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PII: S0024-3205(20)31733-1

DOI: https://doi.org/10.1016/j.lfs.2020.118973

Reference: LFS 118973

To appear in: *Life Sciences*

Received date: 2 October 2020

Revised date: 22 December 2020

Accepted date: 23 December 2020

Please cite this article as: L.-Y. Lee, G.S.Y. Hew, M. Mehta, et al., Targeting eosinophils in respiratory diseases: Biological axis, emerging therapeutics and treatment modalities, *Life Sciences* (2018), https://doi.org/10.1016/j.lfs.2020.118973

Targeting eosinophils in respiratory diseases: biological axis, emerging therapeutics and treatment modalities

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Abstract

Eosinophils are bi-lobed, multi-functional innate immune cells with diverse cell surface receptors that regulate local immune and inflammatory responses. Several inflammatory and infectious diseases are triggered with their build up in the blood and tissues. The mobilization of eosinophils into the lungs is regulated by a cascade of processes guided by Th2 cytokine generating T-cells. Recruitment of eosinophils essentially leads to a characteristic immune response followed by airway hyperresponsiveness and remodelling, which are hallmarks of chronic respiratory diseases. By analysing the dynamic interactions of eosinophils with their extracellular environment, which also involve signalling molecules and tissues, various therapies have been invented and developed to target respiratory diseases. Having entered clinical testing, several eosinophil targeting therapeutic agents have shown much promise and have further bridged the gap between theory and practice. Moreover, researchers now have a clearer understanding of the roles and mechanisms of eosinophils. These factors have successfully assisted molecular biologists to block specific pathways in the growth, migration and activation of eosinophils. The primary purpose of this review is to provide an overview of the eosinophil biology with a special emphasis on potential pharmacotherapeutic targets. The review also summarizes promising eosinophil-targeting agents, along with their mechanisms and rationale for use, including those in developmental pipeline, in clinical trials, or approved for other respiratory disorders.

Keywords: Respiratory diseases; Pulmonary; Eosinophils; Targeted therapies; Immunity; Lung

1.0 Introduction

1.1 Role of eosinophils in host defense and immunity

In 1879, Paul Ehrlich was the first person to recognize the unique ability of eosinophils to stain using acidophilic dyes (1). For many years, they were recognized as cells with end-stage effector functions in helminth infections and tissue damage (1). However, the plethora of clinical studies carried out in previous years have helped establish the crucial role of eosinophils in host defense, allergic inflammation, innate and adaptive immunity (1,2). Importantly, the interaction of eosinophils with B-cells allows them to process antigens, stimulate T-cells and induce humoral responses (1). Inflammatory and adaptive responses can also be initiated by eosinophils through their bidirectional interactions with dendritic cells (DCs) and T-cells (3). Activated eosinophils are able to release a large assortment of newly synthesized as well as pre-formed mediators, such as cytotoxic granule proteins, cytokines, chemokines, and lipid mediators which contributes to the various activities of eosinophils in inflammatory and infectious responses (4).

Eosinophils are now recognized as regulatory cells with the proven ability to influence and enhance local inflammation, instead of just simply effector granulocytes with cytotoxic activities (5). The immunomodulatory role played by eosinophils include mediating aluminum hydroxide-induced B-cell priming, acting as an antigen-presenting cell (APC) for T-cells, influencing T-cell differentiation (i.e. Th1 or Th2), and recruiting T-cells, DCs and macrophages to inflammatory sites in the host (6,7). Eosinophils were shown to regulate DCs and Th2 pulmonary immune response following an allergen challenge in mouse models (8). Moreover, a unique function of eosinophil is the suppression of DC-mediated Th17 production (8). Thus, eosinophils are important modulatory cells to maintain the equilibrium between DC-mediated Th2 and Th17 signaling pathways following an allergen exposure and subsequently, the allergic airway inflammation progression (8). Recently, it was discovered that eosinophils are necessary for the long-term preservation of plasma cells in the bone marrow and eosinophil depletion induces bone marrow plasma cells apoptosis (9). Wu *et al*., also reported that eosinophils regulate glucose homeostasis by preserving adipose tissue alternatively-activated macrophages (AAMs) via the secretion of IL-4 cytokine (10). As a regulator of innate immune response, eosinophils are also responsible for apoptotic cell clearance in the thymus (11).

Similar to other granulocytes, eosinophils undergo development and differentiation in the bone marrow (12). Upon maturation, eosinophils are distributed in various organs in the body, such as blood, lung, uterus, thymus, spleen, mammary gland, adipose tissue, and gastrointestinal tract (GI), to carry out their physiological functions under homeostasis (6,12). The recruitment of mature eosinophils from the systemic circulation to the inflammatory sites occurs following the overexpression of eosinophil-specific chemokines in response to stimuli (12). Interleukin-5 (IL-5) is the most important cytokine responsible for eosinophil differentiation, priming and survival and its main sources of production are type 2 T-helper (Th2) cells and type 2 innate helper lymphoid cells (ILC2) (6,12).

1.2 Cytokines, chemokines, receptors, and surface markers

A broad variety of cytokines, lipid mediators and other major molecules are expressed and secreted by eosinophils (Figure 1). These molecules are stored in eosinophilic granules and rapidly secreted in response of a specific stimuli, hence altering the external microenvironment and cellular functions. Eosinophils are distinguished from other lymphocytes, such as T-cells and B-cells, due to their ability to store and rapidly release pre-formed cytokines within minutes in response to stimuli (13,14). Besides pre-formed cytokines, eosinophils can carry out *de novo* synthesis and secretion of other immunological factors (14). It was hypothesized that the binding of soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNARES), which are part of the membrane fusion complexes, regulates the final steps of cytokine secretion from eosinophilic crystalloid granules and secretory vesicles (14).

Eosinophils are a major source of IL-5 cytokine, which is important for its differentiation, survival and chemotaxis (15). Other Th2 immunomodulatory cytokines secreted by eosinophils include IL-4 and IL-13 (13). Besides Th2 cytokines, other cytokines with Th1 and regulatory capacities released by eosinophils include IL-6, IL-10, IL-12, TNF-α, TGF-β, and IFN-γ (13). IL-6, IFN-γ, and TNF-α causes tissue damage through their pro-inflammatory actions whereas TGF-β contributes to airway remodeling via its role in epithelial changes, subepithelial fibrosis and microvascular changes (16,17). As for the lipid mediators, eosinophils releases a large quantity of prostaglandins, leukotrienes, and platelet-activating factors (PAF) (16). Eosinophils express a wide variety of receptors and molecular surface markers on their cell surfaces, including IL-5Rα, prostaglandins (CRTH2), CC-chemokine receptor (CCR)-3, sialic acid-binding immunoglobulin-like lectin 8 (SIGLEC-8),

leukotriene B4 receptors (IL-4R, IL-5R, IL-33R, IFN-γR, TGF-βR, CCR1, CCR3, CCR4, and TSLPR), PAF-receptor, FcαR, FcγR, and pattern-recognition receptors (PRRs) (18). The most prominent cytokine receptor present on human and mice eosinophils is IL-5Rα where the main receptors which define the distinct biology of eosinophils are CCR3 and SIGLEC-8 (19). Human eosinophils uniquely express SIGLEC-8 and the binding of antibodies or glycan ligands with this structure leads to apoptosis of eosinophils (20). The PRRs families expressed by human eosinophils include Toll-like receptor (TLR) family (TLR1-5, TLR7, TLR9), C-type lectin receptor (CLR) (Dectin-1), nucleotide-binding oligomerization domain (NOD)-like receptors (NOD1, NOD2), and receptors for advanced glycation end-products (RAGE) (19,21,22). Interaction with eosinophilic PRRs induces eosinophil survival, oxidative burst, adhesion system activation, and mediator release (21).

Integrins are cell surface proteins commonly found on immune cells which functions to merge the intracellular and extracellular domains of the immune system. Eosinophils express several integrins, such as α4β1 (CD49d/CD29), α6β1 (CD49f/ CD29), αLβ2 (CD11a/CD18), αMβ2 (CD11b/CD18), αXβ2 (CD11c/CD18), αDβ2, and α4β7 (23). These integrins interact with not only their respective ligands, but also vascular adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), periostin, laminin, and fibronectin located in the extracellular matrix or other cells (23).

Furthermore, blood eosinophils constitutively expresses selectins, including P-selectin glycoprotein ligand-1 (PSGL-1, CD162) and L-selectin (CD62L) (24). A high level of surface P-selectin results in eosinophilic β_1 integrin activation *in vivo* and thus, stimulates eosinophilic adhesion to VCAM-1 and migration to the airway (24).

Notch receptors and Notch ligands are also expressed on human blood eosinophils, which serve as important components for eosinophil autoregulation (25). A study showed that granulocyte-macrophage colony-stimulating factor (GM-CSF) influences the expression and activation of Notch molecules and thus, affects the activity and survival of eosinophils (25).

1.3 Eosinophilic granule proteins

Eosinophils store pre-formed enzymatic and non-enzymatic cationic proteins in large secondary granules which are selectively secreted in response to specific stimuli (26). The release of these granule proteins have typically be considered as the primary effector mechanism of eosinophils against specific parasites and in allergic inflammation (27). The antibacterial effect of eosinophils *in vivo* is specifically mediated thorough the release of cationic secondary granule proteins (28). This dominant population of cytoplasmic crystalloid

granules containing cationic proteins distinguishes eosinophils from other leukocytes (29). The four notable granule proteins present in human eosinophils include eosinophil peroxidase (EPO), major basic protein (MBP), and eosinophil-associated RNases (EARs) which are eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) (19,29).

EPO is the cationic protein present most abundantly in the matrix of the crystalloid granules (16). EPO contributes to eosinophil function by generating reactive oxidizing species (ROS) as well as acting as a direct toxin against mammalian cells and parasites (16). The promotion of oxidative stress by EPO results in cell apoptosis and necrosis (19,30). A study carried out by Panagopoulos *et al*., reported that EPO and other peroxidases play a part in angiogenesis in addition to cellular proliferation, migration, and invasion (31). Ochkur *et al*., also demonstrated the use of a sensitive and specific ELISA for the detection of EPO (32).

MBP exerts direct toxicity which contributes to its function in altering membrane permeability and enzyme functions in mammalian cells, microbes, and helminths (16,19). As for its role in the pathophysiology of asthma, MBP induces bronchoconstriction and epithelial tissue damage (16). MBP was also reported to have pro-angiogenic effects *in vitro* and *in vivo* (33).

ECP and EDN are part of the ribonuclease A superfamily (16). EDN demonstrates cytotoxicity, neurotoxicity, and antiviral activity (single-stranded RNA virus), but it exerts low toxicity towards mammalian cells and parasites (16). Tsuda *et al.,* recently reported that EDN stimulates matrix metalloproteinase 9 (MMP-9) production from nasal epithelium, thus indicating its potential role in the pathogenesis of eosinophilic chronic rhinosinusitis (ECRS) (34). Conversely, ECP shows distinct toxicity towards a large variety of bacteria, helminths, single-stranded RNA viruses, and host tissues (16). ECP exerts its toxic effects through formation of pores in target membranes (19). Besides its cytotoxic effects, ECP also stimulates airway mucus hypersecretion and mast cell degranulation, as well as suppresses B-cell immunoglobulin synthesis and T-cell proliferative responses (19). EDN is known to possess multifunctional properties and may contribute to innate immunity by either killing or inactivating viral invaders (35). In 1998, the potential of EDN as an antiviral agent against respiratory syncytial virus was demonstrated under *in-vitro* settings (36). Domachowske and colleagues demonstrated a dose-dependent decline in the infectivity of respiratory syncytial virus B after the introduction of eosinophils into the viral suspension (36). Interestingly, the inclusion of ribonuclease inhibitor was found to reverse the antiviral effect observed by eosinophils, suggesting a role of EDN (eosinophil secretory ribonuclease). The suggestive

antiviral role of EDN was confirmed when a 40-fold decrease in infectivity, after the introduction of EDN into the suspension of respiratory syncytial virus B was obtained. Importantly, the inactivated form of EDN was reported to have no antiviral effects. Furthermore, the observed antiviral activity of EDN was found to be the direct ribonucleolytic destruction of extracellular virions. These findings collectively suggest that EDN may also be used as a therapeutic agent for the management of respiratory syncytial virus (36). Furthermore, Rugeles *et al*., reported that EDN was responsible for the majority of anti-HIV-1 activity shown by alloantigen-stimulated factors in the supernatant of mixed lymphocyte reactions (37).

Additionally, another important protein found in primary granules of eosinophils is galectin-10, or Charcot-Leyden crystal protein. Galectin-10 was shown to be strongly associated with sputum eosinophilia and hence, can function as a potential alternative biomarker of eosinophil-associated airway inflammation (38).

1.4 Degranulation

The extracellular release of eosinophilic granule proteins is termed as degranulation. Piecemeal degranulation (PMD) is generally acknowledged as the most prevalent manner of eosinophilic degranulation (16,19) .During PMD, the contents of the granule are selectively packaged into vesicles which are transported across the cytoplasm and fused with the cell membrane in order to extracellularly release the granule proteins at the cell surface (29). Cytokines and chemokines which induces PMD are IFN-γ, CCL-11 (eotaxin-1), and TNF-α (16,39). Following the release of its granule proteins via PMD, the eosinophil remains fully functional and responsive to other stimuli. An example of PMD is the release of IL-4 from eosinophils stimulated with eotaxins, whereby secretory vesicles deliver IL-4/IL-4R α complex, which is initially formed inside the granule membrane, to the cell surface and subsequently released into the extracellular space (19).

Another mechanism of degranulation observed in eosinophils is associated with cytolysis, in which the eosinophil undergoes cell lysis in a manner that morphologically differs from either apoptosis or necrosis, thus releasing intact, cell-free, membrane-bound granule proteins which are fully competent (29). The manner of cell death in eosinophilic cytolysis is termed as extracellular trap cell death (ETosis), whereby the nuclear membrane disintegrates and DNA de-condenses into the surrounding cytoplasm (16). Cytolysis is

acknowledged as a common method for the release and deposition of cell-free eosinophilic granules in eosinophil-associated disorders (29).

Lastly, eosinophil degranulation can also occur by classical exocytosis, in which the fusion of intracellular granules with the plasma membrane precedes the extracellular release of the total granule contents (40). This mechanism of degranulation does not occur commonly in eosinophilic diseases, except in the presence of parasitic helminths or specific fungi (40).

Although degranulation is the primary mechanism in which eosinophils exert their function, this process does not occur during transit in blood circulation. Instead, granules are commonly released when the eosinophils arrive at the site of inflammation (41).

1.5 Hypereosinophilic syndromes

Hypereosinophilic syndromes (HES) comprise of a heterogenous collection of disorders with common features of elevated blood and tissue eosinophils and tissue damage (42,43). The clinical manifestations are variable and may include any organ system, but most commonly the skin (42). Since 1975, the definition of HES includes 3 criteria: (i) blood eosinophil count ≥1500/mm^3 for >6 months (or mortality within 6 months associated with signs and symptoms of hypereosinophilic disease), (ii) insufficient evidence of other causes of eosinophilia (e.g. parasite, allergy), and (iii) presumptive signs of organ involvement (e.g. GI dysfunction, heart failure, central nervous system impairment, weight loss, or fever) (44). However, there are several shortcomings with these definitions of HES. Marked eosinophilia is typically caused by helminth infections, but other non-infectious causes include malignancies, drug reactions, immunologic, allergic and inflammatory diseases (45).

