**Emerging concepts and directed therapeutics for the management of asthma: Regulating the regulators**

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**ABSTRACT**

Asthma is a common, heterogeneous and serious disease, its’ prevalence has steadily risen in most parts of the world, and the condition is often inadequately controlled in many patients. Hence, there is a major need for new therapeutic approaches. Mild-to-moderate asthma is considered a T-helper cell type-2-mediated inflammatory disorder that develops due to abnormal immune responses to otherwise innocuous allergens. Prolonged exposure to allergens and persistent inflammation results in myofibroblast infiltration and airway remodelling with mucus hypersecretion, airway smooth muscle hypertrophy, and excess collagen deposition. The airways become hyper-responsive to provocation resulting in the characteristic wheezing and obstructed airflow experienced by patients. Extensive research has progressed the understanding of the underlying mechanisms and the development of new treatments for the management of asthma. Here, we review the basis of the disease, covering new areas such as the role of vascularisation and microRNAs, as well as associated potential therapeutic interventions utilising reports from animal and human studies. We also cover novel drug delivery strategies that are being developed to enhance therapeutic efficacy and patient compliance. Potential avenues to explore to improve the future of asthma management are highlighted.

**Keywords:** Asthma, molecular mechanisms, vascularisation, microRNA, novel drug candidates, targeted drug delivery

**Introduction**

Asthma is a major international health issue affecting >330 million people worldwide. There have been significant increases in worldwide prevalence at an annual rate of 1.4% (4.2 million) in children and 2.1% (6.3 million) in adults (Genuneit et al. 2017). Latin America, Australasia, Europe, North America and South Africa have the highest prevalence (>20%), whereas Asian countries have relatively low rates (2-4%) (Asher and Ellwood 2014; Asher et al. 2006; Beasley 1998; Janson et al. 2001; Zock et al. 2006). Although children make up the majority of asthma patients, they have relatively low mortality rates (0.02% in-hospital asthma mortality). Older patients are more susceptible to asthma exacerbations and mortality risk increases with increasing age, and the elderly (>75 years) have the highest mortality (1.9% in-hospital mortality) (Krishnan et al. 2006).

**Characteristic features of asthma**

Asthma pathogenesis is underpinned by the principal components of airway inflammation and airway remodelling that combine to induce key symptoms like shortness of breath, chest tightness, cough, wheezing and airway hyperresponsiveness (AHR) (Hansbro et al. 2017). These events are linked to excessive reactions to normally innocuous allergen(s) that induce airway inflammation, AHR and reversible airway obstruction (Cahill et al. 2017; Galli 2017). Asthma symptoms are worsened by environmental and physical factors, such as infection, air pollution, smoke, climate change and physical exercise (Kim et al. 2015; Starkey et al. 2013b). When exacerbated by risk factors, patients have accelerated loss of lung function, and some develop irreversible airway obstruction. These exacerbations activate multiple parallel pathways that initiate both inflammation and tissue remodelling that can also induce resistance to mainstay corticosteroid treatments (Galvão et al. 2020; Kim et al. 2015). These events narrow the airways and further deteriorate lung function (Figure 1) (Wisnivesky et al. 2017).

Asthma is now considered a complex syndrome rather. Over the last decade we have moved to categorising patients from generic symptoms towards patient-specific symptoms and/or severity based on clinical phenotypes and inflammatory endotypes (Kaur and Chupp 2019; Lötvall et al. 2011). Almost 20+ years ago, Wenzel *et al*. categorised asthma into T2-high or T2-low based on airway eosinophil counts (Wenzel et al. 1999). Currently, asthma endotypes include T2-high or non-T2, eosinophilic, neutrophilic, granulocytic and paucigranulocytic amongst other classifications. T2-high asthma is further categorised into atopic, late onset or aspirin-exacerbated respiratory disease (Kuruvilla et al. 2019). Non-T2 high asthma is subdivided depending upon the type of stimuli, with smoke exposure, non-atopic asthma, obesity-related asthma and asthma associated with old age (Kuruvilla et al. 2019). Improved understanding of underlying mechanisms of asthma phenotypes and endotypes will enable the optimisation of the therapeutic options available to clinicians and patients.

**Airway Inflammation**

Through the interaction of multifactorial processes, numerous cell types compromise the respiratory system in asthma. These include neutrophils, macrophages, dendritic cells (DCs), mast cells, and airway epithelial cells (AECs), although eosinophils are thought to be pivotal in allergic asthma (Djukanovic 2002; Shukla et al. 2019). During the development of asthma, a myriad of inflammatory mediators, mostly cytokines and chemokines, are secreted and induce the influx of inflammatory cells to the airways (Djukanovic 2002; Shukla et al. 2019) .

T-helper (Th) cells have established roles in asthma pathogenesis. It is proposed that subsets of innate lymphoid cells (ILCs) and DCs are induced that promote the development of Th type-2 (Th2) cells, which then elicit uncontrolled immune responses in the lungs (Romagnani 2000; Starkey et al. 2019). This is supported by a distinct change towards a Th2 cytokine profile in mild to moderate forms of the disease (Barnes 2001; Larché et al. 2003). Activated Th2 cells are widely accepted to cause tissue remodelling and AHR in eosinophilic asthma (Hansbro et al. 2017).

Several external stimuli, including cigarette smoke and other environmental exposures, and bacterial and viral infections skew the immune response to more pro-inflammatory Th1/Th17-dominant responses through a range of different mechanisms that characterises more severe corticosteroid-resistant asthma (Essilfie et al. 2015; Essilfie et al. 2012; Kim et al. 2017b). Exposure to cigarette smoke has been linked to neutrophilic subtypes of asthma with pronounced airway remodelling and non-responsiveness to corticosteroids (Polosa and Thomson 2013). Several studies suggest that repeated exposures to other inhalants, such as diesel exhaust, occupational chemicals and fumes, and air pollutants (e.g., PM2.5) could also result in neutrophilic asthma (Douwes et al. 2002; Esteban-Gorgojo et al. 2018; Simpson et al. 2006). Indeed, although asthma is classically categorized by eosinophilic inflammation and can be managed by corticosteroids, asthma driven by non-eosinophilic inflammation is often resistant to corticosteroid treatment, which is collectively known as non-eosinophilic corticosteroid-resistant asthma (Esteban-Gorgojo et al. 2018). This phenotype is often presented with similar symptoms that occur in other asthma patients, however, their severity is increased higher and including more severe lung function impairment (Adcock et al. 2008; Barnes and Adcock 2009). Although the origin of this particular type of asthma is yet to be fully elucidated, bacterial infections are thought to be another underlying cause (Essilfie et al. 2011; Essilfie et al. 2012; Horvat et al. 2010a; Horvat et al. 2010b). Respiratory pathogens, such as *Chlamydia muridarum, Chlamydia pneumoniae,* and *Haemophilus influenzae*, can induce respiratory symptoms that are co-related with this phenotype, including neutrophilic airway inflammation, airway hyperresponsiveness, and poor response towards steroid-based therapy (Essilfie et al. 2011; Essilfie et al. 2012; Horvat et al. 2010a; Horvat et al. 2010b).

