Annals of Clinical Medicine and Research

Review Article
Published: 11 Sep, 2020

Clinical Features and Management of Paucigranulocytic Asthma

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Abstract

Asthma is a heterogeneous chronic airway disease with several distinct phenotypes characterized by different immunopathological pathways, clinical presentation, physiology, co-morbidities, biomarker of allergic inflammation, and response to treatment. Asthma can be categorized into four inflammatory phenotypes using quantitative induced sputum cytometry. The four phenotypes of asthma include eosinophilic asthma, neutrophilic asthma, paucigranulocytic asthma, and mixed cellularity asthma. Paucigranulocytic Asthma (PGA) is the most common asthma phenotype in adults, and children with stable asthma. It is characterized by less severe refractory asthma compared with eosinophilic and neutrophilic asthma, and significantly better lung function than the other asthma phenotypes. Patients with PGA have lower levels of biomarkers of eosinophilic inflammation, such as fractional exhaled nitric oxide, and serum periostin; and neutrophilic inflammatory responses, including lower serum levels of neutrophil elastase, metalloproteinase-9, and interleukin-8. Additionally, PGA patients have poor response to corticosteroids, and anti-interleukin monoclonal antibodies. The pathophysiology of the paucigranulocytic phenotype involves uncoupling of Airway Hyper Responsiveness (AHR) from inflammation, and is characterized by excessive Airway Smooth Muscle (ASM) hyperplasia and hypertrophy, leading to persistent airflow obstruction. PGA patients require exploration of alternative therapeutic options targeting ASM hypertrophy, and AHR, such as long-acting muscarinic antagonists, phosphodiesterase 4 inhibitors, stem cell factor (protein kinase, c-kit) receptor inhibitors, and bronchial thermoplasty.

Keywords: Paucigranulocytic asthma; Airway smooth muscle; Monoclonal antibodies; Phosphodiesterase 4 inhibitors; Bronchial thermoplasty

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Received Date: *22 Jul 2020*
Accepted Date: *26 Aug 2020*
Published Date: *11 Sep 2020*

Citation:
Syabbalo N. Clinical Features and Management of Paucigranulocytic Asthma. Ann Clin Med Res. 2020; 1(3): 1011.

Introduction

Asthma is a significant public health problem, affecting more than 358 million people globally [1], and its prevalence has been increasing during the last 40 years, and by 2025, there will be about 400 million people suffering from the disease [2,3]. It is the most common chronic respiratory disease in children in the developed countries [4], and its prevalence is steadily increasing in the developing world [5].

Asthma is a heterogeneous chronic airway disease with several distinct phenotypes characterized by different immunopathological pathways, clinical presentation, physiology, co-morbidities, biomarker of allergic inflammation, and response to treatment [6-10]. Asthma can be categorized into four different inflammatory phenotypes using quantitative induced sputum cytometry. The four phenotypes of asthma include eosinophilic asthma, neutrophilic asthma, paucigranulocytic asthma, and mixed cellularity asthma [7,11]. Patients with eosinophilic asthma have an eosinophil count ≥ 3% [12-14], whereas patients with neutrophilic asthma have elevated sputum neutrophil count between ≥ 61% [14] and ≥ 64% [15], depending on the study. Mixed cellularity phenotype is characterized by increase in both eosinophils (>3%), and neutrophils (>61% or >64%) [15]. Paucigranulocytic phenotype embraces patients with very few eosinophils (<3%), and neutrophils (<61% or <64%) in induced sputum [15]. Non-eosinophilic asthma is the term used to classify patients with low eosinophil numbers (<3%), which include neutrophilic asthma, and paucigranulocytic phenotype [16].

Approximately half of the patients with stable asthma have eosinophilic phenotype, whereas the remaining half have non-eosinophilic phenotype [6,17-20]. Eosinophilic asthma is a severe, persistent phenotype of asthma, characterized by recurrent exacerbations, hospitalizations, and worse quality of life and prognosis. Th2 cytokines secreted by type 2 helper (Th2) lymphocytes (CD4+), and Innate Lymphoid Cells group 2 (ILC2), such as Interleukin-5 (IL-5), IL-4, IL-13, IL-

25, IL-33, and TSLP play an important role in the recruitment, and activation of eosinophils, basophiles, and mast cell in the airways. Th2 interleukins play a key role in the pathophysiology of eosinophilic asthma.

Patients with eosinophilic asthma have poor response to the standard treatment, including high-dose Inhaled Corticosteroids (ICS), Long-Acting β2-Agonists (LABAs), and Leukotriene Receptor Antagonists (LTRA) [21-25]. This phenotype of asthma responds favorably to biologics targeted against IgE (omalizumab); and interleukin monoclonal antibodies, such as mepolizumab, benralizumab, reslizumab, dupilumab, and tezepelumab) [26-28].

Neutrophilic asthma is an adult-onset phenotype that is very severe and persistent [29-31], with frequent exacerbations, hospitalizations, and intubations [32]. It is characterized by fixed airway obstruction (low FEV1), and less hyper responsiveness to methacholine bronchoprovocation tests [33,34]. The cytokines secreted or associated with Th17 cells, including IL-17, IL-17F, IL-6, IL-8, IL-21, IL-23, IFN-ϒ, and TNF-α play a pivotal role in the pathophysiology of neutrophilic asthma.

Neutrophilic asthma is associated with co-morbidities such as respiratory infections [35-39], chronic rhinosinusitis and nasal polyps [40-42], obesity [43-50], gastroesophageal reflux disease [41,53-54], and obstructive sleep apnea [41,55-63], which may contribute to the severity of the disease, and complicate the management [64]. Neutrophilic asthma is unresponsive to corticosteroids, and to the current biotherapeutics with anti-interleukin Monoclonal Antibodies (mABs) targeted at eosinophilic asthma [31].

Paucigranulocytic asthma is the most common phenotype in both adults and children with stable asthma, however, it is rare in patients presenting with acute severe asthma. It is portrayed as a less severe phenotype of asthma compared with other phenotypes, although a sub-group of patients with PGA experience severe persistent asthma. PGA is characterized by low levels of biomarker of eosinophilic inflammation, such as FeNO, serum periostin, and dipeptidyl dipeptidase 4; and low expression and concentrations of biomarkers of neutrophilic asthma, including neutrophil elastase, metalloproteinase-9, and IL-8.

Patients with PGA are unresponsive to corticosteroids [65-65], and to anti-interleukin monoclonal antibody therapy. They require exploration of alternative therapies [64], such as novel Long-Acting Muscarinic Antagonists (LAMA), stem cell factor (protein kinase, c-Kit) receptor inhibitors, and bronchial thermoplasty. In this review, we will highlight the clinical features of paucigranulocytic asthma; the underlying mechanisms leading to increase in Smooth Airway Muscle (ASM) hypertrophy, and Airway Hyper Responsiveness (AHR); and possible therapeutic interventions in the management of PGA.

Clinical Features of Paucigranulocytic Asthma

Paucigranulocytic asthma, like neutrophilic asthma is mostly observed in older patients, usually after 20 years, although it may occur in children. PGA is the most common asthma phenotype in both adult patients and children with stable asthma [65-68], with prevalence's ranging from 40% [65] to 51.7% [67]. Paucigranulocytic asthma is less common in adults with acute severe asthma, about 18%, and very rare in children presenting with acute asthma [67]. Neutrophilic asthma is the most common phenotype in adult patients presenting with acute severe asthma (82%), whereas eosinophilic asthma is the most common phenotype in children with acute severe asthma (50%) [67].