2.0. Eosinophils and asthma

2.1 Eosinophils in the Pathophysiology of Asthma

2.1.1 Eosinophilic asthma

Asthma is an inflammatory respiratory disorder outlined by airway hyperresponsiveness (AHR), airway obstruction, mucus hypersecretion, airway inflammation, tissue damage and airway remodeling (16,46–48). According to a study conducted by the Global Burden of Disease (GBD), it was estimated that around 339.4 million people globally were affected by asthma in 2016 (49). Eosinophils have been closely related to the pathophysiology of asthma, as reported from the elevation of eosinophil numbers in peripheral blood and bronchoalveolar lavage fluid (BALF) of asthmatic patients

(16,46). In a certain percentage of patients, eosinophilic airway inflammation represents the occurrence of bronchial asthma (50). Eosinophils are pro-inflammatory granulocytes with major contributions to the inflammatory responses of asthmatic patients, especially in T2-high asthma phenotypes such as severe eosinophilic asthma, by releasing inflammatory mediators to trigger an inflammatory cascade as well as exerting toxic effects directly on host tissues (51). Severe asthma is a heterogenous disorder as different phenotypes exists, such as eosinophilic asthma. Eosinophilic asthma can be clinically characterized by its severity and frequent exacerbations (50). Additionally, other features of eosinophilic asthma includes sputum and airway eosinophilia, elevated blood eosinophil count, adult-onset, and involvement of nasal polyps-associated chronic rhinosinusitis in 50% of the patients (51,52). Conversely, non-eosinophilic asthma can be characterized by low eosinophil counts, with the dominant inflammatory cell type being neutrophils and mixed granulocytes, or very few inflammatory cells (51). Generally, the higher the degree of eosinophilia, the greater the disease severity and exacerbation frequency in eosinophilic asthma (46). Eosinophilic and paucigranulocytic asthma (asthma with very few inflammatory cells) are among the dominant inflammatory asthma phenotypes.

2.1.2 Eosinophil recruitment, migration, development, and survival

In eosinophilic asthma, which is also known as Th2 asthma, eosinophils present as the hallmark as seen in their elevated numbers (Figure 2) (53). The exposure of airway epithelium to allergens or antigens triggers an immunological cascade which attracts eosinophils to the airways by Th2 cytokines and chemoattractants (16). Th2 cytokines secretion contributes to the increase in eosinophil counts in the BALF as they are responsible for eosinophil recruitment, migration and survival (16). The Th2 cytokines which induces eosinophil migration to the airways include IL-4, IL-5 and IL-13, which are produced by activated Th2 lymphocytes and ILC2s (16,53). Among them, IL-5 and the chemokine eotaxin-1 (CCL11) are the primary mediators for the release of bone marrow eosinophils and trafficking of eosinophils to the lungs (16,51). Eotaxin is produced by allergen-challenged endothelial and epithelial cells and enhances eosinophil migration by binding to CCR3 expressed on its surface (16). Besides stimulating eosinophil migration, IL-5 is also an important mediator of eosinophils differentiation, development, activation and survival (52,54). Other major sources of IL-5, other than Th2 cells and ILC2s, are CD34+ progenitor cells, mast cells, invariant natural killer (NK) T-cells, and eosinophils themselves (55). In addition to the regulation of eosinophil numbers, IL-5 produced by ILC2s also regulates their

circadian cycling (55). IL-4 and IL-13 do not directly mediate eosinophil trafficking; IL-4 sustains eosinophil migration by inducing B-cell isotype switching to immunoglobulin (Ig)E for the development of Th2 lymphocytes whereas IL-13 stimulates eotaxin production (53). IL-4 also induces the expression of VCAM-1 and eotaxin by epithelial cells which enhances eosinophil migration to the site of allergic inflammation (16). study by Beckert *et al.*, provides evidence on the effects of the Th2 cytokines, either alone or in combination, on eosinophils in a mouse model (56). C57BL/6 mice were intranasally administered equimolar amounts of single agent or combination IL-4, IL-5 and IL-13. The results demonstrated that IL-4 and IL-13 were correlated with airway eosinophilia, progression of airway hyperresponsiveness, and goblet cell metaplasia (56). However, IL-4 demonstrated weaker effects than IL-13 and no synergism was observed when these two cytokines were administered in combination (56). After the administration of IL-5, it was observed that the eosinophil count in bone marrow and lung tissues were increased but no structural changes of the eosinophils were observed (56). The combined administration of IL-5 and IL-13 significantly increased the number of lung, airway, blood and bone marrow eosinophils, whereas IL-5 and IL-4 combined only increased eosinophils count in lungs and bone marrow (56).

Besides Th2 cells, the damaged epithelial cells also contributes to eosinophils recruitment through the release of IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) (16). These cytokines activate ILC2 from the innate immune system to secrete IL-4, IL-5 and IL-13 (16). Additionally, IL-33 and granulocyte-macrophage colony-stimulating factor (GM-CSF) contributes to eosinophils differentiation and migration as well as mediate their survival in the airways (16). IL-33 directly stimulates eosinophil differentiation from $CD117^{+}$ hematopoietic progenitor cells and contributes to the exacerbation of eosinophilic inflammation by elevating eosinophil, macrophage, lymphocyte, IL-13, TGF-β, CCL3, CCL17, and CCL24 levels in the airways (57). IL-18 was also recently identified as an important cytokine for the production, differentiation, and maturation of ($CD101^{+}CD274^{+}$) pathogenic eosinophils (54).

Fanat *et al*., researched the effects of cells sourced from human airway smooth muscle (HASM) on eosinophils (58). In this study, peripheral blood progenitor cells collected from atopic asthmatics and control subjects with no atopy were cultured together with supernatant collected from HASM cells culture. At the end of the study, it was observed that HASM cell-derived cytokines stimulated eosinophil differentiation *via* the p38 mitogen-activated protein kinase (MAPK) pathway but not the src kinase (srcK) pathway (58). The cytokines released

by HASM cells were identified to be IL-5 and GM-CSF as eosinophil differentiation was inhibited by anti-IL-5 and anti-GM-CSF blocking antibodies (58). Thus, the researchers concluded that HASM cells have the ability to modulate differentiation and maturation of eosinophils from precursor cells including immature eosinophils, that may lead to eosinophilic inflammation and airway remodeling in severe asthmatics (58). However, haemopoietic progenitor cell migration and adhesive response *in vitro* are not influenced by HASM cells. (58).

Lung dendritic cell (DC) subsets also play a role in initial eosinophil recruitment to the site of allergen challenge. A specific sub-type of DCs in the lung, i.e., CD24CD11b DC2s, produce NO synthase which induces CCL17 and CCL22 on lung cDC1s to attract eosinophils for initial eosinophil infiltration. After the allergen challenge, eosinophil recruitment is inhibited by lung CD24 cDC2s through TGF β1 secretion (59). Hence, the impaired expression of CCL17 and CCL22 lead to the response seen during the late phase reaction. It was suggested that different lung APCs secrete specific soluble factors during the memory stage of chronic asthma after an allergen challenge, thus regulating lung cDC1-mediated eosinophil recruitment differently (59).

Several lipids, such as prostaglandins and leukotrienes, are key mediators of the inflammatory response by acting as chemoattractant(s) of eosinophils. For example, leukotriene E4 and prostaglandin D2 (PGD2) induce eosinophil migration to the airways (16). PGD2 acts by binding to CRTH2 receptor (DP2) in eosinophils which induces its recruitment and activation (16).

Nitric oxide (NO) synthesized from epithelial cells and vascular endothelial cells by inducible nitric oxide synthase enzyme is also responsible for the recruitment of eosinophils in addition to its function as a mediator of inflammation, and this correlates with the level of exhaled NO, which is a biomarker of asthma (16).

Following the recruitment phase comes the effector phase. Autocrine secretion of IL-5 by activated eosinophils enables them to survive within tissues in their own capacity as the secreted IL-5 inhibits apoptosis (16,60). Moreover, the adherence of eosinophils to fibronectin stimulates the secretion of other pro-survival mediators, such as GM-CSF and IL-3, by activated eosinophils and hence, promotes their own survival (16,60). The additional effects of these cytokines enable eosinophils to persist in the airways for long periods and prolong their effects.

Eosinophils in inflamed tissues are also activated by antigen-specific IgGs and IgAs, but not IgE, as reported by a study conducted on the effects of antigens and antigen-specific immunoglobulins on eosinophil function (61).

2.1.3 Airway hyperresponsiveness (AHR) and Mucus Hypersecretion

AHR occurs when there is a dysregulation of airway homeostasis. Allergen exposure causes events such as airway epithelium damage, mucus hypersecretion and ASM proliferation which leads to homeostasis imbalance (62). Eosinophils are highly involved in the development of these events through the release of its granule cytotoxic proteins *via* cytolysis, such as MBP, EPO, ECP, and EDN (51,53). The release of these granule proteins was shown to be triggered through chemokines, including eotaxin-1 and interferon-gamma (IFN-γ) (53). MBP presents as two type of homologs, which are MBP-1 and MBP-2, with MBP-1 having a significantly alkaline pH and exerting direct cytotoxic effects on the host cells (51). MBP-1 also has a greater potency in histamine induction and leukotriene C4 release from basophils as compared to MBP-2 (53). MBP and EPO were reported to cause AHR, although ECP and EDN did not (46). It was also reported that MBP induces bronchial hyperactivity by stimulating the release of histamine from mast cells and basophils (46,51). MBP can also cause bronchoconstriction by blocking acetylcholine receptor, M2, which is a negative feedback regulator for acetylcholine (53). Moreover, the combination of EPO, halides and hydrogen peroxide was shown to stimulate the release of mast cell mediators (51).

In addition to cytotoxic cationic proteins, eosinophils secrete several cytokines which can contribute to the hallmarks of asthma. For example, IL-13 is a cytokine released by eosinophils which can enhance the differentiation of goblet cells to promote mucus hypersecretion, as well as cause AHR. Moreover, Th2 cells and ILC2 also produces IL-13 (46).

Additionally, eosinophils also produce lipid mediators in eosinophil lipid bodies, such as leukotrienes, which can also cause AHR and mucus hypersecretion (46). Eosinophilic leukotrienes stimulate bronchoconstriction and activation of basophils and mast cells, which further sustains the ongoing inflammation via the secretion of histamine, prostaglandins, and additional leukotrienes (53).

The role of eosinophils in airway remodeling is further reinforced when mouse studies showed that mice with congenital eosinophil deficiency did not experience collagen and smooth muscle deposition in the airways (46).

2.1.4 Tissue damage and Airway remodeling

Eosinophils cause persistent inflammation in the airways which causes continuous damage to the airway walls (53). During the reconstruction of the damaged airway, airway remodeling occurs as the basement membrane thickens and fibrosis occurs due to airway smooth muscle proliferation, goblet cell hyperplasia, extracellular matrix (ECM) proteins deposition, and new blood vessels formation (angiogenesis) (16,46,53). Airway remodeling is commonly associated with severe asthma phenotypes (63).

Tissue damage occurs as a result of the cytotoxic effects of the eosinophilic granule proteins. The granule proteins exhibit varying strengths of toxicity when tested *in vitro*. MBP, EPO and ECP exert toxicity to several tissues, including bronchial epithelium, skin, heart and brain, whereas EDN only exhibits marginal toxicity (51). MBP, EPO and ECP were found to cause damage to epithelial cells *in vitro* when equivalent concentrations as found in asthmatic patients were administered (16,53). ECP is able to bind to and alter the permeability of cell membranes in the airway (46).

The process of airway remodeling is also contributed by TNF-α, as well as IL-1-β according to recent studies, which activates eosinophils and stimulates the secretion of matrix metalloproteinase-9 (16,53). TNF-α and IL-1-β are also associated with persistent eosinophils recruitment (53). TGF-β is a growth factor secreted by eosinophils which can promote airway remodeling. Bronchial biopsies from asthmatic patients have shown that eosinophils are the primary source TGF-β (46). Studies have shown that TGF-β mRNA expression was upregulated in severe asthmatics and has correlation with the thickness of the basement membrane (16). TGF-β fucntions as a chemoattractant for fibroblast and stimulates fibroblast proliferation and differentiation into myofibroblasts and smooth muscle cells (46,53). Additionally, TGF-β upregulates the synthesis of collagen and glycosaminoglycans leading to ECM production and resulting in tissue remodeling of the airways (46,53).

Besides granule proteins and cytokines, eosinophils also secrete reactive oxidant species (ROS), such as hydrogen peroxide, superoxide anion and hydroxyl radicals, which contributes to airway inflammation and airway remodeling by damaging the cells and tissues of the airways and inducing the fibroblast hyperplasia (16,51,53). EPO stimulates the production of cytotoxic ROS by catalyzing the oxidation process of halides and thiocyanate (46).

Zagai *et al.,* stated that conditioned media (CM) collected from cultured human peripheral eosinophils and eosinophil cationic protein (ECP) extracted from human peripheral eosinophils significantly stimulated the migration of human lung fibroblasts *in vitro* in a concentration- and time-dependent manner (64). Thus, it was suggested that eosinophils affect the fibrotic response in asthmatics. As airway remodeling is associated with fibroblast recruitment, it was proposed that the stimulation of fibroblast migration by ECP may be an important mechanism in the remodeling process of extracellular matrix leading to airway fibrosis in asthmatics.

2.1.5 ASM proliferation

The co-culture of eosinophils isolated from asthma patients and ASM cells demonstrated an enhancement of ASM proliferation, which is inhibited by the presence of leukotriene antagonist, montelukast (46). A mutual relationship exists between eosinophils and ASM cells as ASM cells are able to produce eosinophilic cytokines (46).

Based on the study done by Halwani *et al*., cysteinyl leukotrienes (CysLTs) released from eosinophils when they are in direct contact with airway smooth muscle (ASM) cells enhanced ASM proliferation (65). The requirement of direct cell contact between eosinophils and ASM cells to trigger CysLTs release were further confirmed when the administration of anti-adhesion molecule antibodies resulted in the inhibition of ASM proliferation (66). Additionally, it was proven that the increase in ASM proliferation was dependent on CysLTs released from eosinophils instead of ECM proteins as ASM proliferation was inhibited when leukotriene receptor antagonist (Montelukast) was co-cultured with eosinophils and ASM cells. The researchers concluded that airway remodeling in asthmatic patients can be attributed to eosinophil derived CysLTs which function to increase ASM mass through the enhancement of ASM proliferation.

