinical asthma exacerbations (Study 1, 50% and Study 2, 59%); lung functions was significantly improved; and there were sustained improvement in multiple secondary measures of asthma control in patients with asthma and elevated blood eosinophils who were inadequately

controlled on an inhaled corticosteroid (ICS)-based regimen. Reslizumab reduced the annual frequency of clinical exacerbations by at least half compared to placebo (Study 1, 50% and Study 2, 59% respectively). Lung function also improved in week four and was maintained through one year in both studies. Furthermore, significant improvements were observed in the Asthma Quality of Life, Asthma Control Questionnaire and Asthma Symptom Utility Index scores. Common adverse events reported with reslizumab treatment were comparable to placebo and included worsening of asthma, nasopharyngitis, upper respiratory infections, sinusitis, influenza and headache. Two anaphylactic reactions reported were resolved following medical treatment.

Eosinophilic esophagitis (EoE) is a chronic disorder characterized by the presence of esophageal eosinophilia. Reslizumab therapy has been assessed for adult or pediatric patients with persistant eosinophil esophagistis despite reduction in blood and esophageal eosinophilia. The double-blind, placebo-controlled clinical trials have failed to demonstrate the clinical efficacy of reslizumab in this patient group.

Reslizumab have also been assessed in patients with nasal polyposis. In 50% of the subjects nasal polyp score improved and correlated with IL-5 levels in nasal secretions at baseline [32].

Benralizumab

Target: Antibodies diected against IL-5Rα, inhibits the ligation of IL-5 to its receptor. Benralizumab is an afucosylated humanized mAb (IgG1k) that binds to human IL-5Rα, that inhibits IL-5 mediated cell proliferation by reducing IL-5 binding to its receptor and depletes IL-5Rα bearing cells through enhanced antibody-dependent cell-mediated cytotoxicity [33].

Conditions: Benralizumab is in clinical trials for asthma.

Clinical trial studies: In phase 1 clinical trials in patients with mild asthma, Beralizumab decreased mean peripheral eosinophil counts in a dose-dependent fashion. In the highest dose group, after a single intravenous dose, esoinopenia lasted greater than 12 weeks [34]. Reduction in serum ECP levels were observed 24 hours after the dose. Most frequently reported adverse events were nasopharyngitis, reduced white blood cell counts and increased blood creatinine phosphokinase levels. Benralizumab had an acceptable safety profile and resulted in marked reduction in eosinophil counts within 24 hours after dosing.

Based on results from initial phase 1 trial, the study was extended to a double-blind, placebo-controlled trial. The study demonstrated reduced airway eosinophil counts by 95.8%, sputum eosinophil counts by 89.9%, and blood eosinophilia by 100% in patients with eosinophilic asthma as compared to placebo group [35]. Bone marrow examination in five subjects showed reduced eosinophil precursors. No safety issues were identified in the trials.

Benralizumab administered every 4-8 weeks for 48 weeks in patients with asthma demonstrated a reduction in asthma exacerbations in patients with blood eosinophilia (>300/mL). In patients with acute asthma, a single dose of intravenous benralizumab showed a reduction in the rate and severity of subsequent exacerbations for 12 weeks.

ANTISENSE CCR3 AND COMMON B CHAIN (BC) OF IL-3, IL-5, AND GM-CSF RECEPTORS

TPI ASM8

Target: IL-5 is a key cytokine in eosinophil development, activation, and survival. IL-3 and GM-CSF also have similar actions. IL-3, GM-CSF and IL-5 share a common signaling receptor β chain. The responsiveness to these cytokines is controlled by cell surface expression of the cytokine-associated receptor α-chain. Down regulation of IL-3, IL-5 and GM-CSF by antisense oligonucleotides directed against the mRNA for human CCR3 and of the common chain (βc) of IL-3, IL-5 and GM-CSF receptors, down regulate expression of CCR3 and βc on the cell surface. TPI ASM8 (antisense CCR3 and common β chain) contains 2 phosphoro-thioate 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. TPI ASM8 downregulates the expression of CCR3 and βc on the cell surface.