Paucigranulocytic asthma is characterized by less severe asthma, and moderate exacerbations compared with eosinophilic and neutrophilic asthma; and significantly better lung function in terms of better FEV1, compared with other asthma phenotypes. Ntontsi et al. [67] have reported that patients with PGA have less airflow limitation (FEV1, 81.9% predicted) compared with patients with eosinophilic asthma (FEV1, 74.2% predicted), mixed granulocytic (FEV1, 69.7% predicted), and neutrophilic asthma (FEV1, 72.2% predicted). They have also less acute severe refractory asthma (21.7%), compared with patients with eosinophilic (41.6%), mixed cellularity (43.7%), and neutrophilic asthma (25%) [67].

Paucigranulocytic asthma patients have better lung function based on higher post-bronchodilation FEV1, and FEV1/FVC ratio compared with patients with eosinophilic, and neutrophilic asthma phenotypes [67]. PGA phenotype is characterized by less atopy, and moderate responsiveness to methacholine bronchoprovocation tests [66]. PGA patients also tend to be less obese compared with patients with the neutrophilic phenotypes, and probably have less associated co-morbidities with asthma, such as obesity, Gastroesophageal Reflux Disease (GERD), and Obstructive Sleep Apnea (OSA). These co-existing diseases are particularly common in patients with neutrophilic asthma [31,41], and require appropriate therapy, including bariatric surgery for obesity, and Continuous Positive Airway Pressure (CPAP) for OSA [31]. Table 1 shows the clinical and diagnostic features of paucigranulocytic asthma.

Patients with PGA have lower levels of biomarkers of eosinophilic inflammation, such as sputum and blood eosinophil counts, Eosinophilic Cationic Protein (ECP), Fractional Exhaled NO (FeNO), serum periostin, and Dipeptidyl Peptidase-4 (DPP-4). Similarly, they have low levels of neutrophilic inflammatory responses, including lower serum levels of neutrophil elastase, Matrix Metalloproteinase-9 (MMP-9), and Interleukin-8 (IL-8) [67,68].

Patients with PGA are unresponsive to the standard care, including high-dose ICS, LABA, and/or LTRA [65]. Their response to anti-interleukin monoclonal antibody biotherapeutics is not clearly documented, but biologics seem to be ineffective. Unlike the eosinophilic phenotype, the paucigranulocytic phenotype does not have precision diagnostic biomarkers, such as FeNO, periostin, dipeptidyl peptidase-4, and osteopontin [31]. Patients with PGA require alternative therapies [64], such as LAMA, phosphodiesterase-4 inhibitors, stem cell factor (protein kinase, c-kit) receptor inhibitors, and bronchial thermoplasty.

Pathophsyiology of Paucigranulocytic Asthma

The mechanisms underlying the pathophysiology of eosinophilic and neutrophilic asthma are well understood, whereas, the immunopathology of paucigranulocytic asthma is less clear. The pathophysiology of the paucigranulocytic phenotype may involve uncoupling of airway hyper responsiveness from inflammation, which is associated with excessive airway smooth muscle hyperplasia and hypertrophy, leading to persistent fixed airflow obstruction [67,68].

Animal experiments have revealed that, the severance between

Table 1: Clinical and diagnostic features of paucigranulocytic asthma.

Late on-set, most cases after 20 years
Moderate atopy compares with eosinophilic asthma
Moderate severe exacerbations compared with eosinophilic asthma
Less co-morbidities, such as chroninic rhinosinusitis, nasal polyps, AD, AERD, and EIA
Sputum eosinophil count <2%-3%; neutrophil count <61%-64%
Low concentrations FeNO<30 ppb
Low serum levels of periostin
Low serum levels of dipeptidyl peptidase-4 levels
Moderate airway obstruction (higher FEV1) compared with eosinophilic asthma
Moderate hyperresponsivenness to methacholine bronchoprovocation tests
CT scan – airway smooth muscle hyperplasia and hypertrophy
Corticosteroid unresponsiveness
Poor response to biologics
Low serum levels of protease tryptase, and metalloprotease-9
Low serum levels of interleukin-8

Abbreviations: IL: Interleukin; FeNO: Fractional Exhaled Nitric Oxide; ppb: parts per billion; FEV1: Forced Expiratory Volume in 1 sec; CT: Computed Tomography; AD: Atopic Dermatitis; AERD: Aspirin-Exacerbated Respiratory Disease; EIA: Exercise-Induced Asthma

airflow obstruction and inflammatory mediators may be due to neuronal factors [69], ASM cell phenotypic changes [68], inflammatory mediators, such as 20-Hydroxyeicosatetraenoic acid (20-HETE), signaling molecules, including pro-contractile G-Protein-Coupled Receptors (GPCR) [71,72], and susceptible genes [73,74].

Neurogenic Mechanisms

The airways and the lung parenchyma are innervated by the parasympathetic nervous system, *via* its cholinergic muscarinic receptors [75,76]. There are five identified muscarinic receptors that belong to the G-protein-coupled receptor family, however; only M1, M2, and M3 have been implicated in the pathophysiology of asthma and COPD [76]. Muscarinic receptors are highly expressed in parasympathetic neurons in airway smooth muscles, submucous glands, airway epithelial cells, and the pulmonary vasculature [77-82]. M3 receptors mediate acetylcholine's effect on airway smooth muscle tone, and mucous secretion from the submucous glands, and goblet cells [76,82]. M2 receptors have an inhibitory auto-regulatory effect on the release of acetylcholine from both pre- and post-ganglionic nerve terminals, thus limiting vagal reflex-induced bronchoconstriction, and mucus secretion [82]. M2 receptors are also expressed by structural cells, such as fibroblasts, and ASM cells [82]. M1 receptors are expressed on post-ganglionic neurons in the ganglia, and airway epithelial cells; they modulate parasympathetic neurotransmission, and regulate water and electrolyte secretion in the airways, respectively [81,83].

Acetylcholine is the predominant parasympathetic neurotransmitter in the airways [84]. It is synthesized from Choline and Acetyl-CoA mainly by the enzyme choline acetyltransferase in parasympathetic neurons [79-81], and to some extent by inflammatory cells [82], and airway epithelial cells [85].

Parasympathetic neuronal activity, *via* acetylcholine signaling is increased in patients with asthma [82]. There are several mechanisms that lead to increased neural activity in asthmatic airways, including loss of epithelial barrier due to inflammation, which exposes the neurons to pro-inflammatory mediators [82]. Inflammatory mediators by direct contact with exposed parasympathetic cholinergic nerve terminals can lead to release of acetylcholine which can trigger vagal reflex-mediated bronchoconstriction [86]. Dysfunction of M2 auto receptors, may lead to increased acetylcholine release, leading to airway hyper reactivity [76,87]. M2 receptor dysfunction has been reported to be induced by respiratory viral infection [87], and is thought to be driven by Major Basic Protein (MBP) secreted by eosinophils [87,88]. The increase in acetylcholine signaling on M3 and M1 receptors, and the M2 receptor dysfunction, may all contribute to the increased bronchoconstriction, mucus hyper secretion, AHR, and airway remodeling [89].

Airway neurons have great plasticity and can undergo remodeling similar to airway smooth muscle cells. They can switch to a more cholinergic isotype, and branch more extensively in response to inflammatory insults, such as respiratory infections, allergens, mechanical stress, and pro-inflammation mediators [89-91]. Neuronal plasticity may be a feature of early-life exposure to allergens, resulting in the induction of nerve growth factors, such as Neurotrophin (NT)-4, which mediate neuronal remodeling, and persistent hyper responsiveness [92].