2.1.6 Eosinophilic pro-inflammatory cytokines and chemokines

Besides the direct cytotoxic effects of eosinophilic granule proteins on tissues, eosinophils produce and secrete a variety of pro-inflammatory mediators which contributes to the pathophysiology of asthma. For example, eosinophils produce a variety of Th2 cytokines in the airways of asthmatics, including IL-4, IL-5, and IL-13 (51). The recruitment of leukocytes to the airways is also regulated *via* the secretion of eosinophilic chemokines, such as CCL3, RANTES (CCL5), and eotaxin (CCL11) (51). Alternatively, neutrophil recruitment is induced by IL-8 and GM-CSF, which are also expressed by eosinophils following an

allergen challenge (51). Eosinophil activation is also mediated *via* Th1 cytokines released by eosinophils themselves, which is IFN-γ (51). Thus, it is evident that eosinophils exhibit pleiotropic effects, which includes destruction of host tissues, regulation and potentiation of inflammatory pathways, as well as host defense. The severity of asthma symptoms in patients is worsened by these eosinophilic functions. Therefore, therapies which target eosinophils can be a potential intervention for asthma and other eosinophilic inflammation-associated diseases.

2.2 Eosinophils as Biomarkers of Asthma

Heterogeneity exists in asthma with regards to its underlying pathophysiology, clinical signs and symptoms, and treatment response. The use of merely clinical and physiological assessment has become inadequate in the accurate prediction of the underlying mechanism of the disease or the treatment response. Thus, additional testing for biomarkers in conjunction with examination of clinical signs and symptoms of the patient will be able to help identify asthma phenotypes and endotypes, anticipate the disease progression and its prognosis, as well as enhance the precision therapy for asthma (67). Eosinophil counts have become a useful biomarker in the assessment of asthma.

Szefler *et al.*, reported that the measurement of total eosinophil counts and sputum eosinophils as biomarkers for asthma are recommended as supplemental outcome measures (68). Supplemental outcome measures are defined as outcomes of asthma for which standardized definitions and specific methods of measurement exists, and their validity has been proven but they remain as an optional inclusion in funded clinical asthma research (68). The eosinophil measurements are obtained through complete blood counts. In addition to sputum eosinophils, nasal discharge eosinophils with asthma symptoms may also be a predictive factor for persistent asthma (69). As such, testing for nasal discharge eosinophils with asthma symptoms increases with age (69).

The severity of sputum eosinophilia in asthma can be assessed using various biomarkers. However, the biomarkers' diagnostic accuracy might vary between different asthma phenotypes. Westerhof *et al.*, carried out an investigation to determine the accuracy of several biomarkers for the detection of sputum eosinophilia in different phenotypes of adult asthma, such as total IgE, area under curve (AUC), and exhaled nitric oxide fraction (FeNO) (70). Among the different asthma phenotypes of adult patients used for comparison were severe and mild, obese and non-obese, atopic and non-atopic, and smoking/ex-smoker

and non-smoking asthma patients. The findings showed similarities in AUCs for blood eosinophils and FeNO among the various asthma phenotypes whereas total IgE showed higher accuracy in the detection of sputum eosinophilia in non-obese and non-atopic asthmatics as compared to obese and atopic asthmatics (70). The researchers concluded that the measurement of blood eosinophils and FeNO as sputum eosinophilia biomarkers, irrespective of asthma phenotype, showed superior diagnostic accuracy as compared to total IgE (70). A separate study also showed that blood eosinophils can most accurately identify sputum eosinophilia in patients with varying severities of asthma, such as mild, moderate or severe asthma (71). Thus, eosinophils found in blood circulation can be used to facilitate individualized asthma treatments.

Nevertheless, some studies have shown that despite statistically significant associations, blood eosinophil and neutrophil counts, FeNO and IgE levels, FEV1 percentage and age are poor surrogate biomarkers, both singularly and in a combination, for predicting sputum eosinophil and neutrophil percentages (72).

Phenotyping based on airway inflammation severity is often applied to asthmatic patients. According to a study done by Walsh *et al*., the relationship between rates of exacerbations and the phenotype of severe asthma was investigated based on longitudinal measures of sputum eosinophils and neutrophils (73). The results demonstrated that asthmatic patients with persistent eosinophilic phenotype experienced shorter intervals before the first exacerbation and an increased risk of exacerbation over a 1-year period as compared to those with non-eosinophilic phenotype. However, no observable changes were noted in time to first exacerbation or exacerbation risk among neutrophilic phenotypes.

A cohort study carried out in UK also reported that asthmatic patients with blood eosinophil numbers >400 cells/μL experienced a worsening in asthma control and more severe exacerbations, as defined by the increased use of asthma relievers, asthma-related hospitalization, oral corticosteroids use, or antibiotics prescriptions (74).

Petsky *et al*., reported, in a systemic review, that carrying out therapy adjustment according to airway eosinophilic markers, such as sputum eosinophil counts and exhaled nitric oxide, significantly decreased the risk of asthma exacerbations (75). However, no significant impact was observed with regards to dose of daily inhaled corticosteroid, asthma control or lung function.

Periostin is a proposed novel biomarker of eosinophilic airway inflammation. A specific asthma subset as characterized by the expression of Th2 cytokine-inducible genes in bronchial epithelial cells was recently discovered. Periostin is included in this gene signature

which is present in about 50% of asthma patients and is associated with eosinophil-mediated airway inflammation. In a study done by Jia *et al.*, an assay for peripheral blood periostin protein was developed to investigate its potential as a biomarker in order to designate selective therapies for specific groups of asthma patients (76). The patients used for sample collection were asthmatics with persistent symptoms in spite of inhaled corticosteroid administration at maximum doses. The results showed that asthma patients with evident eosinophilic airway inflammation demonstrated a significant increase in levels of serum periostin as compared to those with minimal eosinophilic airway inflammation (76). Serum periostin levels also proved to be a more useful predictor of eosinophilia in airways than other indices, such as peripheral blood eosinophil, IgE and FeNO levels (76). The researchers concluded that the occurrence of eosinophilic airway inflammation can be predicted using periostin as a systemic biomarker in asthma patients and thus, this can potentially be a utility for selecting patients for emerging asthmatic therapeutics, such as ICS and biologics, targeting Th2 inflammation (76). Simpson *et al.*, also reported that serum and sputum periostin levels have high correlation with sputum eosinophil counts (77). Periostin concentrations were also found to be higher in serum than in sputum. However, the ability of periostin levels to predict the presence of eosinophilic asthma was modest.

Besides periostin, eosinophil peroxidase (EPO) level are also reported in several studies to have correlation with sputum eosinophil levels. Rank *et al.*, carried out a study to compare nasal, pharyngeal, and sputum EPO levels with induced sputum eosinophil percentage and found that there was a strong association between nasal and pharyngeal EPO levels and the percentage of eosinophils of induced sputum. Another study carried out by Nair *et al.*, concluded that EPO levels assayed by ELISA was an appropriate surrogate marker of eosinophils and/or eosinophil degranulation in sputum of respiratory patients (78).

ILC2s are recognized as another novel biomarker for eosinophilic airway inflammation. In an investigation carried out by Liu *et al.*, the inherent diagnostic ability of ILC2 was compared to standard biomarkers of eosinophilic asthma, including age, sex, BMI, number of blood eosinophils, IgE, and FeNO (79). The results concluded that ILC2 levels were significantly elevated in patients with eosinophilic asthma and the ILC2 percentage was the most significant component of eosinophilic airway inflammation as compared to the other biomarkers (79). Moreover, ILC2 percentage showed high sensitivity and specificity in differentiating patients with eosinophilic asthma from asthma patients without eosinophilic inflammation (79). Thus, ILC2 is a prospective substitute biomarker for eosinophilic airway inflammation in mild-moderate asthma patients and subsequently, can be used to select

patients for beneficial asthmatic therapies targeting Th2 inflammation. There is a six-gene signature paper that predicts exacerbations in the severe asthma patients, this six-gene sig has been proposed as a marker for severe asthma exacerbations, and more recently in COPD (80,81).

Biomarkers of eosinophilic inflammation appears to differ between severe asthmatic patients with concomitant obesity and normal asthmatic patients. In a study done by Desai *et al*., obese severe asthmatic patients demonstrated significant elevations in submucosal eosinophils and sputum IL-5, however no changes were observed in sputum eosinophils (82). Hence, supplementary investigations should be performed to determine if eosinophil-targeted therapies or diet and lifestyle modifications are more effective in providing anti-inflammatory effects in obese patients with asthma.

2.3. Bacteria and eosinophils

A greater diversity of microorganisms with altered compositions are known to be present in asthmatic patients, for example, more Proteobacteria and less Bacteroidetes as compared to healthy subjects (83). Hence, it was proposed that the composition of airway microbiome has correlations with the type of airway inflammation (84,85). A study carried out by Sverrild *et al.*, revealed that the level of eosinophilic airway inflammation has correlations with the varying airway microbiome constituents in asthma patients (83). A more abundant bacterial profile was obtained from patients with the lowest eosinophil counts, for example, a greater proportion of *Neisseria*, *Bacteroides*, and *Rothia* species and a lower proportion of *Sphingomonas*, *Halomonas* and *Aeribaccilus* species as compared to asthma patients with greater eosinophil counts and healthy subjects (83). The type of bacterial species also affects eosinophil functionality differently. A study conducted by Hosoki *et al*., showed that *Staphylococcus aureus* (SA) stimulated the release of EDN in a dose-dependent manner, but *Haemophilus influenzae* (HI) and *Prevotella sp.* (PS) did not (86). SA, HI and PS all significantly enhanced superoxide generation, but SA had a greater effect which significantly induced a greater eosinophilic TNF-α production as compared to either HI or PS (86). Conversely, HI and PS induced IL-10 production more strongly than SA (86). Thus, it was concluded that SA may be associated with exacerbation of eosinophilic inflammation in asthma whereas HI and PS may be involved in its inhibition.

2.4 Drugs targeting eosinophils in the treatment of asthma

Asthma is conventionally treated with beta-agonists, anti-cholinergics and inhaled corticosteroids (ICS). In addition, biologics are commonly used as an alternative to treat severe and refractory asthma in which conventional treatments show limited efficacy or intolerable side effects. Monoclonal antibodies, such as those targeting IL-5, IL-5Rα, IgE and IL-4Rα, have shown favourable outcomes in clinical trials for the treatment of severe, uncontrolled asthma (87). Patients with severe eosinophilic asthma were also successfully managed with biologics which target IL-5, IL-4, IL-13, and IgE. This is because local eosinophilopoiesis is hypothesized to be the predominant mechanism that results in on-going airway eosinophilia and steroid requirements in patients with severe asthma, and these agents either directly or indirectly targets eosinophils which leads to improved asthma outcomes, such as reduced exacerbations and steroid-sparing effects. Among these biologic therapies, anti-IL-5 drugs, including reslizumab, mepolizumab, and benralizumab are most used and supported as adjuncts for severe eosinophilic asthma therapy and poor asthma control. Studies have shown that treatments that target IL-5/IL-5Rα in severe eosinophilic asthma patients resulted in significant decrease of blood and sputum eosinophilia, reduction of exacerbation episodes, along with major improvement in clinical symptoms (15). Patients on anti-IL-5 therapies experienced approximately half of the asthma exacerbation rates (88). However, improvements in health-related quality of life (HRQoL) scores and lung function could not be confirmed due to insufficient evidence (88). With regards to safety, patients taking reslizumab, mepolizumab or benralizuamb did not experience any serious adverse effects (88). Moreover, novel therapies targeting other eosinophil-associated factors and their receptors have also been studied, including those that target CC-chemokine receptor 3 (CCR3), prostaglandin D2 (PGD2), thymic stromal lymphopoietin (TSLP), as well as novel oligonucleotide therapies.

2.4.1 Reslizumab

Reslizumab is a humanized, neutralizing anti-IL-5 monoclonal antibody which acts by binding to IL-5 to block its interaction with its receptor (89,90). IL-5 receptors (IL-5R) can be found on the surfaces of eosinophils. IL-5 functions to increase the activation and maintenance of eosinophils, which drives eosinophilic inflammation (89). As such, the blocking of IL-5 binding with its receptor on eosinophils leads to the disruption of eosinophil maturation and promotes apoptosis (91). Many studies have shown the beneficial outcomes from reslizumab treatment in patients with severe eosinophilic asthma. For example, in an

experiment comparing reslizumab and placebo use in asthma patients, the reslizumab group experienced significantly less asthma exacerbations as compared to the placebo group (91). The results of the study concluded that reslizumab can be effectively used for treating asthma patients with persistent eosinophilia who are inadequately controlled with corticosteroids (91). Another study reported that patients that were administered 3.0 mg/kg of IV reslizumab every month experienced better asthma control as well as an improved lung function (90). The study also highlighted that patients with an increased eosinophil load that is inadequately managed with moderate-high doses of ICS benefited the most from reslizumab therapy (90). When compared to benralizumab, which targets IL-5Rα, it was shown that reslizumab confers higher efficacy than benralizumab in patients with eosinophilic asthma (89).

2.4.2 Mepolizumab

Another anti-IL-5 monoclonal antibody with the same mechanism of action as reslizumab is mepolizumab, which has shown to be useful for treating eosinophilic asthma in several studies. In a research carried out by Haldar *et al*., the efficacy of mepolizumab in the treatment of patients with refractory eosinophilic asthma and history of recurrent exacerbations was investigated. who were administered mepolizumab therapy for 1 year experienced reductions in exacerbation rates as well as improvements in the Asthma Quality of Life Questionnaire (AQLQ) scores (92). At the end of the study, the patients who undergone 1 year of therapy reported less exacerbations, scored higher in the Asthma Quality of Life Questionnaire (AQLQ) and showed significant reductions in levels of blood and sputum eosinophils (92). However, mepolizumab showed no significant distinction in symptoms, post- bronchodilator FEV_1, or airway hyperresponsiveness when compared to placebo and other anti-IL-5 therapies (92). Another study demonstrated the effects of mepolizumab in prednisone-sparing. Nair *et al*., reported that mepolizumab administered to asthma patients with high sputum eosinophils despite continuous prednisone therapy effectively lowered blood and sputum eosinophil counts and thus, proved the prednisone-sparing effect of mepolizumab (93). Furthermore, Sehmi *et al*., demonstrated that subcutaneous mepolizumab therapy significantly attenuated systemic differentiation of eosinophils in severe eosinophilic asthma patients, as observed from the decrease in blood eosinophils and elevated eosinophil-lineage-committed progenitors (EoP) counts in the mepolizumab group (94). However, mepolizumab had no significant effects on mature eosinophils, sputum EoP counts or maintenance dose of prednisone, indicating that mepolizumab did not inhibit differentiation of local airway eosinophils to mature cells (94).

Thus, it is evident that airway eosinophilia and severe eosinophilic asthma can be optimally controlled by targeting IL-5 pathway-associated local airway eosinophil differentiation. In a comparison study on the effectiveness of anti-IL-5 therapies, mepolizumab showed superiority in reducing the frequency of asthma exacerbations and improving asthma control over reslizumab or benralizumab in severe eosinophilic asthma patients with identical baseline levels of blood eosinophils (95).