**Airway obstruction**

AHR and airway obstruction in asthma causes premature closure of the large airways and, hence, increases airway resistance that reduces the expiratory flow rate and the capacity to expel air (Hansbro et al. 2017). The obstructive effects are challenging to overcome but the body can compensate for these alterations by dynamic hyperinflation. This helps to increase blood oxygen levels but reduces the blood concentration of carbon dioxide, causing respiratory alkalosis (Fireman 2003; Frieri 2005). Hyperinflation may also generate high intra-pleural and intra-alveolar pressures, reducing blood oxygenation rate and distorting the pulmonary circulation (Fireman 2003). Persistent lung hyperinflation progressively reduces blood oxygen concentration and leads to hypoxia (Fireman 2003; Frieri 2005). Failure to adequately treat asthma exacerbations can cause collapse of the respiratory system as a consequence of all of these events, increasing mortality risk.

**Inflammatory cascades**

Allergic asthma can be categorised into three distinct phases: induction-, early- and late-phase asthmatic reactions (Shastri et al. 2014). It is well accepted that airborne antigens, such as allergens, microbes, and viruses, act as stimulants and irritate AECs (Cahill et al. 2017; Galli 2017). Asthmatic inflammation results from a cascade of events (Figure 2). Briefly, inflamed AECs secrete thymic stromal lymphopoietin (TSLP) and cytokines, such as interleukin (IL)-33, that activate DCs (Mitchell and O’byrne 2017), which are vital in polarising naive Th cells through the presentation of immunogenic antigens (Kaiko et al. 2008a; Kaiko et al. 2008b). DCs also interact with interstitial lung macrophages and T-cells through complex interconnected networks involving major histocompatibility complexes and T-cell receptors (TCRs) (Frieri 2005; Yang et al. 2012), leading to the release of IL-4, which triggers the activation of Th2 cells. Th2 cells further activate Th9 and B-cellsvia the release of IL-4 and IL-13 (Hansbro et al. 2017). IL-4 and IL-13 promote remodelling of the asthmatic airways involving mucus hypersecretion, smooth muscle proliferation, and myofibroblast differentiation (Barnes 2001; Shastri et al. 2015a). Notably, IL-13 downregulates the production of pro-Th1 cytokines, such as IL-12 (Starkey et al. 2013a). It also induces a CD40-dependent switch from immunoglobulin G (IgG) to IgE and, hence, increases IgE synthesis in B-cells (Romagnani 2000). Both Th9- and B-cells activate mast cells via IL-9 and IgE production. Binding of IgE to its’ receptors on mast cells triggers their degranulation, leading to the release of pro-inflammatory mediators, including histamine and leukotrienes (Holgate 2000). Th2 cells also secrete IL-5, which activates and recruits eosinophils to the airways, and promotes their survival (Brusselle et al. 2013; Shastri et al. 2015b). Activated eosinophils can further elicit inflammation by secreting pro-inflammatory cytokines and leukotrienes (Brusselle et al. 2013). These factors induce AHR and constrict the airways (Brusselle et al. 2013). Activated DCs and naive Th cells can also activate Th17 cells via the release of inflammatory mediators, including IL-23 and IL-6 (Hansbro et al. 2017), and these cells in turn recruit and activate neutrophils. Neutrophils are also activated by damaged AECs through the secretion of the chemokine CXCL1 (Ennis 2003; Hallstrand et al. 2014). Neutrophils are the most abundant leukocytes in the airway mucosa and have a major role in tissue remodelling.

Ongoing inflammation results in the late-phase asthmatic response characterised by permanent structural changes, including deposition of extracellular matrix (ECM) proteins around the airway smooth muscle (ASM), resulting in ASM hypertrophy and hyperplasia, sub-basement membrane fibrosis and mucus cell metaplasia (Liu et al. 2017). These changes are collectively termed airway remodelling. Various ECM proteins are present at abnormal levels in asthmatic patients and contribute to airway remodelling including collagen, fibronectin, tenascin, fibulin, and periostin (Lau et al. 2010; Liu et al. 2016; Liu et al. 2017). Differences in the composition of ECM proteins may distinguish specific type(s) and severity of asthma, and predict responses of patients to monoclonal antibody (mAB) treatment.

**Impact of airway vascularisation**

The presence of abnormal vasculature in the pulmonary sub-epithelial vascular network of the airways may also play pivotal roles in asthma pathogenesis (Grigoras et al. 2012). Increases in the amount, density, and area of microvessels occur in the sub-epithelial zone of asthmatic airways (Chetta et al. 2003; Grigoras et al. 2012; Hashimoto et al. 2005; Hoshino et al. 2001a; Hoshino et al. 2001b; Huang et al. 2015). Moreover, studies have revealed the involvement of pro-angiogenesis factors, including vascular endothelial growth factor (VEGF) in sputum, bronchoalveolar lavage (BAL) fluid and bronchial tissue in asthma (Table 1) (Abdel-Rahman et al. 2006; Asai et al. 2003; Meyer and Akdis 2013). VEGF induces the proliferation and growth of endothelial cells, and is produced by various inflammatory cells, including eosinophils, macrophages, and mast cells (Bakakos et al. 2016). There are different isoforms of VEGF; VEGF-A, VEGF-B, VEGF-C, and VEGF-D (Ferrara 2007). Moreover, various receptor tyrosine kinases are known to bind VEGF and induce angiogenesis, including VEGF receptor (VEGFR)1 and VEGFR2. Both are expressed in most endothelial and haemopoietic stem cells, but they have different cellular functions (Meyer and Akdis 2013). VEGFR2 is the primary receptor that promotes angiogenesis; whereas VEGFR1 is proposed to act as a competitive inhibitor that binds to VEGF but does not promote angiogenesis, hence reducing VEGF-VEGFR2 binding (Meyer and Akdis 2013). The degree of vascularisation in asthmatic airway tissue is also increased and is dependent on the severity of exacerbations (Hashimoto et al. 2005; Salvato 2001). Notably, there is also a concomitant relationship between percentage vascularisation, lung function, and severity of asthma exacerbations (Grigoras et al. 2012; Hoshino et al. 2001a; Hoshino et al. 2001b). Understanding the underlying mechanisms leading to increased vascularisation may help elucidate its role in airway inflammation and altered lung function in asthma.