Additionally, acetylcholine *via* muscarinic M3 receptors exerts pro-inflammatory effects through chemoattraction of inflammatory cells, promote survival, and release of pro-inflammatory mediators from bronchial epithelial cells, and immune cells, such as macrophages, mast cells, monocytes, neutrophils, and eosinophils [82,87-89].

Dysregulation of neuronal control of airway smooth muscle contractility by Nerve Growth Factors (NGF) may promote airway hyper responsiveness without secretion of inflammatory mediators, thus, uncoupling airway obstruction from airway inflammation [69]. Animal experimental studies have demonstrated that mice treated with nerve growth factor induced airway hyper responsiveness to the same degree as allergen-sensitization [69]. There is evidence that NGF may be involve in activation of inflammatory cells, and release of cytokines, which further orchestrates hyper responsiveness, and airway remodeling [93].

Muscarinic antagonists block the action of acetylcholine at the

muscarinic M3 receptors of airway smooth muscle cells, inducing ASM relaxation and bronchodilatation [89,94]. Muscarinic antagonists have also anti-inflammatory actions by inhibiting the release of pro-inflammatory cytokines from immune cells and epithelial cells [95,96]. In animal models, pre-treatment with tiotropium reduces eosinophilic inflammation in response to allergen exposure [95]. Tiotropium partially prevents airway remodeling, such as, inhibition of submucous gland hypertrophy, and decreases the number of MUC5AC-positive goblet cells. Tiotropium has also been reported to reduce airway smooth muscle thickening [96], a hallmark of paucigranulocytic asthma.

Clinically, muscarinic antagonists, particularly, Long-acting Muscarinic Antagonists (LAMA), such as tiotropium are effective as add-on therapy for the treatment of asthma, and Chronic Obstructive Pulmonary Disease (COPD) [97-99].

Airway Smooth Muscle

The tracheobronchial tree is lined by airway smooth muscle from the trachea down to the respiratory zone which relaxes and constricts the airways, and is the major determinant of airway caliber. Bronchoconstriction is the most severe symptom of an asthmatic attack. Increase in the number (hyperplasia), and size (hypertrophy) of ASM cells cause a faster strong contraction, and impair relaxation of ASM in patients with asthma compared with healthy subjects.

Airway remodeling characterized by hyperplasia and hypertrophy of ASM cells has been long recognized in the lungs of asthmatic patients [100]. Paucigranulocytic asthma is characterized by airway remodeling and airway hyper responsiveness independent of airway inflammation [68]. Airway remodeling is a complex pathophysiological process involving structural changes, such as ASM cell migration, hypertrophy and hyperplasia; thickening and subepithelial basement membrane fibrosis; submucous gland, and goblet cell hyperplasia; thickening and shedding of the airway epithelium [101-107]. Table 2 summarizes the pathophysiology of airway remodeling in patients with severe asthma, including paucigranulocytic asthma.

Patients with paucigranulocytic asthma have increase in ASM mass, especially patients with severe disease [108-112]. The changes in ASM may develop independent of airway inflammation [68]. ASM hyperplasia and hypertrophy, and subepithelial basement membrane fibrosis are the major determinant of allergen-, pollutant-, or cytokine-induced bronchoconstriction, persistent airflow obstruction, and decrease in lung function in patients with asthma [101,102,113-115]. Thickening of the subepithelial basement membrane is due to increased deposition of collagen I and III, tenascin, and fibronectin produced by myofibroblasts [116]. Similarly, thickening of airway epithelium due to edema, and neovascularization and vasodilatation may result in fixed airway narrowing and AHR with minimal inflammatory mediator release [117].

The mechanisms by which ASM cells are modified in patients with asthma, particularly the paucigranulocytic phenotype are complex and poorly understood [104,107,118,119]. Several stimuli, such as inflammatory cytokines, chemokines, pollutants, and mechanical strain can prime ASM to become nonspecifically hyper responsive to contractile agonists [120]. The role of signaling molecules in dysregulation and hypertrophy of ASM, such as Regulator of G protein Signaling (RGS5); pro-contractile G Protein-Coupled Receptor (GPCR); and abnormalities of ASM contractile properties are discussed in detail in references [68,71,117]. Murine studies have also implicated involvement of caveolin-1 (cav-1), a transmembrane protein [121], and transcription factors involved in cell growth, such as early growth response-1 (Egr-1) in the pathophysiology of airway hyper responsiveness [72].

Overexpression of genes present in chromosome 17q21, a chromosome linked with asthma susceptibility, such as gasdermin B (GSDMD) [68,122], and Orosomucoid-Like (ORMDL3) [68,123], has been shown to promote subepithelial fibrosis, ASM cell proliferation, AHR, and remodeling. This may be due to increase in expression of mediators known to promote airway remodeling, such as TGF-β1, metalloproteinase-8, and disintegrin. These proteins selectively activate growth factors, such as activating transcription factor-6, and its target gene Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA2b) [123].

Airway smooth muscle cytosolic Ca2+ plays an important role in smooth muscle contraction. The SERCA pump uses energy from ATP hydrolysis to remove Ca2+ from the cystosol back into the cisterns of the sarcoplasmic reticulum, where it is stored until released by the next action potential and contraction. Experimental studies have shown that the spontaneous return to baseline of the cystosolic Ca2+ concentration is dramatically delayed in asthmatic ASM cells [124]. Dysregulation of SERCA2b pump expression has been reported to contribute to airway remodeling and AHR in bronchial asthma [125].

Table 2: Pathophysiology of airway remodeling in patients with paucigranulocytic asthma.

Epithelial injury due to allergens, pollutants, and respiratory viral infections
Release of cytokines, chemokines, growth factors, and adhesion molecules
Airway epithelial thickening, shedding, and further release of “alarmin” cytokines
Submucous glands and goblet cell hyperplasia, and mucus hypersecretion
Airway hyperresponsiveness
Subepithelial basement membrane fibrosis
Deposition of extracellular matrix proteins
Mast cell infiltration of airway smooth muscle cells
Activation of myofibroblasts, and fibroblasts
Airway smooth muscle hyperplasia, and hypertrophy
Neoangiogenesis, and exaggerated vasodilatation
Airway remodeling
Corticosteroid-resistance

Airway smooth muscle cells are very productive secretory cells, and display plasticity depending on inflammatory milieu. They can secrete a variety of cytokines (IL-1β, IL-8, IL-5, IL-6, IL-8, IL-11, and IL-10); chemokines (eotaxins, and Gro-α); growth factors (EGF-1, FGF, PDGF; VEGF, and IGF-1); and angiogenic factors (angiogenin, and angiopoietin) [108,126,127]. In addition, ASM cells from asthmatic patients have a distinct hyperreactive "primed" phenotype, which is characterized by increased release of pro-inflammatory cytokines, chemokines, and growth factors [108]. The growth factors may act in an autocrine fashion to promote ASM cell migration, hyperplasia and hypertrophy, which further aggravates the bronchoconstriction. Patients with PGA are potential candidates for smooth muscle reduction therapies, such as bronchial thermoplasty, and mast cell targeted therapies [128].

Inflammatory Cells

Paucigranulocytic asthma is characterized by ASM dysfunction, airway remodeling, and AHR without obvious airway eosinophilic, and neutrophilic inflammation [68]. Zhang, et al. [129] have suggested that the lack of airway eosinophilia and neutrophilia in association with airway remodeling is a possible consequence of "burnt-out" inflammation. This may indicate exhaustion of the inflammatory cells during the course of the allergic responses, which ultimately manifests as paucity of eosinophils and neutrophils [68]. This may manifest as persistent airflow limitation and less variability in asthma [129].