Despite these studies that showed a positive outcome from mepolizumab therapy, there exists some concerns regarding the potential risk of 'rebound' eosinophilia following treatment cessation (55). Another study has also showed that mepolizumab treatment cessation resulted in rapid blood eosinophilia and subsequent worsening of asthma symptoms and exacerbations (55). Thus, further studies should be carried out to ensure the safety of eosinophil-targeted therapies. Nonetheless, mepolizumab is approved by both FDA and EMA to be used in patients >6 years of age with severe eosinophilic asthma. The primary benefits of mepolizumab include improvements in the lung function (forced expiratory volume) and marked reductions in asthma exacerbations (96)

2.4.3 Benralizumab

Benralizumab is a humanized, afucosylated, IgG1κ monoclonal antibody which prevents the receptor interaction of IL-5 by binding to IL-5Rα via its Fab domain. As a result, eosinophilia is reduced in blood, tissue and bone marrow due to the inhibition of eosinophil differentiation and maturation. In addition, benralizumab also depletes eosinophils in tissues and systemic circulation via antibody-dependent, cell-mediated cytotoxicity as it can bind to the RIIIa region of the Fcγ receptor on NK cells, neutrophils and macrophages through its afucosylated Fc domain (97,98). The dual-function of benralizumab allows for a more rapid and sustained reduction of eosinophilia than other IL-5-targeted monoclonal antibodies (97). Benralizumab therapy has shown positive outcomes in both moderate-severe asthma patients as well as eosinophilic chronic obstructive pulmonary disorder (COPD) patients (99). Sridhar *et al*. conducted a research in which eosinophilic asthma and eosinophilic COPD patients were given benralizumab subcutaneously and the results demonstrated that benralizumab could selectively modulate eosinophil- and basophil-associated blood proteins and genes (99) Following benralizumab administration, eosinophil chemokines (eotaxin 1 and 2) were upregulated significantly in both the asthmatic and COPD patients (99). Furthermore, the expression of eosinophil- and basophil-associated genes were significantly reduced following benralizumab therapy (99). Thus, benralizumab is shown to

selectively modulate blood proteins and eosinophil- or basophil-associated genes, with alterations occurring prominently in eosinophil-high patients rather than eosinophil-low patients (99). Randomized clinical trials have also provided evidence on the optimal safety profile of benralizumab, as well as its efficacy in reducing asthma exacerbations, steroid-sparing and improvement of lung function (98). When compared to reslizumab, benralizumab was associated with a significantly improved lung function in asthma patients with severe eosinophilia (≥400 cells/μL) (95). Benralizumab is approved by FDA as an add-on maintenance therapy for use in patients with severe eosinophilic asthma aged over 12 years. The primary benefits include significant reductions in the asthma exacerbations, improvements in lung function parameters (forced expiratory volume in one second) and marked reduction in prescription of inhaled corticosteroids (100).

2.4.4 Omalizumab

Bronchial asthma is not only associated with elevations in pro-inflammatory Th2 cytokines, but also high levels of IgE. Besides the roles of Th2 cytokines in eosinophil differentiation, maturation, migration, and survival, they also contribute to the production of IgE. Omalizumab is an anti-IgE monoclonal antibody that can effectively reduce peripheral blood eosinophil (PBE) counts as well as airway eosinophil counts in asthmatic patients (101). Massanari *et al.*, reported that omalizumab had an inhibitory effect on eosinophils as observed from the lowered PBE counts in moderate-severe persistent allergic asthma patients who were on moderate-high doses of ICS (102). It was observed that patients who were administered omalizumab therapy demonstrated a significant decrease in PBE counts from baseline, with patients that have post-treatment free IgE levels < 50 ng/mL showing a larger decrease (102). In both steroid-reduction and steroid-stable phases of the studies, larger decreases in PBE counts were observed as compared to placebo (102). Thus, omalizumab treatment groups consistently experienced a pattern of lowered PBE counts and favourable clinical outcomes. Omalizumab is approved by both FDA and EMA to be used in patients over 12 years with moderate allergic asthma demonstrating raised levels of IgE. Omalizumab reduces the asthma exacerbation rates and, in some cases, reduces the corticosteroid prescriptions (103).

2.4.5 Dupilumab

Dupilumab is a novel anti-cytokine biologic drug used in the treatment of asthma. Therapies targeted at IL-4 and IL-13 are also beneficial in asthma treatments as they are Th2

cytokines that can stimulate eosinophil recruitment and migration to local airways. IL-4 and IL-13 contributes to the pathophysiology of the typical characteristics of asthma, such as chronic airway inflammation, tissue remodeling and airway hyperresponsiveness (104). Dupilumab is a monoclonal antibody that can inhibit the interaction between IL-4 and IL-13 with IL-4Rα. In a recent study, dupilumab significantly attenuated Th2 cell-associated inflammatory biomarkers, which is correlated with an improvement in lung function and airway symptoms as well as reduction of asthma exacerbations in patients with difficult-to-control asthma (104). Dupilumab is approved to be used as an add-on therapy in patients over 12 years of age and who demonstrate a poorly controlled moderate-to-severe asthma phenotype. Dupilumab has been shown to prevent asthma exacerbations and improvements in lung function (105).

2.4.6 MEDI-563

In addition to the commonly used anti-IL-5 drugs, MEDI-563 is a novel humanized anti-IL-5R α monoclonal antibody that has been developed with an enhanced effector function in the management of asthma (106). Kolbeck *et al*., reported that MEDI-563 caused both eosinophils and basophils to undergo antibody-dependent cellular cytotoxicity (ADCC) *in vitro* (107). MEDI-563 administered to non-human primates also decreased peripheral blood and bone marrow eosinophil counts (107). Thus, this study suggests that the focus of anti-asthma biologic therapies should be shifted from the passive removal of IL-5 to the reduction of eosinophil and basophil counts via ADCC mechanism.

2.4.7 Novel CCR3 receptor antagonist

Agents which target eosinophil cell surface-structures, for example CC-chemokine receptor 3 (CCR3), have also showed efficacies in decreasing blood and tissue eosinophil levels (108). Ki19003 (4-[[5-(2,4-dichlorobenzylureido) pentyl][1- (chlorophenyl) ethyl] amino] butanoic acid) is a novel CCR3 antagonist. CC-chemokine ligands (CCL), such as CCL11, CCL24 and CCL26, are associated with eosinophil chemotaxis (109). A study done by Komai *et al*., administered Ki19003 to ovalbumin-induced BALB/c mice to study its effects on airway remodeling in a mouse model of allergic asthma (109). It was observed that Ki19003 inhibited antigen-induced elevations in the number of eosinophils found in bronchoalveolar lavage fluid (BALF), but other cells were not affected (109). Furthermore, a dose-dependent increase in TGF-β1 production in BALF and hydroxyproline amount in lungs were observed following Ki19003 administration (109). Ki19003 treatment also

resulted in the attenuation of allergen-induced subepithelial and peribronchial fibrosis (109). Hence, it can be concluded that CCR3 antagonism can prevent eosinophil airway infiltration as well as the progression of subepithelial and peribronchial fibrosis following an allergen challenge. Therefore, the process of airway remodeling, which is a prominent feature of allergic asthma, can potentially be prevented using CCR3-targeted treatments.

2.4.8 PGD2 antagonist

Chemoattractant receptor-homologous molecules expressed on T-helper type 2 cells (CRTH2) that mediates chemotactic response to PGD2 are also expressed on eosinophils (110). PGD2 inhibitors, such as timapiprant and fevipiprant, have been studied for their effects on eosinophilia. Timapiprant was shown to improve conditions of mild-moderate eosinophil-mediated allergic asthma patients whereas the administration of fevipiprant therapy to patients with moderate-severe asthma who were not adequately managed with ICS resulted in reduced sputum eosinophil counts and subsequently, attenuation of eosinophilic airway inflammation (111). However, the phase III trial of fevipiprant did not show marked clinical improvements in patients with poorly controlled asthma (112).

2.4.9 Anti-TSLP

TSLP plays a part in eosinophilic inflammation by recruiting eosinophils following epithelial damage. AMG 157 is a human anti-TSLP monoclonal IgG2λ which inhibits the interaction and binding of TSLP to its receptor. A Phase I clinical trial conducted by Gauvreau *et al*., reported that AMG 157 treatment administered to allergic asthma patients of mild severity significantly reduced FeNO in addition to blood and sputum eosinophil levels following and prior to allergen (113). Moreover, the AMG 157 treatment group experienced a greater reduction in maximum percentage decrease in FEV_1 during late response than the placebo group, with a greater reduction occurring as the treatment progressed (113). Thus, the researchers concluded that AMG 157 attenuates bronchoconstriction and other indications of respiratory inflammation pre- and post-allergen challenge.

2.4.10 Novel oligonucleotide therapy

Oligonucleotides, such as siRNA and miRNA, are promising novel therapeutic strategies for the treatment of various respiratory disorders via their gene silencing or RNA interference abilities (114–117). An oligonucleotide-based therapy, TPI ASM8, was studied for its potential beneficial effects in lung function and sputum eosinophilia in patients with

mild allergic asthma. TPI ASM8 consists of two phosphorothioate antisense oligonucleotides (AON) which are modified, with one targeting CCR3 receptor and another targeting the beta chain (βc) which is common among IL-3, IL-5 and GM-CSF receptors. According to a study carried out by Imaoka *et al*., patients with mild allergic asthma who were administered nebulized TPI ASM8 following an allergen challenge showed reductions in sputum eosinophils, early and late asthmatic responses, as well as airway eosinophil progenitor cells (118). Thus, it was concluded that the accumulation of eosinophils and their progenitor cells in airways of asthmatics can be inhibited successfully via TPI ASM8-mediated CCR3 and βc expression blockade. Therefore, further efforts of developing novel therapies that inhibit accumulation of airway progenitor cells should be taken.

2.4.11 Corticosteroids

Oral corticosteroids (OCS) are one of the mainstay therapies for the long-term management of severe asthma, which is characterized by persistent asthma symptoms and airway inflammation despite maximum efforts using anti-asthma treatments. Corticosteroids effectively acts on various components of the Th2 inflammatory pathway and its use can result in rapid attenuation of eosinophils associated inflammation as well as long-term reduction in airway hyperresponsiveness (119). As such, corticosteroids confer better responses in asthma patients with eosinophilic inflammation as compared to neutrophilic inflammation (120). However, a major drawback to corticosteroid use is the development of corticosteroid resistance or insensitivity, which frequently occurs in long-term use of corticosteroids. This feature is especially prominent in severe asthmatics with persistent Th2 inflammation despite regular OCS use. Thus, biological therapies are introduced as alternative therapies although a large proportion of asthmatic patients will still require the use of OCS to control their asthma (119). The therapeutic action of OCS on inflammatory cytokines and eosinophils are proven in several studies. In a study conducted by Dente *et al*., short-term courses of OCS reduced both sputum eosinophilia and sputum pro-inflammatory cytokine concentrations, in addition to improving lung function in patients with severe refractory asthma (121). The improvement of pulmonary function was concluded from the significant increase in FEV_1 following OCS treatment/ The prednisone group also exhibited a significant decrease in sputum eosinophil percentages and concentrations of IL-5 and IL-8 (121). It was also noted that prednisone treatment only showed positive effects in patients with baseline sputum eosinophilia, whereas only a significant decrease of sputum IL-8 was observed in non-eosinophilic patients (121). Several studies also reported on the inverse

relationship between CS dose and PBE counts displayed in severe eosinophilic asthma patients. Prazma *et al.*, predicted a model in which reducing the daily dose of OCS by 5 mg/day resulted in a 41% increase in PBE counts (122). Another study reported that ICS dose increment from medium to high dose in patients with uncontrolled asthma resulted in a significant reduction of blood eosinophil concentrations (123).

2.5 Alternative therapies affecting eosinophils in the treatment of asthma

2.5.1 Quercetin

Known to be present as flavonoids in fruits and vegetables, quercetin was shown to be an anti-inflammatory agent with the potential use in the treatment of asthma (124). In this case, the clinical features of asthma were relieved by suppressing the activation of the signaling pathway of NF-κB which results in the reduced production of proinflammatory mediators associated with airway hyperresponsiveness and airway inflammation (124). Two other studies have also insinuated quercetin's role in the prevention of eosinophilic airway inflammation via the attenuation of eosinophil activation, particularly the production of chemokines (125,126). In the study by Cheak Yong *et al.*, quercetin was encapsulated into a lipid crystalline nanoparticle (LCN) to overcome its limitation in terms of bioavailability as well as aqueous solubility which resulted in the successful exhibition of an improved anti-inflammatory effect of quercetin when formulated as such (124).

2.5.2 Curcumin

Curcumin is a constituent originating from the spice, turmeric, that is thought to be a potential remedy for the management of asthma (127,128). Similarly, to quercetin, curcumin possesses anti-inflammatory properties which relieve airway inflammation of allergic nature as well as airway hyperresponsiveness through the suppression of the activation of the signaling pathway associated with NF-κB and the attenuation of eosinophil production (128). Its clinical application is also limited by the same reasons seen in quercetin (128,129). Hence, Ng *et al.*, sought to load curcumin into liposomes as liposomes were noted to be able to modify a system's pharmacokinetic and biodistribution profiles (128–130). The study managed to overcome the limitation of curcumin's unfavourable solubility as well as demonstrate the sustained release properties attained through the encapsulation of curcumin in a liposome, indicating that curcumin loaded liposomes could be an appealing option for the treatment of asthma (128).

3.0 Eosinophils and COPD

Chronic obstructive pulmonary disease is described by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as a disease that can be commonly prevented and treated (131–134). However, COPD is noted to be among the most substantial causes of morbidity and mortality globally and this has caused a significant burden which is ever growing on the economy and society (131). This disease is characterized by the poorly reversible limitation of airflow which is commonly progressive and the elevated response to chronic inflammation that occurs in the peripheral airways and lung parenchyma due to the inhalation of noxious particles or gasses which is primarily (but not exclusively) caused by smoking tobacco (131,135–137). Emphysema and small airway fibrosis may result from the chronic inflammation because of the destruction of parenchymal tissue and disruption of mechanisms of repair and defense which causes the raise of the characteristic symptoms associated with COPD (131,135). As the disease progresses, so does the degree of inflammation which results in the elevation of neutrophil, macrophage and lymphocyte levels within the airway lumen (137). The increase in numbers of neutrophil and macrophages occur due to the activation of pattern recognition receptors which precipitates an innate immune response that also gives rise to the activation of airway epithelial cells as well as mucus secretion (137,138). An increase in T lymphocyte and B lymphocyte numbers in the lungs is seen when adaptive immunity is activated in the latter course of the disease which might result in the amplification of neutrophilic inflammation due to the increment in CD4+ T helper 17 cells present in the lungs (137). Although various inflammatory cells are implicated in COPD, neutrophils are by far the most abundant and has been extensively associated with the pathogenesis of COPD (139–143). However, it has been reported that some COPD patients without asthma have elevated numbers of eosinophils in their airways instead during exacerbations and clinical stability (Figure 3) (137,141,144,145). This is commonly seen in asthmatic patients where eosinophilic inflammation is known to be a distinguishing feature of the disease but the presence of a subdivision of COPD patients with eosinophilic airway inflammation has been reported (141,143,144,146–148). COPD patients with this phenotype are known to show the most significant response to corticosteroid treatment as well as an increased risk of exacerbation relapse and hospital readmissions (140,142,143,146,149,150).