Conditions: TPI ASM8 is developed for atopic asthma.

Clinical trial studies: In animal models TPI ASM8 equivalent has been shown to downregulate its targets and the resultant airway hyperresponsiveness and inflammation after allergen challenge. When administered to healthy subjects in 2 single-dose phase 1 studies, less adverse events were seen than 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 or placebo for four days to examine the effects of inhaled TPI ASM8 on allergen-induced sputum eosinophil counts; and CCR3 and bc mRNA levels in sputum cells. The study also assessed early and late asthmatic responses in patients with mild asthma after allergen challenge [36]. TPI ASM8 reduced allergen-induced sputum eosinophil counts by 46% on day 3. The allergen-induced (day 2 to day 3) levels of bc 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=5.039). The allergen-induced levels of CCR3 mRNA increased 1.4-fold with TPI ASM8 and 6.4-fold with placebo (p= 5.055). TPI ASM8 also significantly reduced the early asthmatic response (p=5.03). There were no serious adverse events from TPI ASM8 inhalation, and and were similar to placebo. TPI ASM8 was well tolerated. 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. [37].

EOSINOPHIL MODULATING THERAPIES- IGE , IL-4, IL-13, TSLP, IL-25, IL-33

Soluble mediators such as IgE, IL-4, IL-13, thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, are associated with eosinophilic inflammation, which might play a role in pathogenesis of eosinophil associated diseases like asthma and HESs. Biologic therapies targeting variety of such mediators provide promising treatment against many diseases involving these soluble mediators. Many biotherapeutics are currently available and developments are undergoing in preclinical and clinical trials for their ultimate clinical use.

BLOCKING IGE

Omalizumab

Many EADs are associated with elevated serum IgE levels with marked eosinophilia. Such EADs includes allergic asthma, eosinophilic gastrointestinal disorders, and lymphocytic variant HES. Omalizumab (anti-IgE) is a recombinant therapeutic mAb against IgE approved by FDA for use in the treatment of allergic asthma. Omalizumab binds to IgE and prevents its binding to FcεRI, leading to inhibition of mast cell and basophil activation [38].

Target: IgE antibodies are associated mainly with allergic reactions when the immune system reacts to environmental antigens such as pollen or dust mite and parasitic infections. Although the action of anti-IgE on immediate hypersensitivity is well established, less is known about the effects of anti-IgE therapy on inflammatory cells such as the eosinophil. Omalizumab a humanized monoclonal antibody that binds circulating IgE antibody, reduces circulating levels of IgE, FcoRI expression on mast cells, respiratory tissue eosinophilia, and production of GMCSF and Th2 cytokines (IL-4, IL-5, and IL-13) (150). Omalizumab is a treatment option for patients with moderate to severe allergic asthma whose asthma is poorly controlled with inhaled corticosteroids and inhaled long-acting ß2 agonist bronchodilators. Omalizumab reduces IgE mediated airway inflammation and may have a role in airway remodeling.

Conditions: Omalizumab has been developed for treatment of asthma.

Clinical trial studies: In one study, after 16 weeks of omalizumab treatment, both bronchial and sputum eosinophilia were reduced in asthmatic patients, despite a lack of improvement in methacholine PC_{20} [39]. In another study in mild asthma patients; treatment with Omalizumab for 12 weeks, demonstrated a significant reduction in EG2+ cell staining in the lung submucosa along with reduction in sputum eosinophil counts [40].