There are several immune, and structural cells implicated in the pathogenesis of asthma, which may contribute to the immunopathology of paucigranulocytic asthma, such as Innate Lymphoid Cells group 2 (ILC2), mast cells, fibroblasts, myofibroblasts, and epithelial cells. These cells and their mediators are potential targets for therapeutic interventions, especially mast cells, and stem cell factor and its c-kit receptor.

Mast Cells

Mast Cells (MCs) play a protective role against bacterial, parasitic, and viral infections. In addition, they are pivotal in the pathophysiology of asthma, and autoimmune diseases. It is beyond the scope of this review to discuss in detail the roles of mast cells in the pathophysiology of asthma, and for detailed information the reader is referred to references [129-135].

In patients with asthma, mast cells have been identified in the airway submucosa, smooth muscle bundles, and epithelium [136]. Mast cell numbers localized with the bronchial smooth muscles bundles is significantly increased in patients with asthma compared with normal subjects or patients with eosinophilic bronchitis [132,133]. The symbiotic interaction between ASM cells and mast cells may be important in the pathophysiology of airway smooth muscle hypertrophy, remodeling, and AHR. Airway smooth muscles produce a conducible microenvironment, by secreting cytokines, chemokines, and growth factors for differentiation, activation, and survival of mast cell [107,137]. The chemoattractant cytokines, and chemokines, such as TGF-β1, SCF, CXCL8, CXCL9, CXCL10, CXCL10, CXCL11, and CXCC12 play an important role in the recruitment of mast cells into the ASM bundles [138,139]. Mast cell secretory products also promote migration, and proliferation of ASM cells, and prime them for their greater contractility.

There is significant correlation between ASM mast cell numbers and airway hyper responsiveness in patients with asthma [140-143]. In chronic asthma, mast cells within the ASM bundles demonstrate ultrastructural features of activation, with evidence of ongoing mediator release, and cytokine synthesis [140-143]. This suggests that, airway smooth muscle cell infiltration by mast cells is one of the critical determinants of the asthmatic phenotypes [143-145].

Mast cells are tissue resident, innate immune cells with heterogeneous phenotypes modified by cytokines, growth factors, and microenvironmental stimuli [136]. MCs have been traditionally classified into two major types based on the protease granule content, with tryptase representing total mast cells [146]. MCT cells contain primarily tryptase and are predominantly localized in alveolar septae, airway epithelium, and submucosa, as well as in the small intestinal submucosa. They represent the majority of mast cells in the lungs. In contrast, MCTC subtype is identified by the presence of tryptase and chymase, and there is evidence that they also contain carboxypeptidase A3, and hematopoietic prostaglandin D synthase, and cathepsin G [136,146].

Mast cells express CD34, c-kit, and the high affinity IgE FcεRI receptors on their surface [146]. The density of the IgE FcεR1 receptors is upregulated by high levels of IgE, and in the presence of IL-4; thus, enhancing activation of the effector cells [136,147]. The FcεRI receptor on the surface of mast cells, eosinophils, and basophiles is expressed as a tetramer (αβγ2), and in antigen presenting cells, such as dendritic cells are expressed as a trimmer (αγ2). The expression of β chain on mast cells, and basophils results in increased FcεRI surface expression and amplifies the signaling through the receptor. In the classical allergic response, tissue mast cells, and basophiles bound to specific IgE FcεRI receptor, crosslink, and aggregate with IgE Fcε3 receptors on subsequent exposure to the same allergen or related allergen [147]. The α chain of the FcεRI on the surface of the mast cells, and basophiles bind to the Fc portion of the Fcε3 on the IgE molecule. This triggers mast cells, and basophiles to initiate complex signaling events, including phosphorylation of the γ subunit and recruitment of Sky kinase. Sky activates a number of downstream signaling events associated with mast cells and basophile activation [148]. This results in the secretion of biological active mediators by the effector cells [149-153].

Activated mast cells secrete an array of mediators, including granule-associated preformed mediators, such as amines (histamine, and serotonin); neutral proteases including tryptase, chymase, and carboxypeptidase A3; as well as newly formed lipid-derived mediators, e.g., cysteinyl leukotrienes (LTB4, LTC4, LTD4, LTE4), Prostaglandins (PGD2), PAF, thromboxanes; and proteoglycans (histamine and chondroitin sulphate) [154]. The mast cell-derived autacoid mediators including histamine, PGD2, and cysteinyl leukotrienes are potent bronchoconstrictors, secretagogues and induces mucosal edema. Whereas, the mast cell specific serine protease tryptase induces smooth muscle contraction, airway remodeling, and AHR through various mechanisms [132,155].

Activation of Prostaglandin D2 (DP2) receptors amplifies Th2 cytokine production induced by the "alarmin" cytokines IL-25, and IL-33 [156]. Most important, DP2 receptors binding to airway smooth muscle, contributes to increased airway smooth muscle cell proliferation, and migration, which leads to increase in ASM mass, and AHR [156-158].

Mast cells also secrete a milieu of cytokines, such as IL-1α/β, IL-4,

Table 3: Mast cell-derived inflammatory mediators.

Amines	**Cytokines**
Histamine	Interleukin-4 (IL-4)
Serotonin	IL-5
Serine proteases	IL-5
Tryptase	IL-6
Chymase	IL-10
Carboxypeptidase	IL-13
Lipid mediators	IL-13
Leukotriene C4 (LTC4)	IL-17
LTD4	IL33
LTE4	**Chemokines**
Prostaglandin D2 (PD2)	CCL2
Platelet-activating factor (PAF)	CCL3
Thromboxane B_2 (TXB_2)	CCL5
Proteoglycans (chondroitin sulfate)	CCL11
Growth factors	
SCF	CXCL8
TGF-β1	CXCL9
FGF-2	CXCL10
VEGF	CXCL11
NGF	CXCL12

IL-5, IL-6, IL-8, IL-9, IL-16, IL-18, TSLP, and TNF-α; chemokines, including as CCL1, CCL2, CXCL8 (IL-8), and CXL10/CXCR, CXC11, CXC12, Macrophage Inflammatory Protein 1α (MIP-1α), MICP-1β, MCP, and eotaxins; and growth factors, such as GM-CSF, TGF-β1, VEGF, FGF, NGF, and SCF [159]. The cytokine, chemokines, and growth factors are responsible for airway inflammation, smooth muscle contraction, mucosal edema, mucus secretion, AHR, and airway remodeling [147-153]. Table 3 shows the list of inflammatory mediators, and growth factors secreted by mast cells.

Mast cells also infiltrate the submucous glands in patients with asthma, showing features of activation and degranulation. There is a positive correlation between mast cell degranulation with the degree of mucus plugging in the airways, which is one of the key features of severe asthma [133].

The hallmark of paucigranulocytic asthma is increase in ASM mass due to hyperplasia and hypertrophy [105], and infiltration of ASM by mast cells [131,137]. Therefore, targeting airway smooth muscle [160], mast cells, stem cell factor and its c-kit receptors are alternative strategies for the treatment of paucigranulocytic asthma.

Stem Cell Factor

Stem Cell Factor (SCF), also known as mast cell growth factor and its receptor c-kit, [161,162] plays an important role in the developmental processes of mast cells, hematopoietic stem cells, melanocytes, and in gametogenesis [163]. SCF has also been implicated in tumorigenesis and growth of hematological and solid cancers [164,165]. It is produced within tissues by stromal cells, immune cells, ASM cells, and malignant cells.