3.0 Eosinophils and COPD

3.1 Role of eosinophils as biomarkers in COPD

Blood eosinophils have been recognised by several studies to be potential a biomarker of disease severity and clinical outcome in exacerbations of COPD (151–153). The measurement of eosinophils present in the blood, as a proxy for eosinophils present in tissues, has become an essential biomarker for the prediction of risk of exacerbations in COPD patients (154). Elevated counts of blood eosinophil in COPD has been well associated to the clinical features of the disease. Patients with an increment in blood eosinophil levels of ≥ 450 cells/μl during stable disease reportedly reflected a higher rate of exacerbation by 13% in the following year compared to patients with lower numbers (152). Another studied showed, amid COPD patients in the general population, increment of blood eosinophil counts > 0.34×10^9 cells/L were affiliated with a heightened risk of developing severe exacerbations by 1.76-fold (155). However, blood eosinophils singularly were not a dependable biomarker to predict the severity of the disease, the associated risk of exacerbations, or sputum eosinophilia (156). Instead, elevated sputum eosinophil counts acts as a more reliable biomarker compared to elevated concentrations of blood eosinophils for the identification of a subdivision of patients with more disease severity and more persistent exacerbations (156).

Eosinophil concentrations may also be a useful biomarker for the prediction of outcomes in COPD as increased number of blood eosinophil in COPD were related with a longer survival period and a higher incidence of hospital readmissions (157–159). A study also suggested that the deterioration of pulmonary function tests could be predicted according to number of blood eosinophil (158). Low blood eosinophil counts on the other hand, is predictive of a poorer clinical outcome and longer durations of hospital admission (158–160). However, a few studies report that blood eosinophil counts do not appear to be related to mortality (158,161). It is also noted that blood eosinophil concentrations can be utilized as a biomarker for the prediction of readmissions of patients suffering from severe COPD exacerbations (162). Moreover, Bélanger *et al*., suggests a relationship between greater blood eosinophil count upon admission of a COPD exacerbation and increased rate of readmissions (161–163).

3.2 Eosinophils as a guide for COPD treatment

Although airway eosinophilic inflammation in COPD may worsen the stabilization of symptoms, it can be used as to predict the benefit of treatment involving inhaled and oral corticosteroid (157,163,164). A review by Hillas *et al*., states the benefit of corticosteroid use in patients experiencing acute exacerbation of COPD (AECOPD) who have increased blood

eosinophils counts (165). The review also covered a post hoc analysis of three randomized controlled trials (RCTs) which demonstrated that COPD patients with eosinophil counts > 100 cells had a reduced risk of AECOPD of 25% when a combination of budesonide and formoterol was used compared to formoterol alone (165,166). Two other studies also showed results of greater decrease of exacerbation frequency in patients as the blood eosinophil count increased when inhaled corticosteroids (ICS) was added to the therapy alongside a long-acting β-agonist (LABA) whereas patients who were on a single therapy regimen of LABA exhibited progressively increasing rate of exacerbations as the eosinophil counts increased (165,167–169). In addition, Vestbo *et al*., has also demonstrated in his study that patients reflecting blood eosinophil counts ≥ 2% experienced reduced exacerbations of 30% with the use of either a regimen consisting of single inhaler extra fine triple therapy or long-acting muscarinic antagonist (LAMA) (170). Likewise, the 2019 GOLD COPD Strategy document states that blood eosinophil levels are to be used to guide ICS therapy in the treatment of stable COPD patients and frequent exacerbators as well (171).

It was also suggested that ICS withdrawal in COPD patients with eosinophil counts ≥ 4% and a history of exacerbations may increase the possibility of developing AECOPD (172,173). A trial reported that the continuous use of ICS reduced the exacerbations of moderate and severe grades in patients with either ≥ 4% relative eosinophil count or ≥ 300 cells/mL absolute eosinophil count compared to patients who were tapered off ICS (173). Similarly, the SUNSET trial identified that as ICS was tapered off, it led to a heightened risk of exacerbations among patients with an eosinophil count of ≥ 300 cells/mL (174). However, several studies including the Clinical Practice Research Datalink (CPRD) reported that ICS withdrawal among COPD patient did not affect the exacerbation risk of moderate-to-severe severity significantly (172,175–177). The difference in findings might be due to the use of different patient groups where the WISDOM trial had made use of patients with COPD of high severity, a past medical history of exacerbations and prior treatment with ICS before the trial, the SUNSET trial studied COPD patients treated with ICS regimens long-term and did not have persistent exacerbations whereas other studies such as CPRD utilised a patient population not at high exacerbation risk where the usage of bronchodilators and ICS are lesser than clinical trials (172–174). Nevertheless, both the WISDOM trial and CPRD reported a similar finding whereby patients with eosinophil counts more than 6.0% do not have an increased risk of exacerbations on withdrawal of ICS (172,173).

The study by Oshagbemi *et al*., did not report findings of increased all-cause mortality risk among patients with elevated absolute or relative blood eosinophil counts who withdrew

from ICS regimen but there are several studies that have evidence which conflict this finding (172,178,179).

Even though, blood eosinophil counts can be regarded as a guide to treating COPD, it is critical to remember that COPD patients, blood eosinophils are not influenced by ICS use (165,180). This is because there is pronounced variation in blood eosinophil levels (165,181–183). Hence, one measurement of blood eosinophil counts may not be sufficient for the accurate choice of utilizing ICS in a COPD patient's regimen (165).

3.3 Therapeutic targets for eosinophilic inflammation in COPD

COPD treatment regimens are largely associated with use of bronchodilators which treat the symptoms but not the underlying inflammation nor disease progression (135). Biologics targeting immune mediators such as IL-5 are showing promising results in COPD patients with the eosinophilic phenotype (135,184). IL-5, a key eosinophil cytokine mediates its differentiation, proliferation, survival, and activation via the IL-5 receptor (184–187). Humanized monoclonal antibodies, mepolizumab and benralizumab reduces blood and tissue eosinophil counts via the inhibition of IL-5 binding to eosinophil surface receptors and binding to interleukin-5 receptor α respectively (185,186,188–190). These two drugs have been known to successfully reduce eosinophilic inflammation and lower exacerbations rates in asthma (135). However, mepolizumab was not able to obtain the approval from the US Food and Drug Administration (FDA) to be used as an adjuvant to the maintenance regimen for COPD patients with eosinophilic inflammation because one of two phase III studies carried out showed no evidence of efficacy (135,184,186,187). Studies on benralizumab have been inconsistent in which it demonstrated a decrease in rate of exacerbations in a Phase IIa trial consisting of COPD patients with eosinophilic inflammation but failed to reach the primary end point in two phase III trials consisting of COPD patients with exacerbation histories of moderate to very severe grades (135,185,189,190).

Another target of interest in eosinophilic COPD is the transcription factor, GATA3 which plays a part in the activation of Th2 cells and action on the type 2 innate lymphoid cells (ILC2 cells) (191). These interactions result in increased cytokine IL-4, IL-5 and IL-13 production which are thought to moderate airway eosinophilia in non-allergic asthma and COPD (191). SB010 is a drug that consists of the active constituent, DNAzyme hgd40 which specifically binds to and cleaves the mRNA of GATA3 and has been evaluated in human and animal models (191). Turowska *et al.*, reports significantly reduced GATA3 mRNA along with reduction of Th2-specific cytokines production in murine models of allergic airway

inflammation when treated with SB010 (192). Greulich *et al*., were able to prove the involvement of the GATA3 pathway in eosinophilic COPD patients through their phase IIa clinical trial which sought to demonstrate the feasibility to reduce sputum eosinophilia in COPD patients with elevated sputum eosinophil counts through treatment involving the inhalation of GATA3-specific DNAzyme SB010 for 4 weeks and were able to conclude that a decrease in airway eosinophilia is possible to attain via the GATA3-specific DNAzyme as such seen previously in asthmatic patients (191). Furthermore, the trial was able to identify the safety aspects associated with the use of SB010 in the studied COPD population whereby serious adverse events were not reported (191). However, the trial only included a small patient group so more studies including a larger patient groups and longer treatment period will be required for the identification of the long-term clinical effectiveness and safety in this subgroup of COPD patients (191).

4.0 Eosinophils and Cystic Fibrosis

Cystic fibrosis is an autosomal recessive disorder that is an illness which is chronically progressive and life-limiting (193–196). The disease occurs due to a gene mutation of CF transmembrane conductance regulator (CFTR) causing a significant functional deficiency in the CFTR protein which are highly expressed in the tissues of the airways (193–195). In normal circumstances, the CFTR protein functions to regulate the movement of chloride and sodium ions across the epithelial cell membranes but when either one or both copies of the gene is mutated, transport of the ions is deficient, resulting in the accumulation of thick mucus throughout the body which can lead to the development of respiratory insufficiency, consequential deficiencies in host anti-bacterial defences and various other systemic obstructions and abnormalities (193–195). Including being a chronically progressive lung illness, cystic fibrosis is also characterised by extensive inflammation and respiratory failure (195). This could be further exacerbated by the formation of biofilms on the exterior of medical appliances utilized for treatment causing life-threatening infections in these patients (197). Cystic fibrosis is known to affect multiple organs and the common symptoms include breathlessness or wheeze, persistent cough along with frequent respiratory infections, increased appetite and bulky greasy stools (198). Deterioration in pulmonary symptoms and the loss of lung function are the key factors that determine the severity of cystic fibrosis. More than half of the patients may require a lung transplantation during severe form of the disease (199).

Cystic fibrosis associated inflammation is known to be dominated by neutrophils which releases oxidants and proteases such as elastase which can be found in airway secretions preceding the development of bronchiectasis in these patients (194,195,200,201). A few studies have also reported of the correlation between neutrophil elastase with the deterioration of lung function as well as respiratory exacerbations (194,200). However, Zhang *et al*., notes that a type II inflammation associated with an asthma phenotype can often be recognised in cystic fibrosis patients (195). The presence of this type of inflammation is indicated by the increased levels of total immunoglobulin E (IgE), specific IgE sensitization or absolute eosinophil count in the circulation independent of infection by the pathogen, *Pseudomonas aeruginosa* (195,202). This enables the differentiation of the similar symptoms of asthma and cystic fibrosis in terms of variability of lung function and bronchodilator response which in turn aids the recovery of lung function, airway remodeling, and rate of exacerbations in patients with eosinophilic inflammation related exacerbations through the focus of treatment on to the type II inflammatory response (195). Moreover, the study by Zhang *et al*., showed positive effects via a decrease in eosinophil and IgE levels as well as dose of corticosteroids when mepolizumab was used to treat type II inflammation in patients with eosinophilic phenotype cystic fibrosis (195).

Apart from neutrophils that are known to play an important inflammatory role in airway disease associated with cystic fibrosis, eosinophils are also found to be one of the components that contribute to injury during cystic fibrosis. In two separate studies involving 42 and 20 patients with cystic fibrosis respectively, Koller *et al*., showed significantly higher levels of ECP in sputum of patients with cystic fibrosis compared to control subjects (203,204). Collectively, these findings indicated the destructive role played by eosinophils and an interrelationship between the clinical variables and secretory activity of eosinophils in cystic fibrosis. An increase in the propensity of eosinophils to release their granule proteins and a firm correlation between ECP levels and variables of pulmonary function like forced vital capacity has also been identified (204,205). Furthermore, cytokine profiles were found to be responsible for eosinophil activation and degranulation in patients with cystic fibrosis, especially IL-8 and IL-3 were found to be significantly correlated with the levels of ECP in sputa from 32 patients and therefore, these appeared to be responsible for elevated degranulation of eosinophils (206).

However, the mainstay drugs used to treat cystic fibrosis have been noted to cause various side effects (207). Thus, in order to diminish these drug associated side effects experienced by patients, tissue targeting strategies should be utilized to overcome the inadequate drug penetration due to the barrier formed by the accumulation of thick and viscous mucus (208,209).

5.0 Eosinophils and Pneumonia

Pneumonia is a disease which is relatively prevalent and has caused a significant burden towards the global population (210). It is defined by the acute infection of the lung parenchyma and is used as a hypernym to describe a cluster of syndromes caused by not one disease but a group of specific infections, which result in different manifestations and sequelae (210,211). Acute and chronic eosinophilic pneumonia are two common pulmonary eosinophilic disorders which occur due to lung tissue damage by activated eosinophils (212,213). These disorders are characterized by the build-up of eosinophilic infiltrates in the pulmonary parenchyma, often accompanied by peripheral blood eosinophilia, which can be caused by either infectious or non-infectious factors (212,214).

De Giacomi *et al*., reports significantly elevated levels of IL-33 in acute eosinophilic pneumonia (AEP) patients and hypothesized that IL-33 plays an essential role in AEP (213). This is because attraction and activation of eosinophils is amplified when Th2-polarizing cytokines, IL-5 and IL-13 are rapidly produced due to the robust production of IL-33 as well as the enlistment and activation of ILC2 cells in the mucosa of the airway which takes place due to either epithelial or endothelial cell (213). However, the study by Katoh et. al. reports that IL-33 levels were not considerably elevated in the bronchial alveolar lavage fluid (BALF) of eosinophilic pneumonia patients whereas IL-25 and IL-5 levels were significantly heightened in the BALF of chronic eosinophilic pneumonia (CEP) patients but not AEP patients (215). Hence, it was postulated that CEP might be perpetuated by IL-25 which were thought to be produced by eosinophils via IL-5 stimulation (215,216). Despite these reports, the pathophysiology of both AEP and CEP are poorly understood and will require further studies to completely delineate it (213,214).

Treatment of AEP and CEP both utilize corticosteroids as the mainstays of treatment in which AEP is treated with systemic corticosteroids whereas CEP utilizes oral corticosteroid therapy with an aim to diminish the disease progression as well as lessen the risk of relapse (212–214,217–219). As an alternative therapy for CEP, biologic agents have been utilized for the regulation of eosinophilic inflammation (218,219). Studies have shown the effectiveness of omalizumab, an anti-IgE antibody and anti-IL-5 antibody's, mepolizumab and reslizumab in the treatment of CEP through the reduction or discontinuation of corticosteroid use in patients with relapsed CEP (218,219). IL-5 is a common aim in the treatment of CEP because its elevated levels are associated with the release of cytotoxic granular proteins from eosinophils which is postulated to be an important underlying mechanism of CEP (219). However, there is insufficient information supporting the utilization of biologics for CEP treatment as well as the concerns associated with the adaptation and treatment duration of it (218,219). Hence, further studies regarding the use of steroid-sparing therapeutic regimens as an alternative therapy for CEP are required.

6.0 Eosinophils and Lung Cancer

Lung cancer is a common malignancy among both sexes and represents over 10% of all malignancies (220,221). The occurrence of eosinophilia is often attributed to hematological malignancies, with some cases of solid tumour-associated eosinophilia. Approximately 1% of malignant tumours are associated eosinophilia (220). Bone marrow stimulation by IL-5 is theorized to be the primary causative factor of solid malignancy-associated eosinophilia. Paraneoplastic eosinophilia cannot be treated with a specific treatment, besides treating the underlying malignancy using typical therapies, including surgery, chemotherapy, radiotherapy, or even novel treatments such as metformin (222).