Significant improvement in FEV1 and peak flows was demonstrated. In a meta-analysis omalizumab reduced circulating levels of blood eosinophils in asthmatic patients receiving concomitant corticosteroid therapy. In an open-label study with nine patients treated with omalizumab, tissue eosinophilia was not significantly decreased despite a moderate reduction in peripheral eosinophilia and clinical improvements with eosinophilic gastritis and duodenitis [41]. In another placebo-controlled study in 30 patients with eosinophil esophagitis, the study failed to demonstrate an effect of drug on clinical symptoms or tissue eosinophilia. Overall, these clinical studies suggested that omalizumab is able to modulate eosinophil counts in blood and sputum and within the lung. A meta-analysis shows that omalizumab is associated with statistically significant relief in symptoms, decreased rescue medication use, and improvement of quality of life in patients with inadequately controlled allergic chronic rhino-sinusitis (151). In addition, a recent double-blind placebo control study showed that mepolizumab, a humanized monoclonal anti-IL-5 antibody, reduces the size of nasal polyps (152). IgE and/or IL-5 inhibition may be considered as a potential novel therapeutic approach in patients with chronic rhino-sinusitis with severe eosinophilic inflammation (153).

BLOCKING IL-4 AND IL-13

IL-4 and IL-13 are pleiotropic cytokines produced by various cell types including eosinophils. The receptors for IL-4 and IL-13 share a common α chain (IL-4Rα) and are also expressed on eosinophils. Both IL-4 and IL-13 play a major role in eotaxin-mediated recruitment of eosinophils to areas of allergic inflammation and promotion of eosinophil survival. IL-4 is also required for production of IL-5, and eosinophil differentiation in the bone marrow in the presence of IL-5 [42].

Lebrikizumab (MILR1444A)

Target: Lebrikizumab is a monoclonal antibody targeting IL-13

Conditions: Lebrikizumab has been developed for poorly controlled asthma despite ICS therapy

Clinical trial studies: In phase 2 trial, monthly lebrikizumab therapy improved lung function at 12 weeks in patients with poorly controlled asthma despite ICS therapy. This was seen only in a subset of patients with a T_H2 phenotype and elevated periostin levels [43]. However, clinical trial in patients with asthma who were not receiving ICS failed to demonstrate an effect irrespective of serum periostin levels. A Phase 3, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of lebrikizumab is ongoing. This study will evaluate the efficacy and safety of lebrikizumab in patients with asthma whose disease remains uncontrolled despite daily treatment with inhaled corticosteroid therapy and at least one second controller medication. Patients will

be randomized 1:1:1 to receive double-blind treatment with either lebrikizumab ("high" or "low") or placebo in addition to their standard-of-care therapy.

Tralokinumab (CAT-354)

Target: Tralokinumab is monoclonal antibody targeting IL-13

Conditions: Tralokinumab has been developed for poorly controlled asthma despite ICS therapy

Clinical trial studies: Although a trial with tralokinumab failed to meet its primary end point, clinical improvement was observed, especially in patients with increased levels of sputum IL-13 [44]. A phase 3 multicentre, randomized, double-blind, parallel group, placebo controlled, to evaluate the Efficacy and Safety of Tralokinumab in Adults and Adolescents With Asthma Inadequately Controlled on Inhaled Corticosteroid Plus Long-Acting B2-Agonist (STRATOS2) is underway. The primary ourcome include asthma exacerbation rate reduction and evaluation the effect of tralokinumab compared with placebo on the annualised asthma exacerbation rate in adult and adolescent subjects with asthma that is inadequately controlled with inhaled corticosteroid plus long-acting β2-agonist.

Anrukinzumab (QAX576)

Target: Anrukinzumab is an anti-IL 13 antibody.

Conditions: Anrukinzumab has been developed for eosinophil esophagitis.

Clinical trial studies: Anrukinzumab therapy has been studies in patients with eosinophil esophagitis. The data from a recent placebo-controlled study of the anrukinzumab, demonstrated a reduction in esophageal eosinophilia by 60% in patients with eosinophil esophagitis as compared with 23% in patients receiving placebo (p=0.004) [45]. A phase 2, randomised, double-blind, placebo-controlled study of anrukinzumab has ben done in patients with mild, atopic asthma [61]. The primary study endpoint was maximum change in FEV1 post-allergen challenge during early and late asthmatic response (EAR and LAR, respectively), compared with baseline values. Anrukinzumab significantly inhibited EAR and LAR area under the curve (AUC) values compared with placebo. No serious adverse events observed. Another phase 2, randomised, double-blind, placebo-controlled, parallel-arm study was completed in patients with persistent asthma. Results showed that anrukinzumab did not meet the clinical efficacy endpoint, and its development was subsequently terminated in May 2009.