Stem cell factor plays a pivotal role in promoting mast cell growth and differentiation from bone marrow and peripheral blood progenitors [166,167], and in the regulation of several functions of mature mast cells [168,169]. Stem cell factor is involved in important mast cell functions such as survival [167,170], migration, adhesion, and IL-6 production [167,171-173]. SCF also amplifies mast cell activation by FcεRI, ST2, and TLR4 receptors [174-176], and may act as a chemoattractant in compartmentalizing mast cell within their residential areas, for example, ASM bundles, or airway submucosa [134]. Stem cell factor binds to its specific receptor c-kit bound on the membranes of several types of cells, including mast cells, macrophages, and bronchial structural cells [134].

c-kit is a tyrosine growth factor receptor, with a large extracellular domain of five Ig-like domains, a single transmembrane span, and a long cytosolic tail containing a tyrosine kinase domain, and tyrosine phosphorylation sites [177]. C-kit signals *via* phosphorylation of at least eight tyrosine residues, inducing Ca2+ influx, and transcription, which enhances mast cell degranulation, and cytokine production, respectively [178].

Patients with severe paucigranulocytic asthma have airway hyper responsive and poor disease control despite the use of high-dose ICS, LABA, and LTRA. Mast cell modifying biologics, such as c-kit receptors inhibitors seem to be appropriate add-on treatment for patients with paucigranulocytic phenotype.

Treatment of Paucigranulocytic Asthma

The most commended strategy for the treatment of severe asthma is the guideline proposed by the Global Initiative for Asthma (GINA) [2]. The GINA strategy recommends that asthma should be treated according to the severity of the disease, and based on the treatment required to control and reduce symptoms and exacerbations. There are five levels of treatment constituting increasing treatment according to severity. Steps 1 to 3 are classified as mild-moderate asthma, and steps 4 and 5 include patients with moderate-severe disease. The step-wise guidelines recommend initial treatment with Inhaled Corticosteroid (ICS) at step 2, followed by increasing the dosage of ICS up to 800 μg/day, and adding a Long-Acting β2-Agonist (LABA) to achieve control at step 3. In patients with severe asthma, steps 4 and 5, the dosage of ICS is increased up to 2000 μg/day, and therapeutic alternatives, such as leukotriene receptor antagonists, slow-release theophyllines, or Long-Acting Muscarinic Antagonist (LAMA) are added to the regimen [2].

Despite treatment according to guidelines, monitoring adherence, and adequate inhaler technique, a significant proportion of asthma patients do not achieve adequate control of asthma symptoms with the standard care treatment [2,179]. Between 49% and 53% of adults receiving treatment adequately have poorly controlled asthma [180,181], and up to 64% of adolescent patients have asthma that is inadequately controlled by the currently available therapies [182]. Table 4 shows the list of drugs available for the treatment of asthma.

In primary care, it has become clinical practice to phenotype asthma for precision and targeted therapies [10,183], because asthmatic patients respond differently to the standard treatment. Eosinophilic asthma is a well characterized phenotype of asthma, which can be diagnosed using biomarkers, such as sputum and blood eosinophil counts, Fractional exhaled Nitric Oxide (FeNO), Serum Periostin (POSTN), Dipeptidyl Peptidase-4 (DPP-4), and osteopontin [28,184-188]. However, paucigranulocytic asthma has no specific pharmacodynamic biomarkers, and may only be confirmed by paucity of eosinophils and neutrophils in induced sputum, or differentiated from eosinophilic asthma because of low

Table 4: Standard drugs used for the treatment of asthma.

Inhaled β2-agonist
Short acting (salbutamol, levalbuterol, terbutaline, pirbuterol)
Long-acting (salmeterol, formoterol)
Combination of LABA and inhaled corticosteroids
Salmetrol and fluticasone (Advair Diskus)
Formoterol and budesonide (Symbicort)
Chromones
Cromlyn sodium, nedocromil sodium
Inhaled anti-cholinergics
Short-acting (ipratropium bromide)
Long-acting (oxitropium bromide, tiotropium bromide)
New long-acting (umeclidinium bromide, glycopyrrolate)
Corticosteroids
Betamethasone dipropionate
Budenoside, fluticasone, flunisolone
Ciclesonide, mometasone
Oral methylxanthines
Rapid release theophyllines
Sustained release theophyllines (Theo-24, Theocron, Uniphyl)
Phosphodiesterase (PDE)-4 inhibitor (roflumilast)
Leukotriene receptor antagonists
Montelukast, pranlukast
Cinalukast, zafirlukast
5-lipoxygenase inhibitors
Zileuton
Novel therapies
Anti-TNF therapy, e.g., infliximab, etanercept
Prostaglandin D2 receptor antagonists, e.g., fevipiprant, setipiprant
Protein kinase c-kit, Lyn, and Fyn inhibitors, e.g., mastinib, imatinib

levels of eosinophilic inflammatory markers, such as FeNO, POSTN, DPP-4, and OPN.

Paucigranulocytic phenotype is unresponsive to corticosteroids, and to the current biotherapeutics, which makes it very difficult to treat. Patients with paucigranulocytic asthma require targeted therapies targeted against AHR, neuronal dysfunction, airway smooth muscle hypertrophy, and abnormal signaling molecules.

Th2-Directed Biologics

Patients with paucigranulocytic asthma do not respond to the Th2 cytokine biologics targeted against IL-5, IL-4, IL-13, IL-25, IL-33, and TSPL. However, there is a subgroup of patients with paucigranulocytic asthma with higher sputum and blood eosinophil counts than healthy subjects [189]. This sub-group of patients may probably achieve symptoms control on treatment with biotherapeutics, such as omalizumab, mepolizumab, benralizumab, reslizumab, dupilumab, tezepelumab [26-28]. Table 5 shows the list of targeted biologics targeted for the treatment of severe refractory eosinophilic asthma, and neutrophilic asthma (brodalumab and secukinumab).

Epithelial cell activity is intense with epithelial thickening, shedding, and increased secretion of "alarmin" cytokines, such as

Table 5: Monoclonal antibodies, and interleukin antagonists, and their target.

Agent	Target	Indication	Stage of Development
Omalizumab	IgE	EA	Marketed 2003
Mepolizumab	IL-5	EA	Marketed 2015
Reslizumab	IL-5	EA	Marketed 2016
Benralizumab	IL-5R	EA	Marketed 2017
Dupilumab	IL-4α/IL-13	EA	Marketed 2018
Tezepelumab	TSLP	EA	Marketed 2018
Pitrakinra	IL-4α/IL-13	EA	II
Lebrikizumab	IL-13	EA	III
Tralokinumab	IL-13	EA	III
Brodalumab	IL-17RA	NA	II
Secukinumab	IL-17A	NA	II
Fezakinumab	IL-22	EA	II

Abbreviations: EA: Eosinophilic Asthma; NA: Neutrophilic Asthma; IL: Interleukin; TSLP: Thymic Stromal Lymphopoietin. Brodalumab and secukinumab are approved for the treatment of atopic dermatitis

IL25, IL-33, and TSPL. Targeting these cytokines, particularly with the currently approved TSPL monoclonal antibody tezepelumab, may be a reasonable approach to modulate the epithelial inflammation.

It is suggested that, patients with some of the characteristics of eosinophilic asthma, such as biomarkers and co-morbid diseases be considered for treatment with anti-eosinophilic asthma biologics. The GINA [2], and British Thoracic Society, Scottish Intercollegiate [190] guidelines recommend initiation of anti-IgE at step 5. The National Asthma Education and Prevention Program, Expert Panel Report 3 (ERP 3) guidelines [191], also recommend eosinophilic asthma targeted biologics, including interleukin monoclonal antibody therapy at steps 5.