6.1 Function of eosinophils in the pathophysiology of lung cancer

A distinct feature of cancer is sustained low-grade inflammation. Eosinophils have long been associated with cancer as they are one of the regulatory components of the tumour microenvironment (TME) responsible for tumour initiation and development (223). Eosinophils are commonly associated with negative connotations in allergic diseases, however they are able to provide immune protection against helminths, bacterial and viral pathogens (223).

There are evidences showing that the infiltration of eosinophils into tumour cells results in an improved prognosis of cancer (224). Tumour-associated eosinophilia can be

observed in many studies of patients with cancer as well as mouse models of cancer. Studies have reported that activated eosinophils play a vital role in tumour rejection. Activated tumour-honing eosinophils release chemoattractants which induces the migration of tumour-specific CD8(+) T-cells into the tumour, resulting in tumour eradication and thus, an increased chance of survival (225). Tumour rejection is also promoted by the significant alterations in the tumour microenvironment as initiated by the activated eosinophils, such as macrophage polarization and normalization of tumour vasculature (225).

Eosinophil peroxidase (EPO), which is an eosinophilic cationic granule protein, drives cell cycle progression and proliferation at non-cytotoxic levels, thus suggesting the role of eosinophils in tumorigenesis (226). According to a study conducted by Walsh *et al*., EPO increased the expression and phosphorylation of epidermal growth factor-2 (HER2) in a sustained manner, which consequently induced extracellular-regulated kinase 1/2 activation (226). Subsequently, cyclin-dependent kinase inhibitor p27 (kip) entered the cytoplasm from the nucleus in a focal adhesion kinase-dependent manner. Thus, the findings of the study led to the conclusion that EPO can induce the upregulation of cell proliferation via HER2 mediation.

However, the actions of eosinophils and eosinophil mediators varies according to the cancer type as eosinophils have been linked to improved prognosis in certain neoplasias but poor prognosis in others. This is because eosinophils can produce either anti-tumorigenic (e.g. TNF-α, IL-18, granzyme, and cationic proteins) or pro-tumorigenic molecules (e.g. pro-angiogenetic factors) subject to the internal environment (223). Melanoma, oral, gastric, colorectal, and prostate cancers are neoplasias in which eosinophils secrete anti-tumorigenic factors whereas a poor prognosis is expected from the actions of eosinophils in cervical carcinoma and Hodgkin's lymphoma (223).

Although there exist evidences for a positive correlation between eosinophils and better responses in some patients with melanoma, investigations to study the relationship between eosinophils and NSCLC are not frequently conducted. However, a case report showed that a patient with metastatic lung adenocarcinoma (AD), a type of non-small cell lung cancer (NSCLC), presented with shortness of breath, chest pain and associated hypereosinophilia, as defined by an absolute eosinophil count (AEC) of >1500 cells//μL, in the absence of primary bone marrow disorder (220). Additionally, a study done by Lou *et al*., reported that a patient with metastatic lung AD presented with asymptomatic hypereosinophilia following initiation of nivolumab therapy, which is an anti-programmed cell death 1 (PD-1) drug (227). Following a transient discontinuation of therapy, her

eosinophil counts transiently decreased, but increased again following re-initiation. The patient showed a favourable response throughout the therapy. Thus, eosinophils can act as potential peripheral biomarkers of favourable response to immunotherapy in patients with lung carcinoma, which warrants further studies of the role of eosinophils in lung carcinoma for the development of novel treatment strategies. As such, efforts should be focused on the delivery of eosinophil-targeting drugs for lung cancer in the form of nanocarriers in order to improve specificity and reduce adverse effects (228,229).

6.2 Eosinophils as biomarkers of lung cancer

Eosinophils can be used as a peripheral blood marker for detecting tumour-associated protein expression in cancer patients. Tumours cells in primary lung AD express surface immune factors such as indoleamine-2,3-dioxygenase-1 (IDO1) and programmed cell death-ligand-2 (PD-L2) (230). A study showed that AEC, along with absolute monocyte count (AMC), could potentially act as a biomarker to predict IDO1 expression in patients with resected stage 1 to 3 primary lung AD (230). IDO1 is a tryptophan (Trp) catabolic enzyme that catalyzes the transformation of Trp to kynurenine for immunosuppressive functions such as activation of myeloid-derived suppressor cells and T-regulatory cells, inhibition of effector T and NK cell functions, and promotion of neovascularization of solid tumours (231).

Eosinophils can also act as clinical outcome predictors in patients with lung AD. Tanizaki *et al.*, reported that NSLC patients administered nivolumab therapy exhibited higher AECs which were significantly linked to a better progression-free and overall survival (232). Additionally, a cohort study reported that elevated eosinophil counts correlated with reduced mortality risk in specific subgroups of cancer patients, such as colorectal cancer (233).

However, a study showed that only a small percentage of eosinophils (0.3%) were present in the immune infiltrate composition of NSCLC tumours (234).

Besides AEC, another eosinophil-related biomarker of lung AD is EPO. Ye *et al.*, demonstrated that EPO overexpression in lung AD patients can potentially act as a biomarker for poor prognosis (235). During the study, a significantly higher expression of EPO mRNA and protein in lung AD tissues as compared to adjacent normal tissues were observed (235). Moreover, patients with EPO overexpression were significantly associated with pathologic-tumour nodes metastases stage and lymph node metastasis, as well as decreased survival time as compared to patients with low levels of EPO (235).

7.0 Eosinophils and Acute Lung Injury

Acute lung injury (ALI) describes clinical syndromes of acute respiratory failure with prominent mortality and morbidity rates (236). The management of ALI after exclusion of infection consists of primarily supportive measures. Other diagnoses can be ruled out by performing surgical lung biopsy, however this measure has proven to be less useful for predicting therapy methods and outcomes in ALI (237). Peripheral blood and tissue eosinophil counts are often referred to as hallmarks of steroid-responsive acute eosinophilic pneumonia, but are not typically associated with ALI (237). Thus, a study conducted by Willetts *et al.* demonstrated that eosinophil peroxidase-recognizing monoclonal antibody (EPX-mAb) immunohistochemistry can be used as a method to assess eosinophil accumulation or degranulation in the lungs of ALI patients as well as predict the prognosis of survival (237). Therefore, it is suggested that EPX-mAb immunohistochemistry can be utilized as a diagnostic biomarker to identify a proportion of ALI patients with favourable clinical outcomes.

Various clinical trials conducted targeting eosinophils in respiratory diseases are shown in Tables 1-3 and various important drugs targeting eosinophils in respiratory diseases are shown in Figure 4 and Table 4.

Conclusion

Respiratory disorders are chronic conditions requiring long-term therapy to prevent clinical symptoms. Morbidity and mortality are high for many respiratory disorders and existing available treatments are restricted due to lack of effectiveness, severe toxicity or both. Recent efforts to understand eosinophil biology has opened the door to many innovative biological treatments. Various drugs that target eosinophils are currently being tested in clinical trials and needs to be validated prior to medical use. No health issues are raised till date, despite theoretical concerns about the possible toxicity of rapidly declining eosinophil counts and the long-term effects of eosinophil depletion on the immune system and tumor monitoring. We expect to gain a better understanding with more research to recognize new biological factors for these respiratory disorders and eventually the implementation of this knowledge to patients.

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Figure 1: Schematic representation of surface receptors and immunological moieties of eosinophils. Eosinophils are bi-lobed, multi-functional innate immune cells with diverse cell surface receptors, including those crucial for chemotaxis, cell adhesion, activation (via cytokine/growth factors), lipid mediation, and immune modulation (CD40/80/86/siglec-8/Fc/MHC class-II). Eosinophils also comprise of a variety of intracellular functional moieties, such as lipid bodies and granules that play a key role in immune regulation and eosinophil functionality.
Abbreviations: PR – Pattern recognition receptors; MBP – Major basic protein; EPX – Eosinophil peroxidase; MHC – Major histocompatibility complex; CD – Cluster of differentiation; CCR - CC-chemokine receptor; CXCR - CXC-chemokine receptor; FPR - Formyl peptide receptor; C5aR - Complement component 5a receptor; C3aR - Complement component 3a receptor; LFA-1 - Lymphocyte function-associated antigen 1; CR – Complement receptor; LTC4 – Leukotriene C4; LTE4 - Leukotriene E4; Leukotriene D4; PAF - Platelet-activating factor; PAR – Protease activated receptor; PAFR - Platelet-activating factor receptor; CRTH2 - Chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells; DP1 - Prostaglandin D2 receptor 1; EP4 - Prostaglandin E2 receptor 4; LTB_4 - Leukotriene B4

Figure 2: Pathophysiology of eosinophil in asthma Abbreviations: IL (Interleukin); TSLP (thymic stromal lymphopoietin); APC (Antigen-presenting cell); Th0 (naïve T-cell); Th2 (T-helper type 2 cells); MBP (Major basic protein); EPO (Eosinophil peroxidase); ECP (Eosinophil cationic protein); EDN (Eosinophil-derived neurotoxin); NGF (Nerve growth factor); SCF (Stem cell factor); ASM (Airway smooth muscle); AHR (Airway hyperresponsiveness); TGF-β (Transforming growth factor beta); ECM (Extracellular matrix)

Figure 3: Pathophysiology of eosinophil on COPD Abbreviations: CPE - Cytopathogenic effect; $CD4^+TH2$ – $CD4^+$ T helper 2 cells; IL – Interleukin; PAF – Platelet activating factor; MBP – Major basic protein; EPO – Eosinophil peroxidase; ECP – Eosinophil cationic protein

Figure 4: Current drugs acting on eosinophils

Abbreviations: IL (Interleukin); TSLP (thymic stromal lymphopoietin); Th0 (naïve T-cell); Th2 (T-helper type 2 cells); ILC2 (Type 2 innate lymphoid cells); IL-5Rα (Interleukin-5 receptor alpha); CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells); PGD2 (Prostaglandin D2); GM-CSF (Granulocyte-macrophage colony-stimulating factor); IL-4Rα (Interleukin-4 receptor alpha); IgE (Immunoglobulin E); FcεRI (High-affinity immunoglobin E receptor); DC (Dendritic cell)

Table 1: List of clinical trials on eosinophils for asthma

S. No	Intervention	No. of recruitments/Country/Phase of study/Ongoing/cancelled	Area of trial	Sponsor	Ref
1.	Saffron	86/Phase 1/Iran/Ongoing	Effect on allergic asthma patient's clinical symptoms, blood pressure, lipid panels, basophils and eosinophils receiving saffron supplementation.	Ahvaz-Jundishapur university of medical sciences	(238)
2.	Mepolizumab	32/Japan	Evaluation of mepolizumab's efficacy and safety in severe eosinophilic asthma patients receiving treatment with it long-term.	Sutoh Hospital	(239)
3.	Vitamin D	86/Mexico	Evaluation of vitamin D's effect as a supplement on the pathogenic bacteria colonization in the upper respiratory tract of patients with allergic asthma.	General Hospital of Mexico	(240)
4	Benralizumab	2681/Phase III/Completed	Effects of baseline factors of patients with severe asthma on the efficacy of benralizumab.	AstraZeneca	(241)
5	Benralizumab	2508/Phase III/Completed	Efficacy of benralizumab based on atopic status and serum	AstraZeneca	(242)

			concentrations of IgE.		
6	Fevipiprant	61/Phase II/United Kingdom/ Completed	Potential reduction of eosinophilic airway inflammation in moderate-to-severe eosinophilic asthma receiving fevipiprant (QAW039).	Novartis Pharmaceuticals	(111)
7	Mepolizumab	651/Phase III/Completed	Assessment of the effectiveness and long-term safety associated with the treatment of severe eosinophilic asthma patients with subcutaneous mepolizumab.	GlaxoSmithKline	(243)
8	Induced sputum	300/Multiple countries/Phase III/Completed	Comparison of different gene and protein expression in sputum samples of severe asthma and non-smoking mild/moderate asthma patients	Academisch Medisch Centrum - Universiteit van Amsterdam (AMC-UvA)	(244)
9	Omalizumab	1071/USA/Phase III/Completed	Evaluation of factors associated with the prediction of response to omalizumab for the purpose of identifying patients with highest potential to attain greatest clinical	-	(245)

			advantage from treatment.		
12	Mepolizumab	621/Multiple countries/Phase II/Completed	Association of asthma exacerbations with enhanced decline in lung function.	GlaxoSmithKline	(246)
13	Tiotropium Respimat	459/Multiple countries/Phase III/Completed	Influence of T2 status on responses to tiotropium Respimat add-on therapy.	Boehringer Ingelheim	(247)
14	Mepolizumab	556/Multiple countries/Phase III/Completed	Assessment of mepolizumab's effect on severe eosinophilic asthma patient's health-related quality of life.	GlaxoSmithKline	(248)
15	-	120/USA/Phase I/Ongoing (Suspensed)	Description of exacerbation-prone asthma's clinical features.	University of Wisconsin, Madison	(249)
16	Omalizumab	-	Benefit and persistence of response in subjects continuing or withdrawing from long-term omalizumab treatment.	-	(250)
17	Benralizumab	211/Multiple countries/Phase III/Completed	Assessment of benralizumab as a treatment for mild-to-moderate persistent asthma patients in terms of its safety and efficacy.	AstraZeneca	(251)
18	Mepolizumab	580/Japan/Phase III/Completed	Description of mepolizumab's safety and efficacy in the	GlaxoSmithKline	(252)

			treatment of Japanese patients with severe eosinophilic asthma from the MENSA trial.		
19	*Nigella sativa*	80/Saudi Arabia/Phase II/Completed	Evaluation of nigella sativa oil benefits as a supplement in the treatment of asthma based on clinical and inflammatory parameters	University College, London	(253)
20	Oral corticosteroids	20/Japan/Phase 1/Completed	Identification of the relationship between asthmatic patients FeNO levels and blood eosinophils.	Japanese Society for the Promotion of Science and Wakayama Medical Award for Young Researchers	(254)
21	-	112/Phase I	Assessment of the relationship between type 2 inflammation and risk of virus-induced asthma exacerbations	Bispebjerg University Hospital, the University of Copenhagen	(255)
22	Mepolizumab	580/Multiple countries/Phase III/Completed	• Assessment of the relationship of baseline blood eosinophil counts and mepolizumab's efficacy.	GlaxoSmithKline	(256)
23	Dupilumab	776/Multile countries/Phase II/Completed	• Assessment of dupilumab's efficacy and safety aspects as	Sanofi-Genzyme and Regeneron Pharmaceuticals	(257)

			an add-on therapy in uncontrolled and persistent asthmatics on medium-to-high doses of ICS/LABA therapy, disregarding the baseline eosinophil counts		
24	Personalised therapy	300/USA/Phase III/Completed	Personalization of asthma therapy	Milton S. Hershey Medical Center	(258)
25	Lebrikizumab	1068/Multiple countries/Phase II/Completed	Assessment of lebrikizumab's efficacy and safety aspects when used for the treatment of uncontrolled asthmatics despite ICS and at least a second controller medication.	Hoffmann-La Roche	(259)
26	-	483/Multiple countries/Phase IV/Completed	Addressment of unanswered fundamental queries associated with biomarkers of asthma. Assessment of the relationship between biomarkers of asthma and health outcomes associated with disease.	Hoffmann-La Roche	(260)
27	-	259/Finland/Phase	Evaluation of	Seinajoki	(261