Two types of vascular systems exist in respiratory tissues, the pulmonary system (low pressure, undertakes gas exchange) and the bronchial circulation (high pressure system that supplies nutrients and oxygenated blood) (Zanini et al. 2010). The bronchial circulation consists of the inner vascular plexus in the *lamina propria* and the outer plexus in the adventitia (Zanini et al. 2010). The vascularisation phenomenon in lungs is restricted to microvessels or capillaries (Asai et al. 2003; Hashimoto et al. 2005; Kanazawa et al. 2007; Kanazawa et al. 2004). Emerging evidence demonstrates the presence of abnormal vascular structure in the internal plexus within the sub-epithelial, sub-mucosa, and *lamina propria* (Asai et al. 2003; Hashimoto et al. 2005; Kanazawa et al. 2007; Kanazawa et al. 2004). The vasculature in the outer plexus is poorly studied due to the difficulty in isolating such tissues. Angiogenesis is an important mechanism leading to vascularisation. Physiological challenges to the airways may increase the expression of pro-angiogenic mediators, like VEGF, thereby promoting angiogenesis in affected tissues (Kim 2017). Endothelial cells in airway tissues also release endogenous proteases, such as matrix metalloproteinases (MMPs), which distort vessel membranes and induce vasodilation (Carmeliet 2000; Carmeliet 2005). This leads to the influx of plasma proteins and cells into the tissues, which promote the formation of endothelial tip cells (Carmeliet 2000; Carmeliet 2005). This process leads to the creation of new vessels, and the establishment of additional vascular networks (Chung and Ferrara 2011; Hellström et al. 2001; Silva et al. 2008; Yoo and Kwon 2013). Further studies need to identify other potential mechanisms of vascularisation in asthmatic airways, such as vasculogenesis, which occurs in chronic obstructive pulmonary disease (COPD) and pneumonia.

The formation of extra microvessels provides an additional route for inflammatory mediators to translocate to the airway epithelium and lumen, resulting in sustained inflammation and aggravation of airway obstruction (Harkness et al. 2015; Narayanan et al. 2016). The excess production of mediators and influx of inflammatory cells induces vasodilation and plasma engorgement (Page et al. 2017). Vascularisation may also alter tissue structure (Chakir et al. 2003; Niimi et al. 2003). Consequences of these events include airway fibroblast hyperactivity, mucus hypersecretion, and ASM hypertrophy (Benayoun et al. 2003; Harkness et al. 2015; Zanini et al. 2010). In combination, these responses thicken the airway walls, further worsening lumen narrowing and declines in lung function.

**Targeted therapeutic strategies**

Despite major advances in understanding the pathophysiology of asthma, morbidity rates continue to rise, and current therapies, such as corticosteroids, have adverse effects. Most importantly, a significant population of asthmatic patients do not respond to corticosteroids (Green et al. 2002). However, recent progress in understanding the cellular and molecular mechanisms have shed new light on the development of novel therapeutic strategies for the management of severe asthma (Nixon et al. 2017).

Among various therapeutic strategies, the use of new biological agents, mostly discovered using mouse models and which target key inflammatory mediators, demonstrates significant potential. To date, omalizumab and mepolizumab, which are neutralising monoclonal antibodies (mAbs) against IgE and IL-5, respectively, are approved by the US FDA and EMA (Pelaia et al. 2012; Wenzel 2012). Similarly, therapeutic strategies against other inflammatory mediators involved in asthma pathogenesis are in clinical trials (Table 2). Indeed, there are numerous novel asthma therapies that are either available or under clinical trials.

**Anti-IgE**

IgE has been a target for the treatment of allergic diseases for many years (Ishizaka and Ishizaka 1967; Pelaia et al. 2008). After allergen-challenge, antigen-activated IgE binds to Fc receptors on mast cells and promotes their activation (Pelaia et al. 2012). Consequently, mast cells undergo degranulation and release preformed pro-inflammatory mediators (Pelaia et al. 2012). Omalizumab (anti-IgE mAb) reduced asthma exacerbations showing that IgE suppression may be beneficial in asthma. Omalizumab is a recombinant antibody containing a complementarity-determining region, which is obtained from an anti-IgE antibody in mice (Presta et al. 1993). High-affinity binding of omalizumab to IgE constrains the interaction of the antibody with mast cells, thus preventing mast cell degranulation (Shields et al. 1995). In clinical studies, omalizumab treatment reduced free serum IgE concentrations by 99%, and suppressed new IgE production (Tomkinson et al. 2001). Furthermore, it also decreased the efficacy of antigen-presenting cell interactions with naïve Th cells (Novak et al. 2003). Recently, omalizumab was found to be effective in reducing asthma exacerbation rates across a wide range of eosinophil levels (Hanania et al. 2018). Similar beneficial effects were also observed after the administration of omalizumab in children with severe asthma (Szefler et al. 2018). Interestingly, a recent study demonstrated the efficacy of omalizumab in improving IFN-α and IFN-λ release in patients with influenza A virus- and rhinovirus-induced severe allergic asthma, highlighting the additional potential of omalizumab in exacerbations (Wark et al. 2018). Furthermore, data from a recent phase III clinical trial (NCT01328886) showed that long term therapy with omazulimab is safe and effective in children with severe uncontrolled allergic asthma (Odajima et al. 2017).

**Inhibition of type 2 responses**

TSLP and IL-33 blockade

TSLP and IL-33 are produced by AECs in response to exogenous pro-inflammatory stimuli and are involved in the activation of DCs and the associated cascade of inflammatory events (Hallstrand et al. 2014). Gauvreau *et al*., revealed that a human anti-TSLP mAb (AMG157/MEDI19929; also known as tezepelumab) reduced airway inflammation and relieved allergen-induced bronchoconstriction in patients with mild asthma in a phase I study (NCT01405963) (Gauvreau et al. 2014). In a Phase II trial (NCT02054130), tezepelumab reduced the exacerbation rate in patients with uncontrolled asthma (Corren et al. 2018). Another antibody, ANB020 (anti-IL-33 mAb) cleared Phase I trials and showed a good pharmacokinetic, pharmacodynamic, tolerability and safety profile in healthy volunteers receiving one or multiple doses (Londei et al. 2017). Results from Phase II trials are anticipated soon. Although anti-TSLP and anti-IL-33 antibodies have clinical potential, carefully controlled trials are needed to evaluate their true pharmacological applicability and efficacy in asthma. carefully controlled trials are needed to evaluate their true pharmacological applicability and efficacy in asthma