Long-Acting Phosphodiesterase Inhibitors

Phosphodiesterase (PGE) inhibitors, such as the non-selective PDE inhibitor theophylline have been used for the treatment of asthma and COPD for several decades. PDE enzymes metabolize the second intracellular messengers, including Adenosine Monophosphate (cAMP), and cyclic Guanosine Phosphate (cGMP), which play important roles in intracellular signaling in the regulation of multiple cellular metabolisms [192,193]. The PDE super family of enzymes contains 11 gene families (PDE1 to PDE11), most of which contain several PDE genes [192]. The PDE4 isoenzymes are a family of four encoded by different genes (PDE4A-4D), which specifically hydrolyze the 3'5' phosphodiester bond of cAMP to yield 5'adenosine Monophosphate (5'-AMP) [194,195]. PDE4B isoenzyme is associated with bronchodilatation, and anti-inflammatory effects, while PDE4D is associated with gastrointestinal side effects, such as nausea and vomiting, due to its high presence in the vomiting center in the brain [194,196].

The cAMP-specific PDE4 is highly expressed in cardiovascular tissue, smooth muscle, keratinocytes, brain cells, and immunological cells, such as T cells, monocytes, macrophages, dendritic cells, neutrophils, and eosinophils [194]. Inhibition of PDE-4 can lead to increase in the levels of cAMP, and subsequent modulation of inflammatory responses, and maintenance of the immune balance [197]. Therefore, PDE4 inhibitors are effective therapeutic strategy for the treatment of inflammatory respiratory diseases, characterized by bronchoconstriction. Increase in the levels of cAMP can activate

downstream phosphorylation pathways [198], which lead to relaxation of airway smooth muscle cells, bronchodilatation; and suppression of airway inflammation [199,200].

PDE4 is the most studied subfamily, and pharmacological research has wielded several pharmacological agents for the treatment of many chronic inflammatory diseases, such as asthma, Chronic Obstructive Pulmonary Disease (COPD) [197,201,202] psoriasis [203], atopic dermatitis [204], rheumatoid arthritis [199,200], Inflammatory Bowel Disease (IBD) [199,205], and neuropsychiatric disorders [206].

Roflumilast (Daliresp) is the only approved long-acting selective PGE4-inhibitor for the treatment COPD and asthma, and was approved for these indications by the European Union (EU) in 2010 and in the USA in 2011. Roflumilast has a significantly higher PDE4B affinity (low Km) than the prototypic drugs, such as rolipram and cilomilast; therefore, the effective Inhibitory Concentration (IC50) of roflumilast is lower than the other PDE4 inhibitors [206,207].

Patient with inflammatory diseases have higher expression of PDE4 than healthy individuals [209]. Inhibition of PDE4 results in increase in intracellular cAMP, and subsequent activation of PKA, cyclic nucleotide-gated ion channels, and Epac1/2. These signaling pathways are involved in the regulation of pro-inflammatory and anti-inflammatory cytokine synthesis [210].

In vitro inhibition of PDE4 has been shown to decrease expression of cell surface markers in many inflammatory cells, such as T cells, and decreased release of cytokines, such as TNF-α, IL-1β, and IL-10 in many types of cells [207,211]. *In vivo* inhibition of PDE4 also leads to a broad spectrum of effects, such as inhibition of cell trafficking, and cytokine and chemokine release from inflammatory cells, such as neutrophils, eosinophils, macrophages, and T cells [212]. In addition, PDE inhibitors promote apoptosis of these cells [207,213]. Animal studies have shown that roflumilast reduced accumulation of neutrophils in Bronchoalveolar Lavage (BAL) fluid following exposure of cigarette smoke in guinea pig and mice [214,215]. Cortijo et al. [216] have also reported that roflumilast prevented bleomycin-induced infiltration of neutrophils and macrophages in mice lungs.

In vitro study has shown that roflumilast and its active metabolite roflumilast N-oxide inhibited neutrophil secretion of IL-8, Leukotriene B4 (LTB4), Matrix Metalloproteinase-9 (MMP-9), and neutrophil elastase [207,217]. PDE4 inhibitors have also been reported to inhibit Interleukin-4 (IL-4), and IL-13 generation by human basophiles [218]. Furthermore, roflumilast and roflumilast N-oxide reduced liposaccharide-induced release of chemokines (CCL2, CCL3, CCL4, and CXL10), and TNF-α from human lung macrophages in a dose-dependent fashion [219].

In vitro study has shown that roflumilast N-oxide in combination with formoterol significantly enhanced the effect of dexamethasone, by potentiating formoterol-induced expression of protein Kinase Phosphatase 1 (MKP-1). Addition of formoterol to roflumilast enhances *in vitro* anti-inflammatory activity. It results in significantly increase in the inhibitory effect of roflumilast on LPS-induced release of cytokines from human lung tissue [220]. Clinically, roflumilast and other PDE4 inhibitor have been shown to provide additive protection in asthmatic patients when added to corticosteroids and LABA [221].

In vitro study has shown that roflumilast antagonized profibrotic activity of fibroblasts stimulated by TGF-β [222,223]. Hence, PDE4 inhibitors have the potential to prevent progressive subepithelial basement membrane fibrosis, and pulmonary fibrosis [222].

Roflumilast suppresses TNF-α release from airway epithelial cells, and exerts anti-inflammatory and immunomodulatory effects [224]. PDE4 inhibitors, such as cilomilast and roflumilast have been shown to decrease MUC5AC expression induced by Epidermal Growth Factor (EGF) [225], and roflumilast has been reported to improve ciliary function, and mucociliary clearance [226].

Phosphodiesterase inhibitors are appropriate as add-on therapy for patients with paucigranulocytic asthma, because they suppress immune cell trafficking, activation, and degranulation. They also suppress the release of cytokines, chemokines, and growth factors which promote subepithelial membrane fibrosis, ASM cell proliferation, and airway remodeling [206]. Long-acting selective PDE4 inhibitors, such as roflumilast have been shown to significantly reduce airway hyper responsiveness [227], which is a key feature of PGA. Similarly, oral roflumilast 500 μg morning or evening is useful as add-on treatment for the fixed airflow limitation in patients with increased ASM mass, AHR, and airway remodeling [228].

Several clinical studies have demonstrated that roflumilast helps improve efficacy of other anti-inflammatory agents and bronchodilators, such as corticosteroids, LABA, and LTRA. Roflumilast and its active derivative roflumilast N-oxide have been shown to enhance activity of the glucocorticoid receptor activity and glucocorticoid-dependent gene transcription in peripheral blood mononuclear cell of asthmatic patients compared with control [229]. The combination of roflumilast and fluticasone significantly reduced AHR compared with roflumilast dosage alone [228]. Roflumilast has also been shown to significantly reduce the eosinophil counts (42%), neutrophil numbers, and cytokine levels; thus, alleviating airway inflammation, airway remodeling, and symptoms in patients with the asthma-COPD overlap syndrome [230].

Several clinical trials have documented that roflumilast improves symptom control, exacerbations, lung function, and quality of life [207,231-233]. Roflumilast can be used as an add-on treatment to ICS, and LABA, and/or LTRA therapies [234], and is beneficial in reducing gradual decline in lung function associated with increase in ASM hypertrophy, and airway remodeling. The GINA guidelines [2] recommend addition of slow-release theophyllines, including the only approved long-acting PDE-4 inhibitor roflumilast for the treatment of asthma at step 3. Roflumilast has better selectivity and tolerance.