INHIBITION OF BINDING OF IL-4 AND IL-13 TO IL-4R

Dupilumab and AMG 317

Target: IL-4, IL-13 and common α chain (IL-4Rα) of the receptors for IL-4 and IL-13 expressed on different cells, including eosinophils. Dupilumab (REGN668) and AMG 317 (Amgen) are antibodies to IL-4Rα that inhibit signaling of both IL-4 and IL-13.

Conditions: Asthma, nasal polyposis, atopic dermatitis

Clinical trial studies: In a phase 2b, placebo-controlled trial in patients with eosinophilic asthma, weekly duplimab treatment decreased asthma exacerbations and improved lung function after the withdrawal of inhaled corticosteroids and long-acting beta-agonist therapies [46]. Duplimab also improved clinical symptoms in placebo controlled trials in patients with atopic dermatitis.

Phase 2 clinical efficacy trial of AMG 317 in patients with moderate to severe asthma failed to demonstrate efficacy. It was observed that subjects with more severe disease were more likely to respond and a dose effect was observed [36].

A consistent finding in studies targeting IL-4 and/ or IL-13 has been the lack of effect on peripheral blood eosinophilia [43–44, 46]. A reduction in fractional exhaled nitric oxide which is a surrogate marker of sputum eosinophilia has correlated with treatment response in some studies [43]. This suggests a relatively greater effect of IL-4/IL-13 blockade on airway eosinophils, this has not been confirmed in all studies and could be confounded by the direct effect of IL-13 on nitric oxide synthase.

Pascolizumab (SB 240683) and Altrakincept (NUVANCE)

Target: Pascolizumab is monoclonal antibody targeting IL-4. Altrakincept is a monoclonal antibody targeting IL-4 receptor.

Conditions: Pascolizumab has been developed for asthma.

Clinical trial studies: In vitro studies confirm the specificity of pascolizumab for IL-4. In vitro, the antibody inhibits the downstream events associated with IL-4 stimulation, including IgE and CD23 up-regulation and T-cell proliferation. Pascolizumab may therefore be of potential benefit in the treatment of chronic asthma. Further clinical trials of pascolizumab (monoclonal antibody targeting IL-4) or altrakincept (monoclonal antibody targeting IL-4 receptor) have been disappointing. The anti-IL-4 antibody pascolizumab did not show any clinical benefit in a six month clinical trial in steroid-naïve patients with uncontrolled asthma [11].

Pitrakinra (AER001; BAY 16-9996; IL-4/IL-13 receptor antagonist)

Target: Pitrakinra is a recombinant variant of IL-4 with two 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 and 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.

Conditions: Pitrakinra has been developed for treatment of asthma

Clinical trial studies: Two randomised controlled trials were conducted using different routes of administration. In the first trial, 24 patients with atopic asthma received pitrakinra 25mg or placebo once daily by subcutaneous injection. In the second, 32 patients with atopic asthma received pitrakinra 60mg or placebo twice daily by nebulisation. Inhaled allergen challenge was carried out at baseline and after four weeks of treatment [47]. The decrease in forced expiratory volume in one second (FEV1) during the late phase response to allergen challenge was attenuated after four weeks of treatment with inhaled pitrakinra compared with placebo [48]. In the subcutaneous pitrakinra group there was a non-significant decrease in FEV1. There were also fewer asthma attacks requiring beta agonist rescue in the subcutaneous pitrakinra group. Pitrakinra had no effect on the early response to allergen challenge. There was no effect on the early response or airway hyper responsiveness. There was a significant reduction in baseline exhaled nitric oxide levels after treatment with pitrakinra but no effect on post challenge exhaled nitric oxide levels, sputum or blood eosinophilia, or total IgE levels.