		I/Completed	the 12-year prognosis in patients who develop asthma as adults and the aspects related to its prognosis.	Central Hospital	)
28	-	571/Netherland	Investigation of factors related to frequent exacerbations in non-smoker asthma patients and asthma patients with a history of smoking.	GlaxoSmithKline	(262)
29	Benralizumab	106/South Korea & Japan/Phase I/Completed	Evaluation of benralizumab's effect as a treatment of uncontrolled eosinophilic asthma who experienced 2-6 exacerbations in the previous year and treated with medium/high dosages of ICS and LABA.	MedImmune LLC	(263)
30	-	315/Multiple countries/Phase II/Completed	Comparison between asthma associated with high and low airway reversibility in terms of function of lungs, biomarker panel, and control of disease.	Amgen	(264)
31	SB010	-	Evaluation of SB010 in terms of its safety and efficacy in the	-	(191)

			treatment of allergic asthma patients with sputum eosinophilia.		
32	Salmeterol/ fluticasone propionate Salmeterol	-	Evaluation of efficacy of combination therapy in the treatment of cough variant asthma.	-	(265)
33	Mepolizumab	580/Multiple countries/Phase III/Completed	Determination of mepolizumab use in the treatment of severe eosinophilic asthma to potentially reduce frequent corticosteroid usage in these patients.	GlaxoSmithKline	(266)
34	-	132/China	Identification of the association between levels of FeNO and possible factors in children without asthma.	-	(267)
35	Benralizumab	964/Multiple countries/Phase II/Completed	Assessment of benralizumab's in terms of its effectiveness and safety in the treatment of adult patients with uncontrolled eosinophilic asthma.	MedImmune LLC	(268)
36	OC000459	40/United Kingdom/Phase II/Completed	Determination of effect associated with lower doses of OC00459 given once daily.	Chiesi Farmaceutici S.p.A.	(269)

			Defined phenotype of patients with highest response to OC00459 treatment.		
37	Mepolizumab	621/Multiple countries/Phase II/Completed	Identification of subgroups of severe asthma patients at risk for exacerbations with distinct characteristics. Determine each patient subgroup's response to treatment.	GlaxoSmithKline	(270)
38	-	-	Investigation of $P2Y_{12}$ and gpr99 expression by eosinophils and their LTE_4 response capacity.	Merck Pharmaceuticals	(271)
39	-	169/Europe	Assessment of phenotype stability based on biomarkers or physiological variables.	-	(272)
40	Inhaled cationic airway lining modulator	-	Evaluation of the role of enhancing epithelial barrier in reducing inflammation of the airway due to inhaled particles.	-	(273)
41	Omalizumab	850/Multiple countries/Phase III/Completed	Assessment of FeNO, peripheral blood	Genentech, Inc	(274)

			eosinophils count, and serum periostin as potential biomarkers of Th2 inflammation and potential predictors of omalizumab treatment outcomes.		
42	Azithromycin	109/Belgium/Phase 4/Completed	Benefit of macrolides in neutrophilic airway disease.	University Hospital, Ghent	(275)
43	Bacillus Calmette-Guérin Moreau vaccine (BCG-Moreau)	-	Analysis of impact of different BCG-Moreau strain administration routes and application periods on inflammation of the airways and lungs as well as remodelling in an allergic asthma murine model.	-	(276)
44	Oral prednisolone	233/Sweden/NA/Active	Assessment of the practicality to predict oral prednisolone response based on patient's medical history, physiological variables and biomarkers.	Karolinska Institutet	(277)
45	-	0/USA/Phase 3/Withdarwn	Determination of non-eosinophilic asthma phenotype's prevalence and clinical	Milton S. Hershey Medical Center	(278)

			features.		
46	Allergen	-	Identification that loss of asthma control is associated with increased eosinophilic inflammation of the airway and airway responsiveness to bradykinin due to exposure to allergens causing exhaled NO levels to be elevated	-	(279)
47	Aerobic training	58/Brazil/Phase 3/Ongoing	Evaluation of aerobic training effects on eosinophil inflammation and nitric oxide of moderate or severe persistent asthma patients.	Instituto de Investigação em Imunologia	(280)
48	Allergen	-	Examination of potential to ameliorate asthma-like pulmonary inflammation via induction of oral tolerance to cockroach allergen. Determination of mechanisms associated with the effectiveness of oral tolerance.	-	(281)
49	Simvastatin	-	Enhancement of anti-inflammatory effects of corticosteroids by statins.	-	(282)

Table 2: Clinical trials on eosinophils acting in COPD

S. No	Intervention	No. of recruitments/Country/Phase of study/Ongoing/cancelled	Area of trial
1	Inhaled corticosteroids (ICS)	1144/Korea	Evaluation of ICS prescription status accord the revision of the 2017 GOLD guidelines
2	Benralizumab	29/Finland/Phase II	Effect on bacterial load in the airways assoc with the decline in eosinophilic airway inflammation when treated with benralizum
3	Budesonide	1200/USA/Phase III/Completed	Establishment of characteristics that determi risk of exacerbation and clinical response to treatment with ICS in COPD patients using modelled continuous variable, eosinophil co
4	LABA-ICS/ LAMA	645/Germany/Completed	Comparison of efficacy and safety of treatm initiation guided by blood eosinophils with LABA-ICS or LAMA in COPD patients.
5	Unfractionated heparin	-	Demonstration of improve lung function in pulmonary rehabilitation COPD patients rec unfractionated heparin. Demonstration of the novel, safe and effecti aspects of unfractionated heparin when used

			treatment of COPD.
6	Roflumilast	158/Multiple countries/Phase III/Completed	Assessment of roflumilast's anti-inflammato effects on bronchial mucosal inflammation i moderate-to-severe COPD patients and chro bronchitis patients
7	ICS-LABA LABA-LAMA	3362/Multiple countries/Phase III/completed	Evaluation of blood eosinophil's value as a predictor of responsiveness in the treatment COPD exacerbations using ICS/LABA vers LABA/LAMA therapy.
8	Salmeterol/Fluticasone propionate Salmeterol	60/Italy/NA/Completed	Determination of ICS effects on microbial lo airways of COPD patients. Evaluation of the underlying inflammatory mechanisms associated with the colonisation microbiome of airways.
9	Fluticasone propionate/ formoterol	0/NA/Phase III/Withdrawn	Effect of fluticasone propionate/formoterol (FP/FORM) in COPD
10	Mepolizumab	19/Canada/Phase III/Completed	Determination of mepolizumab's potential t decrease percentage of sputum eosinophil in cigarette smoke associated COPD patients w persistent sputum eosinophilia. Assessment of mepolizumab's effects on cli features of cigarette smoke associated COPI patients with persistent sputum eosinophilia
11	Eosinophil-guided corticosteroid-sparing therapy	318/Denmark/Phase IV/Completed	Determination of potential reduction of syst corticosteroid use in the treatment of AECO without affecting the outcome.
12	Losmapimod	72/United Kingdom/Phase IV/Completed	Evaluation of exacerbation reduction by losmapimod in moderate-to-severe COPD p
13	Indacaterol-Glycopyrronium Salmeterol-Fluticasone	3362/Multiple countries/Phase III/Completed	Identification of the role of LABA-LAMA r in COPD patients with at least one exacerba the past year.
14	ICS withdrawal	444/Denmark/Phase IV/Ongoing	Identification of relationship between baseli blood eosinophil count and rate of lung func decline. Evaluation of risk/benefit ratio in COPD pat receiving ICS therapy through the prediction patient response to ICS using a biomarker
15	Fluticasone furoate/vilanterol	1635/Multiple countries/Phase III/Completed	Identification of COPD patient clusters with potential to attain benefit from ICS/LABA treatment compared to LABA singularly.

	Vilanterol		Validation of COPD clusters identified in th
16	Losmapimod	604/Multiple countries/Phase II/Completed	Analysis of losmapimod's effect as a treatm reduce moderate/severe exacerbation rates i subgroups of COPD patients with a baseline and >2% blood eosinophils.
17	Prednisolone	-	Investigation of the functionality of blood eosinophils in the direction of corticosteroid treatment during COPD exacerbations.
18	Hypertonic saline Mannitol challenge	-	Examination of the association between airv hyperresponsiveness to mannitol and the inflammatory markers in the collected sputu induced by hypertonic saline, blood and exh

Table 3: Clinical trials on eosinophils acting in other respiratory diseases

S. No	Intervention	Condition	No. of recruitments /Country/Phase of study/Ongoing/cancelled	Area of trial
1	RPC4046	Eosinophilic esophagitis	100/Multiple countries/Phase II/Completed	Evaluation of RPC4046 in eosinophilic esophagitis p terms of efficacy and safety
2	Albendazole	Helminth infection	-	Effect of infection by helminths on the status of activ granulocytes.
3	Fractionated exhaled nitric oxide (FeNO) testing	Eosinophilic esophagitis	120/United States/NA/Ongoing	Utility of FeNO in predicting severity of eosinophilic activity.
4	Obesity	Sinonasal disease in asthma	-	Determination of the association between obesity and severity of sinonasal disease, and/or effects on treatm nasal corticosteroid response in patients with asthma.
5	Mepolizumab	Eosinophilic granulomatosis with Polyangiitis	136/Multiple countries/Phase III/Completed	Comparison between mepolizumab and a placebo as regimen in relapse or refractory eosinophilic granulo patients with polyangiitis in terms of its efficacy and a duration of 52 weeks.
6	Mepolizumab	Nasal polyps	160/United states & Europe/Phase	Assessment between mepolizumab and a placebo in t safety and efficacy for treatment of severe bilateral n polyposis.

			III/Ongoing	
7	Aspirin	Aspirin-exacerbated respiratory disease	-	Investigation of the relationship between aspirin thera associated clinical outcomes and levels of plasma eic aspirin-exacerbated respiratory disease.
8	Aspirin	Aspirin-exacerbated respiratory disease	-	Determination of change ILC2 levles in peripheral bl nasal mucosa in aspirin-exacerbated respiratory disea COX-1 inhibitor-induced reactions.
9	-	Aspirin-Exacerbated Respiratory Disease	-	Identification of the mechanistic basis for mast cell a
10	Prednisolone	Chronis eosinophilic pneumonia	50/Japan/Phase IV/completed	Comparison between short-term and long-term cortic treatment in chronic eosinophilic pneumonia patients its safety and effectiveness.
11	Aspirin	Aspirin-exacerbated respiratory disease	-	Investigation of aspirin's capacity to trigger activatio eosinophils and mast cells in aspirin exacerbated resp disease.
12	Aspirin	Aspirin-exacerbated respiratory disease	-	Investigation of mechanism associated with the activ eosinophils. Identification of novel inflammatory mediators throu utilization of proteomics.
13	Reslizumab	Eosinophilic esophagitis	227/United States & Canada/Phase II/Completed	Evaluate the effectiveness of reslizumab in children a adolescent eosinophilic esophagitis subjects.
14	Budesonide/ Montelukast Double-dose budesonide	Non-asthmatic eosinophilic bronchitis	63/China/Phase IV/Unknown	Comparison of effectiveness and tolerance between m as an add-on therapy to budesonide and double dose budesonide for the suppression of airway eosinophili reduction of severity of cough in non-asthmatic eosin bronchitis.
15	Allergen	Allergic rhinitis	-	Determination of eosinophilia in the mucosa, apoptot eosinophils, general cell apoptosis, cell proliferation and CCL11 expression in allergic airway tissues of h resolution of symptomatic eosinophilic inflammation
16	Montelukast	Bronchiolitis	146/Iran/NA/ Completed	Investigation of montelukast's effect on degranulatio eosinophils and recurrent episodes of wheezing in pa post-respiratory syncytial virus bronchiolitis.

Table 4: Current drugs acting on eosinophils in respiratory diseases

S. No.	Drug	Route of administration	Indication	Mechanism of action	Biological effects	Clinical outcomes	Reference(s)
1.	Reslizumab	IV	Adjunctive treatment in severe eosinophilic asthma	Humanized anti-IL-5 monoclonal antibody	• Binds and sequestrates to IL-5 which inhibits receptor binding on eosinophil surface • Decreases survival and activity of eosinophils	• 50% reduction in asthma exacerbation rates • Improvement in lung function, asthma control and QOL	(89–91,317)
2.	Mepolizumab	SC injection	Adjunctive treatment in refractory eosinophilic asthma and	Humanized anti-IL-5 monoclonal antibody	• Binds and sequestrates to IL-5 which inhibits recept	• Reduction in blood and sputum eosinophil counts • Reduction in	(55,92–94,317,318)

			severe eosinophilic COPD		or binding on eosinophil surface • Decreases survival and activity of eosinophils • Attenuates systemic differentiation of eosinophils	asthma exacerbation rates • Improvement in lung function, asthma control and QOL • Steroid-sparing effect • Reduction in annual rates of moderate or severe COPD exacerbations	
3.	Benralizumab	SC injection	Adjunctive treatment in severe eosinophilic asthma	Humanized afucosylated anti-IL-5Rα monoclonal antibody	• Blocks IL-5 interaction with its receptor on eosinophils • Inhibits eosinophil differentiation and maturation in bone marrow • Induces Ab-	• Rapid reduction of serum and tissue eosinophils • 50% reduction in asthma exacerbation rates • Favourable results on lung function, ACQ and QOL • Steroid-sparing	(97,317)

					dependent cell-mediated cytotoxicity via afucosylation which results in eosinophil apoptosis and depletion	effect	
4.	Omalizumab	SC injection	Adjunctive treatment in moderate-severe allergic asthma	Humanized anti-IgE monoclonal antibody	• Binds to free IgE to prevent interaction with FcεRI on mast cells, dendritic cells, and basophils • Decreases pro-inflammatory mediators release from mast	• Significant reduction in PBE counts in patients receiving concomitant ICS • Improved clinical outcomes • Improves asthma control and QOL	(87,102)

					cells and basophils • Inhibits allergen-induced T-cell differentiation		
5.	Dupilumab	SC injection	Adjunctive therapy in severe eosinophilic asthma	Humanized anti-IL-4/IL-13 monoclonal antibody	• Binds to IL-4Rα to block IL-4 and IL-13 signaling pathway • Inhibits B-cell IgE class switching • Reduces airway recruitment of eosinophils	• Reduction in asthma exacerbation rates • Improvement in AQLQ, ACQ and FEV_1 • Transient increase in peripheral eosinophil counts (especially in patients with baseline blood eosinophil counts of ≥300 eosinophils/μL)	(87,104,110)