Long-Acting Muscarinic Agents

Parasympathetic neuronal activity, via acetylcholine signaling is increased in patients with asthma [82]. Long-Acting Muscarinic Antagonists (LAMA) lead to bronchodilation by blocking acetylcholine receptors in the airways. Tiotropium Respimat (hereafter referred to as "tiotropium") is a cholinergic M3 selective LABA, effective as add-on therapy for the treatment of asthma, and COPD [97-99]. Tiotropium has a higher selection for M3 receptors than for M2 receptors, and dissociates very slowly from M3 receptors [236]. It antagonizes the effect of acetylcholine on cholinergic M3 receptors to cause relaxation of ASM and bronchodilatation [94,99]. Tiotropium is the only approved LAMA for the treatment of asthma in adults, adolescents, and children aged ≥ 6 years with a history of exacerbations. Its long duration of action (36 h) may provide additional option in the treatment of asthma as an add-on to ICS alone or ICS plus LABA maintenance therapy [99].

Long-term use of SABA and LABA may lead to loss of their efficacy due to tachyphylaxis [235]. The US Food and Drug Administration (FDA) recommended that LABA should not be given for long term therapy even in combination with ICS [236]. Therefore, there is need for alternative therapies for the 5% to 10% individuals who cannot achieve disease control on the standard combination therapy, such as tiotropium [237].

Tiotropium has been shown to be effective in all asthma phenotypes. It has been recommended by the Global Initiative for Asthma (GINA) 2015 treatment strategy for individuals with poorly controlled asthma at steps 4 and 5 [2].

Several studies have demonstrated the beneficial effect of tiotropium as an add-on treatment to ICS or ICS and LABA therapies. The first well-powered larger trial of tiotropium bromide (TALC study (NCT00365560), compared the efficacy of tiotropium 18 μg as add-on to ICS with doubling the dose of beclomethasone 160 μg, and with twice-daily salmeterol 50 μg as an add-on to ICS in 210 patients with symptomatic asthma [238]. This clinical trial showed that adding tiotropium to ICS improved lung function, and symptom control compared with doubling beclomethasone dose. Adding tiotropium to ICS was non-inferior to salmeterol. Re-analysis of the data from the TALC study revealed that improvement in lung function (FEV1, and PEF), following treatment with tiotropium was more significant in patients with higher cholinergic tone, identified by a lower heart rate, and increased airway obstruction [239].

Kerstjens et al. [240] compared the efficacy and safety of tiotropium 10 μg and 5 μg, each as an add-on to high-dose ICS plus a LABA (NCT00365560) in 107 patients with severe symptomatic asthma. Tiotropium significantly improved lung function (FEV1, FVC, and PEF), mini-Asthma Quality of Life Questionnaire scores, and rescue medication use, which were sustained for over 24 h.

One of the first dose-ranging, crossover studies of once-daily tiotropium 5, 2.5, and 1.25 μg as an add-on to medium-dose ICS in 149 adults with moderate asthma, identified 5 μg as the optimum therapeutic dose. Patients receiving 5 μg showed statistically significant improvement in lung function, and ACQ-7 scores compared with placebo [241]. Two other replicate trials in 912 patients with severe symptomatic asthma, receiving tiotropium 5 μg or placebo as add-on to high dose ICS plus a LABA over 48 weeks have reported significant improvement in lung function in patients receiving tiotropium compared with placebo [242]. These trials have also reported a decrease in the rate of exacerbations, and improvement in the quality of life [242]. A retrospective study in Turkey of data from 64 patients with severe asthma has reported that tiotropium add-on to high-dose ICS was found to significantly decrease the percentage of patients with uncontrolled asthma, the number of emergence department visits, oral corticosteroid use, and antibiotic treatment for upper respiratory tract infection [243].

The first analysis of the cost-effectiveness of tiotropium add-on therapy, from the perspective of the UK National Health Service, was performed by Willson et al. [244]. Analysis of data from two trials in adult patients with severe asthma (NCT00772538 and NCT00776984) showed that, tiotropium provided a cost-effective treatment option for patients with severe symptomatic asthma despite treatment with high-dose ICS plus LABA therapy [244].

In summary, tiotropium provides an efficacious add-on to ICS and LABA therapy in patient with severe persistent asthma, including the paucigranulocytic phenotype. It has good safety profile and tolerability, and has been proved to be beneficial in the treatment of patients with several phenotypes of severe refractory asthma. The GINA strategy recommends tiotropium as an alternative add-on treatment at step 4 and 5 in patients 12 years and older with history of exacerbations [2].

C-kit Inhibitors

Patients with severe asthma have hypertrophic ASM cells infiltrated by a high mast-cell burden, which is a feature of AHR, and airway remodeling compared with patients with mild asthma [245,246]. This suggests the role of mast cells and ASM interaction in the pathophysiology of severe persistent asthma, including the paucigranulocytic phenotype.

Stem cell factor and its receptor c-kit proto-oncogene receptor tyrosine kinase play a very important role in mast cell development, functions, and survival [247]. Patients with asthma have elevated serum levels of stem factor which correlates with the severity of asthma [248]. Gleevec (hereafter referred to as imatinib) has been termed as a miracle drug or called a silver bullet. It was initially approved for the treatment of Chronic Myeloid Leukemia (CML) by the US Food and Drug Administration (FDA) in May 2001. It is also approved for the treatment of several gastrointestinal stromal tumors, dermatofibrosarcoma protuberans, systemic mastocytosis, Kaposi's sarcoma, and many other tumors [249]. The use of imatinib for the treatment of CML was engineered by Brian Druker, an oncologist at the Dana-Farber Institute [250].

Imatinib inhibits the tyrosine activity of c-kit receptors on mast cell [251], which results in marked reduction in bone marrow mast-cell numbers, and an increase in serum levels of tryptase in patients with CML [252]. The proteases tryptase and chymase play several functions in inflammation, airway remodeling, and AHR [246]. Tryptase levels in BAL fluid have been reported to be elevated in patients with difficult-to control asthma, particularly after discontinuing inhaled corticosteroids [253]. Cahill et al. [246] in 62 patients with severe, refractory asthma have reported that imatinib treatment reduced airway hyper responsiveness to a greater extent compared with placebo, as demonstrated by a greater increase in the methacholine PC20. Imatinib also reduced the levels of serum tryptase a biomarker of mast-cell activation compared with placebo (decrease of 2.02 ± 2.32 *vs.* 0.56 ± 1.39 ng/mL). The patients who were treated with imatinib had fewer exacerbations, greater reduction in airway-wall thickness, and higher morning, and evening PEF, and better improvement in patient-reported ACQ-6, and AQLQ compared with placebo treated controls [254].

The key feature of paucigranulocytic asthma is AHR, airway remodeling, and thickening of airway wall, due to subepithelial fibrosis, and ASM hypertrophy. IIges et al. [255] studied the efficacy of imatinib on airway remodeling using Multidetector Computed Tomographic (MDCT) imaging. Treatment for 24 weeks with imatinib revealed significant improvement in Wall Thickness percent (WT%), and Wall Area percent (WA%) compared with placebo. Post-hoc analysis of segmental airways in patient who had airflow obstruction at baseline (FEV1<80% predicted) treatment with imatinib demonstrated an even greater improvement in (WT%), and (WA%) [255]. The SCF-c-kit pathway is a promising future target for biotherapeutics aimed at attenuating airway remodeling and AHR in patients with several phenotypes of severe refractory asthma, including the paucigranulocytic phenotype.