Anti-IL-4

IL-4 contributes significantly to asthma pathophysiology, primarily in the early development of allergy (Humbert et al. 1997; Kotsimbos et al. 1996). It promotes differentiation of naive Th cells into Th2 cells and their proliferation, and also contributes to airway tissue remodelling (Barnes 2006; Barnes 2008; Schipf et al. 2003). Most anti-IL-4 therapies, such as pascolizumab (anti-IL-4 mAb), are highly effective in suppressing asthma features *in vitro* and in animal models (Hansbro et al. 2013). However, these antibodies are typically found to be clinically ineffective in established asthma in humans (Corry et al. 1996; Zhou et al. 1997). Altrakincept (soluble humanised IL-4 inhibitor) blocked airway eosinophil infiltration and mucus hypersecretion in allergen-challenged mice (Henderson et al. 2000). It is safe in moderate asthma patients and reduces inflammation (Borish et al. 2001; Borish et al. 1999). However, again the respiratory function of asthma patients was not improved (Borish et al. 2001; Borish et al. 1999). Further studies are warranted to improve the anti-IL-4 medications for asthma, but it is likely more effective as a preventative rather than a treatment.

Anti-IL-5

IL-5 has important roles in allergen-induced asthma as a mediator of the activation, proliferation, and maturation of eosinophils (Stirling et al. 2001). Animal studies show that anti-IL-5 mAb, TRFK-5, reduced eosinophil influx into mouse airways after allergen challenge (Garlisi et al. 1999), and suppressed AHR in mouse models of asthma (Mauser et al. 1995). Early clinical trials in mild and chronic asthma with a similar anti-IL-5 mAb, mepolizumab showed that it is safe (Holgate 2008; Leckie et al. 2000; Tanaka et al. 2004), but therapeutic efficacy was inconsistent (Leckie et al. 2000; Mauser et al. 1995; Tanaka et al. 2004). Some patients responded well, those with elevated IL-5/eosinophils, and the levels of eosinophils were significantly reduced, but likely not sufficiently so, and overall it did not improve functional endpoints, such as lung function and asthma symptoms. Interestingly, in a phase II trial (NCT00292877) intravenous administration of mepolizumab to chronic corticosteroid-resistant asthma patients demonstrated clinically reduced blood and sputum levels of eosinophils, and improved asthma symptoms (Haldar et al. 2009; Nair et al. 2009). A later phase III clinical trial (NCT01000506) in patients with severe, uncontrolled asthma with eosinophilic inflammation, mepolizumab met its primary and secondary endpoints by reducing the number of exacerbations, increasing the time to first exacerbation, and improving FEV1 and ACQ scores (Pavord et al. 2012). The drug is now approved by FDA and EMA as an add-on maintenance treatment.

Another anti-IL-5 mAb, benralizumab, had a good therapeutic profile in treating asthma. Recently, a Phase III trial (NCT02417961), showed that it significantly reduce eosinophil levels as well as exacerbation rates in asthmatic patients (Ferguson et al. 2018). Another Phase III study (NCT01928771) revealed that it also significantly improved lung function in patients with uncontrolled asthma receiving high-doses of inhaled corticosteroids and long-acting β2-agonists (Bleecker et al. 2016). Together these studies show that long-term administration of anti-IL-5 therapies may be beneficial in asthma.

Anti-IL-13 and Anti-IL-4Rα

IL-13 is an important inducer of airway tissue remodelling, mucus hypersecretion, and B-cell proliferation (Doucet et al. 1998; Grünig et al. 1998). In initial clinical trials, tralokinumab (anti-IL-13 mAb) was safe for intravenous administration, with little or no adverse effects (Hansbro et al. 2011; Singh et al. 2010). A phase II placebo-controlled study of this mAb (NCT00873860) reported acceptable safety profiles with no serious adverse effects (Piper et al. 2013). Recently, two phase III clinical trials with tralokinumab, STRATOS 1 (NCT02161757), and STRATOS 2 (NCT02194699) also reported good safety profiles when administered to patients with severe uncontrolled asthma (Panettieri et al. 2018). Unfortunately, both STRATOS 1 and STRATOS 2 studies showed inconsistent effects in reducing exacerbation rates in asthma, raising questions of their efficacy as treatments (Panettieri et al. 2018). Further trials are warranted to clearly define the effect of tralokinumab in asthma. Lebrikizumab is another anti-IL-13 mAb which decreased exacerbation rates and improved FEV1 in asthma, and it also reduced late-phase responses and serum IgE concentrations by 48% and 25%, respectively (Hanania et al. 2016; Scheerens et al. 2014). However, in a subsequent phase III trial (NCT01868061) various issues with lebrikizumab treatment were reported (Hanania et al. 2016). Serious adverse events, including aplastic anaemia and eosinophilia, were reported, and consistent reduction in exacerbation rates was not observed in asthmatic patients (Hanania et al. 2016). Similar findings were made in another phase III trial (NCT02104674) where lebrikizumab treatment did not significantly improve lung function, raising further efficacy questions on specific targeting of IL-13 (Korenblat et al. 2018).

An anti-IL4Rα mAb, dupilumab, that blocks both IL-4 and IL-13 activity, was found to be effective in preventing ICS-withdrawal-induced asthma exacerbations and improving FEV1 (Wenzel 2013). Noteworthy observations from anti-IL-13 or anti-IL4Rα trials were that blood eosinophil counts were moderately increased in patients. This may indicate that blockade of IL-13 signalling results in the inhibition of eosinophil-recruiting chemokines and, hence, reduces the migration of these cells from the blood to the lungs (Corren et al. 2017; Hanania et al. 2016; Nixon et al. 2017; Wenzel 2013). Dupilumab has been recently approved by the FDA as a treatment for patients with moderate to severe atopic dermatitis, and recently was found to have similar therapeutic benefit in asthma. In a phase III trial (NCT02414854) in patients with uncontrolled asthma, dupilumab significantly reduced exacerbations compared to placebo, and also improved lung function (Castro et al. 2018). Moreover, both phase IIb (NCT01854047) and phase III studies (NCT02528214) reported that dupilumab improved lung function and reduced severe exacerbations in patients with uncontrolled persistent asthma as well as corticosteroid-dependent severe asthma irrespective of baseline eosinophil counts (Rabe et al. 2018; Wenzel et al. 2016).

Novel agents should be developed and tested against other key proteins and cells, including mast cells and neutrophils, that are known to play critical roles in asthmatic inflammation, airway tissue remodelling and severe asthma. Also, drugs that target ECM proteins such as fibulin-1c, which has been shown to be increased in asthma, should also be assessed (Lau et al. 2010). Its inhibition in mouse models prevented both inflammation and airway remodelling (Liu et al. 2016).