Based on these two 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 [48]. There was a significant reduction in exacerbations in a subgroup of patients with a high peripheral blood eosinophil count. Pitrakinra also demonstrated a significant interaction between anti–IL-4R therapy and IL4R gene variation, identifying a pharmacogenetic subgroup that was more responsive to therapy with this antagonist.

TSLP, IL-25, AND IL-33

The CD4+ T helper cells (T_H2 cells) mediate the activation and maintenance of humoral or antibody-mediated, immune response against extracellular parasites, bacteria, allergens, and toxins. T_H2 cells mediate these functions by producing various cytokines such as IL-3, IL-4, IL-5, IL-6, IL-9, IL-13, and IL-17E (IL-25) and thymic stromal lymphopoietin (TSLP) that are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses [49]. Epithelial cells in response to allergen or parasitic infestation release a number of mediators like TSLP, IL-33, and IL-25, which play an important role in driving T_H2-mediated immune-inflammatory responses including increased production of eosinophils. Many cell types have been shown to be capable of responding to TSLP. These include dendritic cells, CD4 and CD8 T-cells, B cells, mast cells, basophils, eosinophils, and NKT cells. IL-33 has emerged as an important mediator in the immunopathogenesis of

allergy and asthma. IL-33 exacerbates eosinophil-mediated airway inflammation. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma [50]. IL-25 is a member of the IL-17 family of cytokines. IL-25 promotes T helper (Th 2) responses. IL-25 also regulates the development of autoimmune inflammation mediated by IL-17–producing T cells.

AMG 157

Target: Single-nucleotide polymorphisms in TSLP are associated with increased and decreased susceptibility to asthma, atopic disease, and EoE [51]. AMG 157, is a monoclonal antibody that inhibits the activity of TSLP.

Conditions: Asthma, atopic disease, and EoE

Clinical trial studies: In a phase 1, multicenter proof-of-concept trial in 31 patients with asthma, treatment with AMG 157 (antieTSLP antibody; MEDI9929) significantly reduced peripheral and sputum eosinophilia, allergen-induced bronchoconstriction, and airway inflammation [51]. AMG 157 resulted in statistically significant reductions in early asthmatic responses (EAR) and late asthmatic responses (LAR) in the airways following allergen challenges in patients with allergic (atopic) asthma. The data also showed statistically significant decreases in baseline markers of inflammation in the airways. Overall, adverse events were similar across treatment and placebo groups with no serious adverse events occurring in the study.

Brodalumab

Brodalumab (Amgen), a human mAb against IL-17 receptor-A which also blocks the effects of IL-25. In clinical trials it did not reduce clinical symptoms, airway reactivity, or eosinophilia in patients with severe asthma [52].

BLOCKING CCR 3 (CCL-11 AND EOTAXIN)

The chemokine receptor CCR3 is expressed primarily on eosinophils and basophils and has multiple ligands, including CCL-11, -24, and -26 (eotaxins). In contrast to IL-5Rα, the expression of CCR3 on eosinophils is positively correlated with disease severity in patients with asthma [53]. This lead to the interest in developing targeted therapy against CCR3. But despite reduction of airway eosinophilia with anti-CCR3 antibody treatment in a murine model, human trials have not been initiated.

Bertilimumab

Target: The chemokine receptor CCR3 ligands including CCL-11, -24, and -26 (eotaxins). Bertilimumab is a humanized anti-CCL11 IgG4 antibody (CAT-213) in clinical trials for ulcerative colitis and bullous pemphigoid, disorders characterized by elevated eotaxin-1 levels. Studies in severe asthma are planned.

Conditions: Developed for asthma

Clinical trial studies: Planned in asthma

INDUCTION OF APOTOSIS IN EOSINOPHILS

Siglec-8 (sialic acid binding immunoglobulin-like lectin 8) and other inhibitory receptors.