Masitinib is a tyrosine kinase inhibitor targeting stem cell factor (c-kit), and Platelet-Derived Growth Factor (PDGF) receptors, which are expressed on several cells, such as mast cells and bronchial structural cells, respectively. Masitinib treatment for 16 weeks has been shown to significantly reduce the Asthma Control Questionnaire score by 0.99 unit compared with 0.43 unit in the placebo arm ($P<0.001$) [256]. Patients receiving masitinib had a more reduction in the use of oral cortictosteroids compared with placebo (median reduction of ≈ 78%, and ≈ 57% in the masitinib group and placebo arm, respectively) [255].

In a recent study, Chanez et al. [257] in Clinicaltrials.gov number, NCT01097694, have reported that masitinib treatment another tyrosine kinase inhibitor, was associated with a significant reduction in severe asthma exacerbation rate of 35%, in patients with severe asthma uncontrolled by oral corticosteroids. They concluded that masitinib treatment over a longer time may provide a new treatment option in severe asthma, irrespective of baseline eosinophil count [257]. Blockade of mast cell activity or its c-kit tyrosine kinase receptor or other KIT receptors on mast cells may prove very useful in the treatment of severe persistent corticosteroid-dependent asthma, and severe persistent corticosteroid resistant asthma, including the paucigranulocytic phenotype.

Bronchial Thermoplasty

Corticosteroids are the mainstay of asthma treatment; however, they do not suppress ASM hyperplasia and hypertrophy which is the histopathological features of paucigranulocytic asthma responsible for airway hyper responsiveness [258]. One of the strategies for the treatment of paucigranulocytic asthma is to target airway smooth muscle [68,105,258,259].

Bronchial Thermoplasty (BT) is a bronchoscopic treatment for subjects aged 18 and above with severe persistent asthma not responding to high-dose ICS, and LABA. Bronchial thermoplasty delivers targeted radiofrequency energy to bronchial airway wall *via* the Alair™ catheter electrode, and results in reduction in hypertrophied airway smooth muscle that is responsible for bronchoconstriction [161,260-264]. The procedure also decreases subepithelial fibrosis, submucous glands, airway nerve terminals, and neuroendocrine cells [264]. Bronchial thermoplasty may lead to functional denervation, thus, reducing sensory neural axonal reflex bronchoconstriction [265].

Bronchial thermoplasty is given over three bronchoscopy sessions at approximately 3-week intervals, one for each lower lobe and one for both upper lobes [263,266]. Radiofrequency electrical energy delivered by a Radiofrequency (RF) generator (Alair™ Bronchial Thermoplasty system) is applied to the airways distal to the main stem bronchi between 3 mm and 10 mm in diameter throughout the tracheobronchial tree, except the middle lobe [263,266,267]. The Alair™ Bronchial Thermoplasty System (Boston Scientific, Marlborough, MA, USA) uses continuous feedback to tightly control the degree of tissue heating to avoid bronchial perforation, scorching, and stenosis [268-269]. The Alair™ catheter delivers radiofrequency energy in order to warm the airway wall to a targeted temperature of 65ºC, which reduces the ASM mass by approximately 50% after 3 to 6 weeks of the procedure [263,266]. Reduction in airway smooth muscle hypertrophy could lead to lesser severe bronchoconstriction.

Bronchial thermoplasty is a safe procedure, however, it is associated with a short-term increase in asthma-related symptoms, such as cough and sputum production, exacerbations, and hospital admissions for asthma during the treatment phase [266,270-273]. Thereafter, the procedure is beneficial with improvement in asthma control, and lung function.

Several randomized controlled trials, and prospective multicenter studies in Australia, Canada, France, Japan, UK, and USA in patients with severe persistent asthma have documented improvement in asthma control, fewer exacerbations, and hospitalization, and better quality of life score which persist for several years following bronchial thermoplasty [263,265,271-274]. The AIR2 (Asthma Intervention Research 2) pivotal trial which compared BT with a sham procedure reported significant improvement in AQLQ scores, reduced frequency of severe exacerbations, and decreased emergency department visits, and days lost from work or school in the year after bronchial thermoplasty [274].

Chupp et al. [275] compared the outcome of BT after a follow-up of 3 years in 190 PAS2 (Post-FDA Approved Clinical Trial Evaluating Bronchial Thermoplasty in Severe Persistent Asthma) subjects with 190 bronchial thermoplasty-treated subjects in the AIR2 trial at 3 years of follow-up. At year 3 after BT, the percentage of PAS2 subjects with severe exacerbations, emergency department visits, and hospitalizations significantly decreased by 45%, 55%, and 40% respectively [275], resembling the AIR2 results [274]. The PAS2 study showed similar improvements in asthma control after BT compared with the AIR2 trial despite enrolling subjects who had poorer asthma control [275]. After 3-year follow-up, PAS2 subjects were able to significantly reduce their mean ICS dose to 2070 µg/day, whereas, the AIR2 subjects significantly reduced their mean ICS to 1841 µg/day [275]. Previous observational studies on the effectiveness of bronchial thermoplasty for severe asthma have reported improvement in AQLQ scores, reductions in exacerbations, and/or a step-down in treatment in 50% to 75% of patients undergoing the procedure [276-278]. Tan et al. [279] have reported that the improvement in asthma symptoms persist up to 5 years of bronchial thermoplasty. BT requires identification of the right patients, pre-procedure preparation of the patient, implementation of proper bronchial therapy technique, and intense post-procedural care and follow-up [279].

Bronchial thermoplasty has a long-term safety profile, and may be considered for patients with predominant chronic airflow obstruction, and patients who do not respond to anti-IgE, anti-interleukin biologics, or macrolides [261,280]. Patients with paucigranulocytic asthma are suitable candidates for bronchial thermoplasty because they have excessive ASM hypertrophy, hyperplasia and hyper responsiveness, without obvious inflammatory markers. They are also unresponsive to treatment with high-dose ICS, LABA, LTRA, and interleukin antagonists targeted against eosinophilic asthma.

The US Food and Drug Administration (FDA) approved BT in 2010 as a safe procedure indicated for the treatment of severe persist asthma in patients 18 years and older, that is not controlled with high-dose ICS, and LABA [266]. It is also approved in several EU countries, Australia, Canada, Japan, and UK. The GINA guideline recommends bronchial thermoplasty for the treatment of severe corticosteroid-resistant asthma at step 5 [2].

Conclusion

Paucigranulocytic asthma is the most common asthma phenotype in adult patients, and children with stable asthma. It is characterized by less severe asthma compared with eosinophilic and neutrophilic

asthma, and significantly better lung function than the other asthma phenotypes. Patients with PGA have lower levels of biomarkers of eosinophilic inflammation, such as FeNo, periostin, and DPP-4; and neutrophilic inflammatory responses, including lower level of IL-8, and metallopreotease-9. The hallmark of PGA is airway smooth muscle hyperplasia and hypertrophy, infiltrated by mast cells, and heightened AHR. Patients with PGA are unresponsive to high-dose ICS, LABA, and biotherapeutics. Novel add-on targeted treatment for the paucigranulocytic phenotype includes LAMA, long-acting PDE4 inhibitors, and bronchial thermoplasty. C-kit stem cell factor inhibitors are attractive option for the treatment of PGA, because they suppress the development, and survival of mast cells which indirectly contribute to ASM hypertrophy, and AHR. Bronchial thermoplasty is a suitable non-pharmacological treatment for patients with paucigranulocytic asthma because it decreases hypertrophied ASM, submucous glands hyperplasia, and neuronal innervation, which are responsible for the persistent airway obstruction, airway hyper responsiveness, and corticosteroid-resistance. The beneficial effects of bronchial thermoplasty include reduction in severe exacerbations, and hospitalization, and improvement in the quality of life which persist up to 5 years.

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