**Anti-IL-17**

Asthma was classically considered as an allergic inflammatory disorder, however, discovery of non-eosinophilic asthma has revealed the association of neutrophils in severe asthma pathogenesis. IL-17 is a pro-inflammatory cytokine that is produced by TH17 cells. Its’ inflammatory roles have been well studied in multiple inflammatory conditions, including rheumatoid arthritis, COPD, cystic fibrosis, and multiple sclerosis (Miossec et al. 2009). In asthma, IL-17 is involved in airway remodelling, neutrophilic inflammation, and corticosteroid resistance in non-eosinophilic asthma (Chang et al. 2012; Chesné et al. 2014; Fogli et al. 2013; Mizutani et al. 2014; Nadeem et al. 2018; Nakae et al. 2002; Vazquez-Tello et al. 2013; Vazquez‐Tello et al. 2010; Wakashin et al. 2008). Hence, inhibiting IL-17, may be a possible treatment for non-eosinophilic asthma. The use of different mouse models has shown efficacy of anti-IL-17 treatments in the potential management of asthma. Treatment in models of allergic asthma show improvement in pulmonary inflammation with significant reduction in neutrophils, eosinophils, T-regulatory cells, and antigen-presenting cells with administration of anti-IL-17 monoclonal antibody (Camargo et al. 2018; Lovato et al. 2016). Similar effects were observed in a refractory asthma model also treated with anti-IL-17 (Liang et al. 2018). However, targeting IL-17 has not yet yielded satisfactory outcomes in clinical trials. Brodalumab, an IL-17 antagonist, proved to be effective in treating adult patients with moderate to severe plaque psoriasis, but failed to demonstrate any treatment effects in patients with moderate to severe asthma (Beck and Koo 2019; Busse et al. 2013; Khokhlovich et al. 2017). Treatment with secukinumab, a humanized anti-IL-17 monoclonal antibody that showed excellent clinical outcomes in treating plaque psoriasis, psoriatic arthritis, and rheumatoid arthritis, was terminated in a phase-II clinical trial in patients with uncontrolled asthma as it was not effective in the target population (Blanco et al. 2017; ClinicalTrial 2015; Langley et al. 2014; McInnes et al. 2015).

**Macrolides**

Several studies have assessed the use of macrolides for the management of asthma, specifically bacterial infection-associated non-eosinophilic asthma (Black et al. 2001; Esposito et al. 2004). Macrolides are antibiotics used to treat bacterial infection by attenuating bacterial protein biosynthesis and biofilm formation (Xepapadaki et al. 2008). Macrolides also possess anti-inflammatory properties and have been shown to potentiate responsiveness of asthma patients to corticosteroid therapy (Spahn et al. 2001). Treatment with macrolide (clarithromycin) in a bacteria-induced severe steroid-resistant severe asthma mouse model demonstrated antibacterial and anti-inflammatory effects alongside re-sensitization to corticosteroids (Essilfie et al. 2015). Likewise, a clinical study also reported the efficacy of clarithromycin in relieving wheezing in asthma patients co-infected with *Chlamydia pneumoniae* (Kraft et al. 2002). Moreover, a randomised, double-blind, placebo-controlled clinical trial on asthma patients receiving macrolide therapy (azithromycin) revealed its immunomodulatory efficacy by reducing asthma symptoms in non-eosinophilic asthma patients (Gibson et al. 2017). Notably, administration of azithromycin (500 mg, thrice per week, for 48 weeks) significantly reduced asthma exacerbations (including severe exacerbations) and sputum eosinophil levels (Gibson et al. 2017). Although recent evidence suggests largely beneficial effects of macrolides, their immunomodulatory functions for asthma management and disease progression is require further investigation and may induce antibiotic resistance in pathogens.

**Phosphodiesterase (PDE) inhibitors**

PDE is an essential enzyme that inhibits cellular signalling molecules like cyclic adenosine monophoshate (cAMP) and cyclic guanosine monophosphate (cGMP) by degrading their phosphodiester bonds (Gao et al. 2017; Karish and Gagnon 2006). Thus, by inhibiting PDE, it is possible to prolong cellular activity initiated by cAMP or cGMP. In asthma, the biosynthesis of one of the hallmark inflammatory mediators, TNF, is inhibited by cAMP, which is regulated by PDE (Shah et al. 1995). Hence, inhibiting PDE with inhibitors (PDEIs), could prolong the activity of cAMP leading to a reduction in the biosynthesis of TNF. Using *in-vivo* inflammation models, it was demonstrated that PDEI was able to reduce TNF concentration by up to 85% compared to sham treatment (Bundschuh et al. 2001; Murad et al. 2017). There are different types of PDEI available on the pharmaceutical market such as, roflumilast, cilomilast, rolipram, BAY19-8004, MEM1414, and GSK256066 (Karish and Gagnon 2006). Among them, only roflumilast is approved for clinical use in treating patients with COPD and was shown to reduce severe exacerbations and improve lung function (Calverley et al. 2009; Luo et al. 2016). However, it is not recommended for patients with asthma due to undesirable clinical outcomes. In multiple clinical trials, PDEI (roflumilast) administration improved lung function in mild to moderate asthma patients, but failed to have any bronchodilator effects and did not reduce the allergen-induced inflammation in the early asthma phase (Bateman et al. 2006; Bousquet et al. 2006; Louw et al. 2007). Furthermore, adverse events, such as headache and nausea, were reported with treatment (Bateman et al. 2006; Bousquet et al. 2006; Louw et al. 2007).

**Anti-histamines**

Histamine is a chemical mediator secreted by mast cells in response to an allergic reaction or event (Thangam et al. 2018). Under normal conditions, histamine is produced and stored within mast cells or basophils (Thangam et al. 2018). Upon release, it binds to histamine receptors expressed in the airways and pulmonary tissues, and subsequently initiates multiple allergic reactions, leading to mucus hypersecretion, broncho- and vascular constriction (Thangam et al. 2018). However, for these events to occur, the amount of histamine accumulated within the tissues must overwhelm its counterpart, histamine N-methyl transferase (HMT) (Salomonsson et al. 2019; Yamauchi and Ogasawara 2019). HMT metabolises airway histamine and has a significant role in regulating histamine effects on the airways (Yamauchi et al. 1994). Both histamine and HMT are regulated in a balanced state, and the downstream cascade is only initiated when the accumulated histamine overwhelms the HMT capability to degrade excess histamine (Yamauchi et al. 1994). Pharmacological inhibition of HMT with an inhibitor (SKF91488) exacerbate the contractile response of bronchi towards histamine, hence showing HMT as a negative regulator of histamine effects on the respiratory system (Curry 1946; Yamauchi et al. 1994).