Target: Siglec-8 is an inhibitory receptor that is highly expressed on mature eosinophils, mast cells, and basophils [54]. Antibody to Siglec-F, the murine orthologue of Siglec-8, reduces blood and tissue eosinophilia in mice. Anti Siglec-8 antibody induces apoptosis of human eosinophils in vitro.

Conditions: Asthma

Clinical trial studies: Anti-Siglec-8 antibodies are still in preclinical development. They have some unique advantages as therapeutic agents for EADs due to their mechanism of action. First, adverse effects due to release of eosinophil granule proteins and other mediators are unlikely to occur with apoptotic cell death of eosinophils, even in patients with marked eosinophilia. Second, efficacy may be increased in patients with activated eosinophils. Finally, Siglec-8 inhibits degranulation of mast cells. This may be useful in disorders such as eosinophil esophagitis in which tissue mast cells may play a role in disease pathogenesis [55].

DEPLETION OF EOSINOPHILS THROUGH ENHANCED ANTIBODY-DEPENDENT CELL CYTOTOXICITY (ADCC)

EMR1 (human epidermal growth factor like module containing mucin-like hormone receptor 1)

EMR1 is a surface receptor of unknown function that belongs to the EGF-7 transmembrane family of G protein coupled receptors. Human EMR1 expression is restricted to mature blood and tissue eosinophils [56]. Afucosylated monoclonal anti-EMR1 antibody dramatically enhances natural killer cell mediated destruction of eosinophils.

CD 52 (Direct destruction by ADCC)

Alemtuzumab.

Target: CD52 is a cell surface glycoprotein present on eosinophils, monocytes, T and B cells, macrophages and natural killer cells. Alemtuzumab (Campath-1H; anti-CD52) is an IgG1k mAb reactive with CD52.

Conditions: It is currently approved for the treatment of B-cell chronic lymphocytic leukemia. Clinical trials has been conducted on HESs patients.

Clinical trial studies: Alemtuzumab has been tested in hypereosinophilic syndrome. Potential mechanisms of action of alemtuzumab in HESs include direct destruction of eosinophils by means of ADCC or opsonization with removal by the reticuloendothelial system or indirect reduction of eosinophilia through its effect on lymphocytes, or both. 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. A 39-year old woman had eosinophilia, pruritic rash, fever, and malaise [57]. After bone marrow transplantation, 30 mg alemtuzumab was administered subcutaneously every 3 weeks. Alemtuzumab therapy controlled and maintantined eosinophilia with beneficial effects observed for 2½ years [58]. Alemtuzumab were also found to be beneficial in patient with HESs and multiple myeloma for two years, but the patient became resistant afterwards. Two brief case reports reported that alemtuzumab reversed encephalopathy associated with HESs and resolved cardiac wall thickening and tethering of the mitral valve associated with HESs. A case series described 11 patients with eosinophilia who were treated with alemtuzumab; two patients were diagnosed as chronic eosinophilic leukemia [59]. These patients had received glucocorticoids, imatinib, IFN-α, dasatinib, and nilotinib previously and were then treated with alemtuzumab. Ten of the 11 patients achieved complete hematologic remission at a median time of two weeks, which was maintained for a median time of three months, with a range from 1.5 to 17 months. Of the patients achieving complete hematologic remission, seven relapsed and subsequently complete hematologic remission was achieved in 2 of these seven patients after retreatment. 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.

CD2- INHIBITION OF COSTIMULATION AND ACTIVATION OF T CELLS

Alefacept

Target: CD2 is a cell adhesion molecule expressed by T cells and natural killer cells. It has also been called T-cell surface antigen T11/Leu-5, LFA-2, LFA-3 receptor. [1] It helps T cells adhere to antigen-presenting cells, and initiates signal transduction pathways that enhance signalling through the T cell receptor for antigen. CD2 is a specific marker for T cells and NK cells. Alefacept (Amevive; ASP0485; CD2-binding fusion protein) is a fusion protein composed of the first extracellular domain of lymphocyte function–associated antigen 3 (CD58) and the human IgG1 Fc domain [60]. Binding of the lymphocyte function–associated antigen 3 fragment to CD2 blocks costimulation and activation of T cells. Furthermore, by binding to CD2 and the FcgR receptors, particularly FcgRIII (CD16), alefacept mediates cognate in-teraction between T cells and NK cells, resulting in T-cell apoptosis [61].