6.	MEDI-563	IV	Asthma	Humanized anti-IL-5Rα monoclonal antibody	• Binds to epitope on IL-5Rα near IL-5 site of catalysis • Potently induces ADCC of eosinophils and basophils *in vitro*	• Depletion of peripheral blood and bone marrow eosinophils and basophils in non-human primates.	(107)
7.	Ki19003	Oral	Allergic asthma	CCR3 antagonist	• Inhibits CCL11-induced migration of CCR3-expressing murine pre-B cells with IC_{50} values of 0.02 μM • Inhibits CCL11-induce	• Inhibition of allergen-induced airway eosinophilia at an oral dose of 200 mg/kg • Reduction in TGF-β1 production and amount of hydroxyproline in BALF • Attenuation of allergen-	(109)

					d migration of eosinophilic granulocytes from bone marrow	induced subepithelial and peribronchial fibrosis	
8.	Fevipiprant, Timapiprant	Oral	Fevipiprant: Mild-moderate eosinophil-dominant allergic asthma Timapiprant: Persistent eosinophilic asthma	Small molecule PGD2 inhibitors	• Antagonist of CRTH2 expressed on eosinophils which mediates chemotactic response to PGD2	• Reduction in sputum eosinophil counts • Improvement of asthmatic symptoms	(110,111)
9.	AMG 157	IV	Mild allergic asthma	Humanized anti-TSLP monoclonal antibody	• Binds to TSLP, which is an alarmin, to prevent receptor interaction and stimulation of eosinophilic inflammation via	• Reduction in maximum percentage decrease of FEV_1 • Reduction of blood and sputum eosinophil counts	(113)

					ILC2 and Th2 pathway		
10.	TPI ASM8	Inhalation	Mild allergic asthma	Antisense oligonucleotides	• Blocks expression of common βc of IL-3/IL-5/GM-CSF receptors • Blocks CCR3 expression	• Reduction in allergen-induced airway sputum eosinophils • Reduction in airway eosinophil progenitor cells	(118)
11.	Prednisone	Oral	Severe asthma	Anti-inflammatory OCS	• Induces eosinophil apoptosis via down-regulation of IL-5 and GM-CSF which promotes eosinophil survival • Inhibits IL-4 and IL-5 transcription	• Reduction of eosinophilic/type 2 inflammation • Reduction in sputum eosinophil counts • Reduction in pro-inflammatory cytokine concentrations • Improvement in pulmonary function	(119,121, 122)

					at genomic level which results in the inhibition of eosinophil maturation, chemotaxis and survival		

Abbreviations: IV (Intravenous); QOL (Quality of Life); SC (Subcutaneous); ACQ (Asthma Control Questionnaire); FcεRI (High-affinity immunoglobin E receptor); PBE (Peripheral blood eosinophil); ICS (Inhaled corticosteroids); AQLQ (Asthma Quality of Life Questionnaire); FEV_1 (Forced expiratory volume); ADCC (Antibody-dependent cellular cytotoxicity); CCR3 (CC-chemokine receptor 3); CCL (CC-chemokine ligand); TGF-β1 (Transforming growth factor beta 1); BALF (Bronchoalveolar lavage fluid); PGD2 (Prostaglandin D2); CRTH2 (Chemoattractant receptor-homologous molecule expressed on Th2 cells); TSLP (Thymic stromal lymphopoietin); FeNO (Fraction of exhaled nitric oxide); ILC2 (Type 2 innate lymphoid cells); βc (Beta chain); OCS (Oral corticosteroid); GM-CSF (Granulocyte-macrophage colony-stimulating factor).

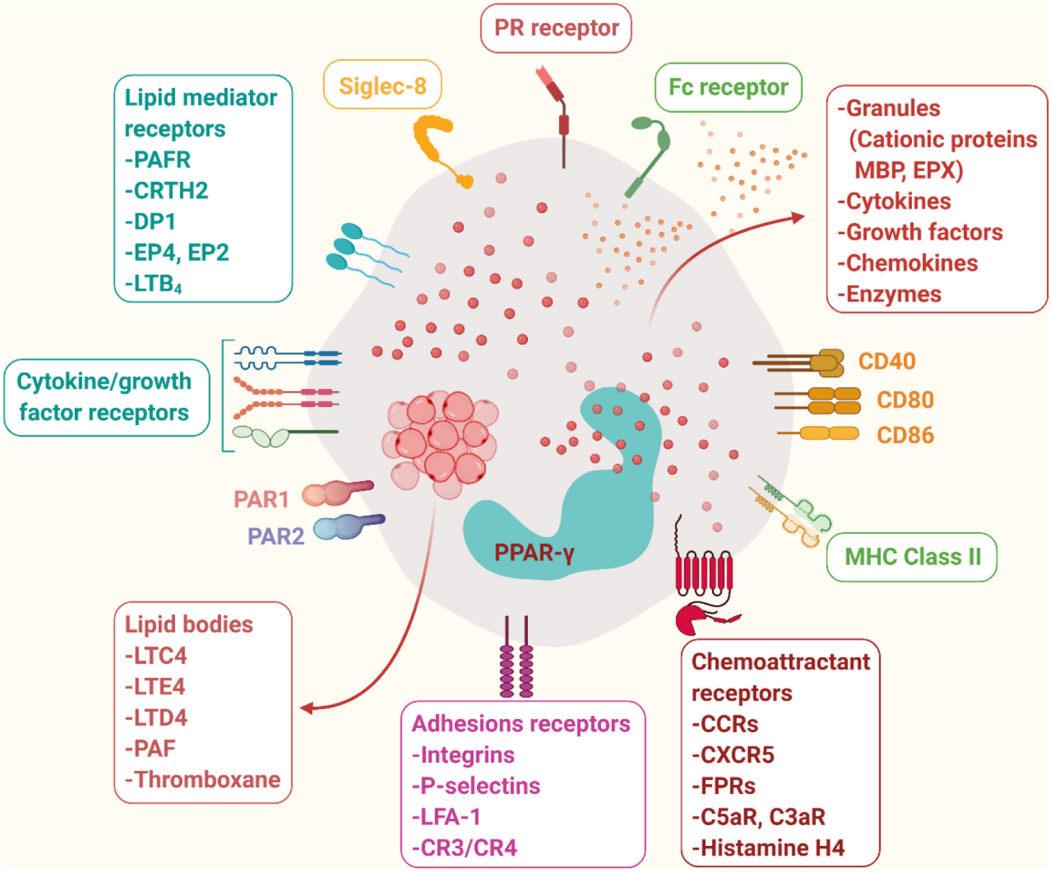
PR receptor
Siglec-8
Fc receptor
Lipid mediator receptors
-PAFR
-CRTH2
-DP1
-EP4, EP2
-LTB4
-Granules (Cationic proteins MBP, EPX)
-Cytokines
-Growth factors
-Chemokines
-Enzymes
Cytokine/growth factor receptors
CD40
CD80
CD86
PAR1
PAR2
PPAR-γ
MHC Class II
Lipid bodies
-LTC4
-LTE4
-LTD4
-PAF
-Thromboxane
Adhesions receptors
-Integrins
-P-selectins
-LFA-1
-CR3/CR4
Chemoattractant receptors
-CCRs
-CXCR5
-FPRs
-C5aR, C3aR
-Histamine H4

Figure 1

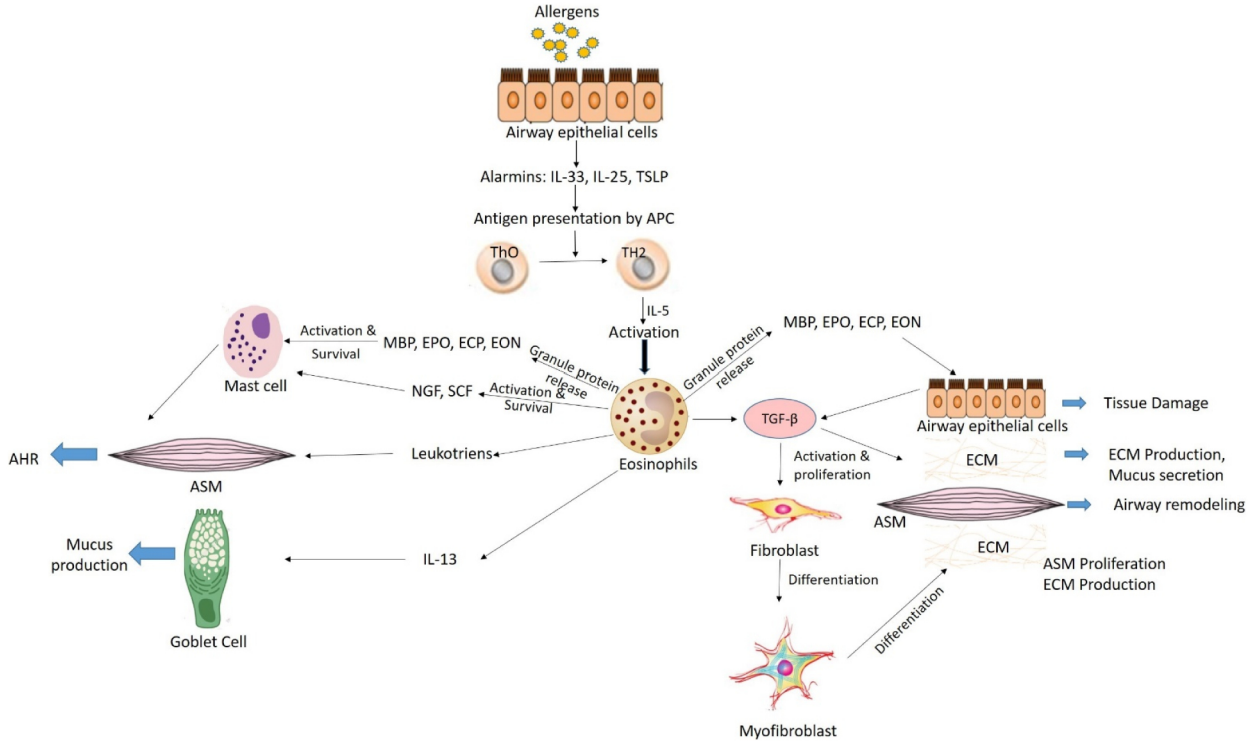
Allergens
Airway epithelial cells
Alarmins: IL-33, IL-25, TSLP
Antigen presentation by APC
ThO
TH2
IL-5
Activation
Activation & Survival
MBP, EPO, ECP, EON
Mast cell
Granule protein release
NGF, SCF
Activation & Survival
Granule protein release
MBP, EPO, ECP, EON
Airway epithelial cells
Tissue Damage
TGF-β
ECM
ECM Production, Mucus secretion
AHR
ASM
Leukotriens
Eosinophils
Activation & proliferation
ASM
Airway remodeling
Fibroblast
ECM
ASM Proliferation
ECM Production
Mucus production
IL-13
Differentiation
Goblet Cell
Differentiation
Myofibroblast

Figure 2

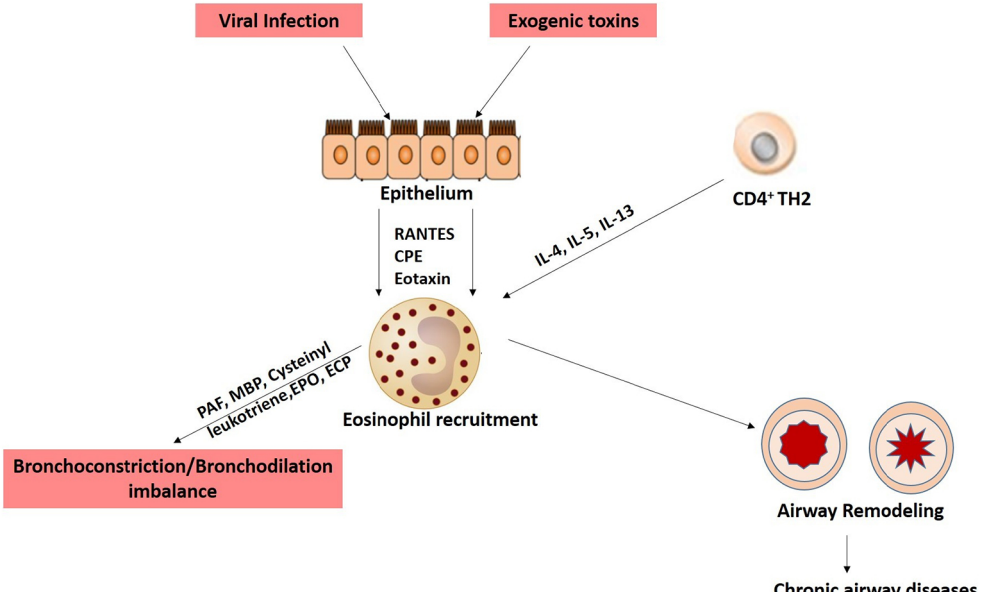
Viral Infection
Exogenic toxins
Epithelium
RANTES
CPE
Eotaxin
CD4+ TH2
IL-4, IL-5, IL-13
PAF, MBP, Cysteinyl leukotriene, EPO, ECP
Eosinophil recruitment
Bronchoconstriction/Bronchodilation imbalance
Airway Remodeling
Chronic airway diseases

Figure 3

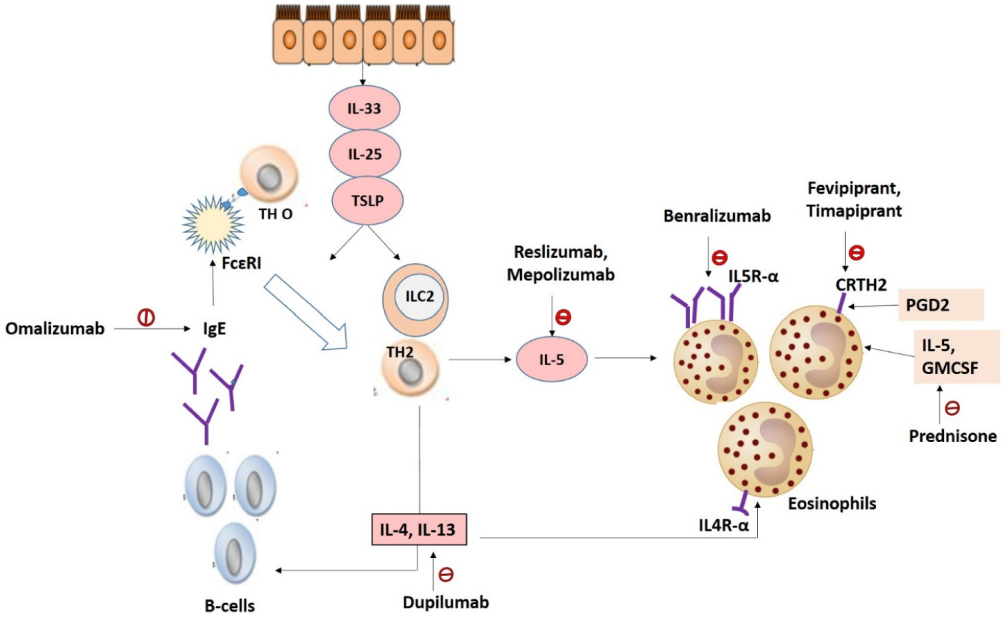
IL-33
IL-25
TSLP
TH O
FcεRI
Omalizumab
IgE
ILC2
TH2
Reslizumab,
Mepolizumab
IL-5
Benralizumab
IL5R-α
Fevipiprant,
Timapiprant
CRTH2
PGD2
IL-5,
GMCSF
Prednisone
Eosinophils
IL4R-α
IL-4, IL-13
B-cells
Dupilumab

Figure 4