There are 4 known types of histamine receptors (H1, H2, H3, H4) in the respiratory system (Ahmed et al. 1982; Ichinose and Barnes 1989; Kay et al. 2018; Tucker et al. 1975). Relevant for asthma H1 receptors mediate the bronchoconstriction of smooth muscle while H2 receptors are responsible for mucus hypersecretion and vascular dilation (Müller et al. 2006). A potential therapy to inhibit H1 receptor activity have been developed in the form of antagonists such as chlorpheniramine and clemastine (Kawauchi et al. 2019; Okubo et al. 2020). Despite being proven to possess strong biological activity and high specificity for H1 receptors, H1 receptor antagonists are not generally recommended for asthma treatment. Instead, inhaled corticosteroids, leukotriene receptor antagonist, and β2-receptor adrenergic agonist are recommended (Kawauchi et al. 2019; Okubo et al. 2020). Asthmatic patients receiving leukotriene receptor antagonist had better recovery in allergen-induced airway obstruction compared to those who received H1 receptor antagonist.

**Anti-vascularisation therapies**

VEGF has a critical role in driving airway vascularisation. As a vascular growth factor, it can increase MMP activity and the translocation and proliferation of endothelial cells, and hence plays major roles in promoting angiogenesis in airway tissues (Harkness et al. 2015). VEGF overexpression in mice leads to prominent airway vascularisation (Baluk et al. 2004). Administration of VEGF inhibitors, such as sunitinib, effectively suppresses eosinophilic airway inflammation and airway remodelling in murine asthma models (Huang et al. 2009; Lee et al. 2002). Moreover, reductions in VEGF levels and peri-bronchial angiogenesis after treatment with immunostimulatory sequences of DNA (ISSD) was observed in an ovalbumin-induced asthma model (Lee et al. 2006). It has been proposed that ISSD binds to Toll-like Receptor 9 and inhibits allergen-induced Th2 immune responses, as well as reversing features of airway remodelling including the development of peri-bronchial fibrosis and increases in ASM thickness (Lee et al. 2006). Additionally, administration of bevacizumab (recombinant humanized anti-VEGF mAb) prior to ovalbumin sensitisation inhibited angiogenesis and reduced airway tissue membrane thickness (Yuksel et al. 2013).

Administration of endostatin, a 20kDA C-terminal fragment derived from collagen-type XVIII, in ovalbumin-challenged mice reduced the progression of sub-epithelial angiogenesis, and relieved pulmonary and lung inflammation (Suzaki et al. 2005). The beneficial effects were reportedly due to the blockade of VEGF/VEGF receptor signalling. Similar effects were also observed after the administration of tumstatin (a protein fragment cleaved from collagen type IV) or synthetic peptides of it (Burgess et al. 2010; Grafton et al. 2014). Tumstatin also suppressed inflammatory cell migration, mucus hypersecretion and angiogenesis in ovalbumin-challenged mice (Hutchings et al. 2003).

Recently, docetaxel, a prodrug (delivered viaαvβ3-targeted nanoparticles) that binds to and stabilises intracellular microtubules, suppressed eosinophil levels and neovascular expansion in the airways of house dust mite-challenged mice (Lanza et al. 2017). It was proposed that docetaxel interacts with tubulin and reduces IL-13 and VEGF production. Likewise, in the same model, the fumagillin-prodrug interacted with methionine aminopeptidase-2 present in proliferating endothelial cells, and inhibited neovascular expansion in the lungs (Lanza et al. 2017).

There is still limited knowledge of the optimal means to prevent or reverse the progression of asthmatic vascularisation. Hence, the development of anti-vascularisation therapies should be considered as novel therapeutic approaches for asthma.

**Targeting microRNAs (miRNAs)**

miRNAs are short non-coding RNAs which control gene expression post-transcriptionally by directly blocking translation of their target mRNAs or by repressing protein production *via* mRNA destabilisation (Dua et al. 2017b; Plank et al. 2015). They regulate many biological processes (cell differentiation and growth, metabolism, cell signalling, apoptosis) related to inflammation. They are involved in altering pro-inflammatory responses and also virus-induced effects in human AECs, which are one of the leading causes of asthma exacerbations (Herbert et al. 2015). Inhibiting the function of specific miRNAs in asthma may be novel therapeutic approaches (Foster et al. 2013; Greene and Gaughan 2013).

A recent study showed roles for miR-23b in controlling TGF-β1-induced ASM cell proliferation by regulating Smad3 and, thereby reducing airway remodelling (Chen et al. 2017). Zhou *et al*.,identified miR-155 as a novel target in allergic asthma (Zhou et al. 2016), which also suppressed chemokine expression (CCL5, CCL11, CCL26, CXCL8, and CXCL10) in human epithelial cells by inhibiting IL-13 signalling (Matsukura et al. 2016). Others showed that this miRNA is increased in an ovalbumin-induced mouse model of allergic asthma but its inhibition with an antagomir did not alter the phenotype, which may be due variable efficacy in uptake of the inhibitor by different cells (Matsukura et al. 2016; Plank et al. 2015). miR-181b-5p has been identified as a potential biomarker for airway eosinophilia, and controls pro-inflammatory cytokine release by targeting the secreted phosphoprotein 1 (SPP1) gene (Huo et al. 2016). It also increases inflammation by promoting nuclear factor-κB signalling via the regulation of p65 and IL-6 (Wang et al. 2015). Similarly, Fan *et al*., showed in asthma patients that miR-145 is involved in maintaining the balance between Th1 and Th2 responses by targeting the runt-related transcription factor 3 (RUNX3), which may be a biomarker for asthma (Fan et al. 2016). miR-196a2 polymorphisms have also been shown to be involved in controlling asthma (Hussein et al. 2016).

An interesting study involving toluene diisocyanate (TDI), a major cause of occupational asthma, demonstrated the involvement of miR-210 *via* inhibitory effects on Treg function, particularly during the sensitisation phase of TDI-induced allergic asthma (Long et al. 2016).