Conditions: Psoriasis, atopic dermatitis

Clinical trial studies: Alefacept decreased the number of memory CD41 and CD81 cells, as well as

activated (CD251) T cells, in lesional skin and synovial tissue, in patients with psoriasis [41] Although alefacept has not been used for the treatment of systemic eosinophilic disease, reduction of blood and skin eosinophilia in 10 patients with atopic dermatitis treated with alefacept was observed [62] Skin biopsy specimens in alefacept treated subjects revealed a significant reduction in dermal infiltrating cell counts and cytokine expression, particularly IL-5 and IL-13. Clinical improvement was observed in all 10 patients. In a second pilot study of alefacept in patients with AD, symptoms were reduced in six of nine patients. Unfortunately, manufacture of alefacept was discontinued in December 2011.

EOSINOPHIL MIGRATION – TARGETING VERY LATE ANTIGEN-4 (VLA-4)

Natalizumab

Adhesion molecules like VLA-4 (a4b1 integrin) has been shown to be critical for eosinophil recruitment into the lung after allergen challenge in mice [63]. In preclinical studies blockade of VLA-4 resulted in significantly reduced tissue eosinophil counts [64] Although natalizumab, 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.

SUMMARY AND CONCLUSIONS

Eosinophils play a central role in pathophysiology of eosinophil-associated diseases (EADs). Some EADs include hyper eosinophilic syndrome (HES), eosinophil myalgia syndrome, eosinophilic gastrointestinal disorders, eosinophil esophagitis, nasal polyposis, eosinophilic granulomatosis with polyangitis and other clinical subtypes which includes allergic disorders such as asthma. Eosinophils are sources of many mediators of inflammation and immune responses, including lipid mediators (eg, leukotriene C4, platelet-activating factor, and eoxins) and eosinophil granules containing principal cationic proteins (ie, Major Basic Protein (MBP), Eosinophil Cationic Protein (ECP), Eosinophil Derived Neurotoxin (EDN), and Eosinophil Peroxidase (EPO)), which can be secreted, causing significant tissue damage. Various strategies have been employed for development of biotherapeutics for eosinophil-associated diseases. IL-5 and IL-5R are ideal targets for the treatment of EADs. IL-5 has a role in differentiation, activation and survival of eosinophils. Targeting IL-5 and IL-5R in preclinical and clinical settings gave promising results. Other modalities used for development of biologic medicines for eosinophil-associated diseases include eosinophil modulating therapies targeting IGE, IL-4, IL-13, TSLP, IL-25, IL-33, destruction of eosinophils by means of ADCC or opsonization with removal by reticuloendothelial system, targeting key eosinophil survival cytokines, IL-5, IL-3 and GM-CSF, and blocking eosinophil migration into inflamed tissues is another promising strategy for the reduction of end-organ manifestation of eosinophilia. Eosinophil-associated disorders are considered "orphan" diseases and nearly all the current treatments for patients with these conditions are used off-label (e.g., alpha-interferon). New therapeutic targets that offer enhanced treatment selectivity and benefit for these conditions need to be identified and developed. Reliable biomarkers of eosinophil involvement and of disease activity are largely lacking. Without this knowledge, the current approach toward disease management is imprecise and therefore, the development of new treatment modalities for these conditions is limited. Furthermore, specific clinical and surrogate outcomes to be used in clinical trials for these conditions are not available.

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Author Contributions

Anubha Singh – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Deepak Kumar Singh – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published
Usha Bhoria – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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