Tang *et al.*, identified roles for miR-21a-3p, miR-449c-5p, and miR-496a-3p in mouse models of asthma, and identified an miR-21/Acvr2a axis in regulating asthma-induced inflammation (Tang et al. 2016). Also, we have shown crucial roles for miR-21 in the pathogenesis of an experimental mouse model of steroid-insensitive asthma. It’s effects occur though the suppression of anti-inflammatory phosphatase and tensin (PTEN) homolog, that increases the phosphoinositide 3-kinase (PI3K) signal, in turn reducing histone deacetylase-2 levels that are required for responses to steroid treatment (Kim et al. 2017a). Elbehidy *et al.*, confirmed miR-21 as a potential novel biomarker for asthma diagnosis in children (Elbehidy et al. 2016). miR-10a has also been identified as a possible therapeutic target in regulating the proliferation of ASM cells *via* the PI3K pathway (Hu et al. 2014). Xiang and colleagues demonstrated the role of miR-487b in activating and regulating macrophages in innate immune responses including pro-inflammatory effects through the induction of IL-33 transcripts (Xiang et al. 2016). Another study showed that antagonising miR-328 in the infected lung enhances the antimicrobial potential of macrophages and neutrophils along with the clearance of Non-typeable *Haemophilus influenzae* (Tay et al. 2015).

A primary pathogenic factor in asthma is the overexpression of IL-13, and most miRNAs implicated in the disease, such as miR-133a, -145, -126, -155 and -146, contribute to its regulation (Chiba et al. 2009; Collison et al. 2011; Greene and Gaughan 2013; Liu et al. 2015; Matsukura et al. 2016). Ho *et al.*, showed in an ovalbumin-induced mouse model of asthma that diallyl sulfide has protective effects due to miR-144, -34a and -34b/c induced Nrf2 activation, which has anti-oxidant effects (Ho et al. 2016). miR-19a has been identified as a potential new therapeutic target for the management of severe asthma, where its downregulation controls epithelial repair (Haj-Salem et al. 2015). Likewise, knock down of miR-106a suppressed airway inflammation, goblet cell metaplasia, sub-epithelial fibrosis and AHR in a mouse asthma model (Sharma et al. 2012).

As well as miRNAs, long non-coding RNAs (LncRNAs), such as LncRNAs BCYRN1, 846, or 4176 have also been implicated in airway inflammation and could be therapeutic targets in asthma (Wang et al. 2017; Zhang et al. 2016).

**Novel drug delivery systems**

The application of novel drug delivery systems is gaining popularity for the treatment of various chronic lung diseases, including asthma (Mehta et al. 2020a; Mehta et al. 2020b; Prasher et al. 2020). These include nanoparticle-based drug delivery, dry powder inhalers, micelle pharmacosomes, liposomes, dendrimers, and antibody-mediated drug delivery systems (Lanza et al. 2017).

*Nanoparticles:* A recent study evaluated the *in vivo* efficacy of biocompatible nanoparticles targeting IL-4Rα. These particles have enhanced permeability, and reduced lung inflammation and improved the immunosuppressive effects of anti-IL4Rα in ovalbumin-sensitised mice (Halwani et al. 2016; Maret et al. 2007). Other studies employed anti-IL-4Rα-blocking antibodies bound to superparamagnetic iron oxide nanoparticles, using polyethylene glycol polymers. These nanocarriers have improved targeting effects on various inflammatory cells (Al Faraj et al. 2016). Another study developed strontium-doped hydroxyapatite porous spheres (SHAS), an adjuvant and carrier in allergen-specific immunotherapy, where they have showed that the subcutaneous injection of allergen (OVA) stimulates both CD4+ and CD8+. The treatment of SHAS-OVA has proven better in efficacy as compared to soluble OVA alone with no necrotic or apoptotic effects (Garbani et al. 2016) .

One of the latest advances are protein corona (the outer layer of proteins adsorbed onto the nanoparticles), which are combined with inhaled nanoparticles to facilitate their movement through the respiratory tract, particularly the lining fluid. The corona contains various innate immune proteins like surfactant protein A, napsin A and complement (C1q, C3) (Shahabi et al. 2015). Inhaled nanoparticles often acquire a layer of protein corona as they pass through the respiratory tract. The identification of individual components of protein corona would improve their use with inhaled nanoparticles in therapeutics. Investigations are underway to identify types of proteins and the mechanisms involved. A recent attempt undertook proteomic and lipidomic analysis to define the composition of the surfactant corona on inhaled nanoparticles (Raesch et al. 2015).

*Liposomes:* Alternative drug delivery modes include liposomes, which are spherical vesicles of lipid bilayers. Maret *et al.*, used all-trans retinoic acid encapsulated liposomes in a mouse model of ovalbumin-induced allergic airways disease, which reduced the synthesis of IgE and airway inflammation (Maret et al. 2007). Similarly, the efficacy of budesonide in stealth liposomal formulations is greater than the drug alone at reducing lung inflammation (Konduri et al. 2003). Liposomal formulations encapsulated with procaterol hydrochloride have sustained release and potent pharmacological effects on pulmonary administration (Tahara et al. 2016). Also, various liposomes can combat the problem of bacterial biofilms in asthma (Bandara et al. 2016; Liu and Post 2009). Other studies used liposomal formulations with various other therapeutic moieties, including amphotericin B, ciprofloxacin, topotecan, and calcifediol against different infections including Aspergillosis and Pseudomonas infection (Adhikari et al. 2015; Castoldi et al. 2016; Saraf et al. 2016). Blom *et al.*, developed a triple co-culture model of epithelial cells, macrophages, and DCs to mimic the human respiratory tract to better understand the immuno-modulatory effects of novel drug delivery systems, such as liposomes and virosomes. These advanced drug delivery modes have proven as a great antigen carriers demonstrating lesser inflammation and controlling the mucosal immune responses (Blom et al. 2016).

*Other drug delivery systems:* Mucoadhesion of drugs is an important aspect of drug delivery in airway diseases, particularly asthma. Co-adhesive microspheres of levosalbutamol sulphate were prepared using spray drying techniques. Microspheres demonstrated sustained release of Levosalbutamol Sulphate because of their particle size, swell-ability, and increased mucoadhesion features (Patel et al. 2012). Similarly, chitosan-based microspheres containing montelukast sodium have been used, and have effective physicochemical properties required for optimal pulmonary drug delivery (Dua et al. 2017a; Panchal et al. 2012). Pachuau *et al.*, used solvent evaporation to prepare matrix microspheres with salbutamol sulphate and theophylline for simultaneous delivery to induce prolong and sustained release (Pachuau et al. 2008). Gelatin microspheres are another important category and have improved mucoadhesive and sustained release properties with drugs like salbutamol sulphate (Jayan et al. 2009) . Both of these studies outcomes provide insight into reducing the frequency of drug administration resulting in better patient compliance.

Recent reports highlight the relevance of advanced drug delivery systems, such as liposomes and nano/macro particles, for the pulmonary delivery of heparin (Yildiz-Pekoz and Ozsoy 2017). Yhee *et al.*, postulated that nanoparticle-based drug delivery is an advanced platform to achieve maximum therapeutic efficacy in asthma, COPD, cystic fibrosis, idiopathic pulmonary fibrosis, and lung cancers (Yhee et al. 2016). Another promising means of delivery in targeting and overcoming the mucus barrier is nanocomplexes for gene therapy, which are in clinical trials (Di Gioia et al. 2015). Other novel drug delivery modalities have been investigated in asthma, including chrono-modulated drug delivery, dendrimers, and micelles (Nasr et al. 2014; Peng et al. 2015; Qureshi et al. 2008). All are advancing respiratory drug delivery, allowing translation of therapeutic moieties into clinically effective and patient-friendly drug delivery systems by reducing the associated side effects, reduced frequency of drug administration, targeted effects and better patient adherence to the dosage regime.

**Conclusions**

Mild-to-moderate allergic asthma is underpinned by allergen-induced IgE and type 2 eosinophilic inflammation that causes airway tissue remodelling and AHR. However, neutrophilic and non-eosinophilic severe steroid-resistant asthma is now recognised that is driven infection or other exposures that induce Th1/Th17 dominant responses. Understanding the pathogenesis of these different forms of asthma enables the development of precision therapies that target the different endotypes. Consequently, biological have been developed for allergic asthma that target IgE and type 2 responses during the sensitisation (TSLP, IL-33, IL-4) or developed (IL-5, IL-13, IL-4Ra) phases of disease. New therapies that target more severe neutrophilic steroid non-responsive phenotype that target type I (TNF/PDEI) and neutrophilic inflammation (IL-17) or infection-induced processes (macrolides) show promise but are less well established. Recent advances have revealed the novel roles and significant involvement of vascularisation and miRNAs in asthma pathogenesis. Angiogenesis and vascularisation in the pulmonary system increase and provide vessels for the delivery of more inflammatory cells and greater levels on inflammation. Thus, targeting pro-vascularisation factors (VEGF) or using suppressors (endostatin, tumstatin) may have beneficial effects. Other novel potential therapies target miRNAs that control the expression of genes relevant to asthma. Targeting specific miRNA with inhibitors may also be beneficial in asthma by; reducing specific pro-inflammatory cytokine and chemokine expression, including IL-13 signalling and more broadly by suppressing nuclear factor-κB signalling, altering the balance between Th1 and Th2 responses, improve regulatory T cell function, reduce mucus hypersecretion, ASM proliferation and fibrosis and macrophage responses, and increase steroid responses and epithelial repair in severe asthma. By targeting such factors, new and effective therapeutic strategies can be developed for asthma. Incorporating new therapeutic agents into novel drug delivery systems including nanoparticles, liposomes and other delivery systems could enhance specific targeting of specific cell types to improve disease management and patient compliance.

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**TABLE 1** Evidence for increased vascularisation in asthma

|  |  |  |
| --- | --- | --- |
| **Feature** | **Measures** | **Reference** |
| Increased amount of blood vessels, vessel density and vascular area | Microscopic evaluation of bronchial biopsy specimens revealed significantly higher amounts of microvessels in the *lamina propria* of asthma patients. Increased numbers of mast cells also detected. Control patients had scattered and less microvessel density. Intensity of microvascularization was reduced with high doses of inhaled fluticasone (500μg 2X/day). | (Chetta et al. 2003; Grigoras et al. 2012) |
|  | Bronchial biopsies from asthma patients had a high degree of airway vascularity. | (Hashimoto et al. 2005; Hoshino et al. 2001a; Hoshino et al. 2001b) |
| Elevated levels of pro-angiogenic factors | Elevated levels of VEGF and angiotensin in sputum supernatants of children with asthma exacerbations.  | (Abdel-Rahman et al. 2006) |
| High levels of VEGF in sputum of asthma patients, reduced by inhaled beclomethasone treatment (800μg/day). | (Asai et al. 2003; Meyer and Akdis 2013) |
| High levels of VEGF in BALF and airway tissue of asthma patients. | (Meyer and Akdis 2013; Tuder and Yun 2008) |

**TABLE 2** Potential biological agents in clinical trials/development for asthma treatment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drug** | **Mechanism of action** | **Observed Clinical Effect** | **Trial Phase** | **Reference** |
| Omalizumab | Anti-IgE mAb | Reduces asthma exacerbations  | Approved by FDA and EMA  | (D’Amato et al. 2007; Hanania et al. 2018; Szefler et al. 2018) |
| Tezepelumab (AMG157/MEDI-9929) | Anti-TSLP mAb  | Reduces asthma exacerbations  | Phase II | (Corren et al. 2018; Corren et al. 2017; Gauvreau et al. 2014) |
| ANB020 | Anti-IL-33 mAb | Reduces asthma exacerbations | Phase I | (Londei et al. 2017) |
| Dupilumab | Anti-IL-4Rα mAb | Reduces asthma exacerbationsIncreases lung function | Phase III | (Castro et al. 2018; Rabe et al. 2018; Wenzel et al. 2016; Wenzel 2013) |
| Pascolizumab | Anti-IL-4 mAb | No significant clinical efficacy | Phase II | (Hart et al. 2002) |
| Altrakincept | Anti-IL-4 mAb | No significant clinical efficacy | Phase II | (Hendeles et al. 2004) |
| Mepolizumab | Anti-IL-5 mAb | Improves Forced Expiratory VolumeReduces asthma exacerbation rate | Approved by FDA and EMA  | (Haldar et al. 2009; Pavord et al. 2012) |
| Benralizumab | Anti-IL-5 mAb | Reduces peripheral eosinophil levels | Phase III | (Bleecker et al. 2016; Castro et al. 2014; Ferguson et al. 2018) |
| Tralokinumab | Anti-IL-13 mAb | Inconsistent clinical effects in reducing asthma exacerbation rate  | Phase III | (Panettieri et al. 2018; Piper et al. 2013) |
| Anrukinzumab | Anti-IL-13 mAb | Reduces allergen-induced asthmatic responses | Phase II | (Hua et al. 2015) |
| Lebrikizumab | Anti-IL-13 mAb | Inconsistent clinical effects in reducing asthma exacerbation rateSignificant adverse effects, including aplastic anaemia and eosinophilia | Phase III | (Hanania et al. 2016; Scheerens et al. 2014) |

**Figure Legends**

**FIGURE 1** Comparison between the normal and asthmatic lung. Healthy individuals have normal airway walls and relaxed airway smooth muscle. The airways of asthmatic patients constrict upon exposure to innocuous antigens, over express mucus, are inflamed with swollen walls and tightened smooth muscle.

**FIGURE 2** Cascade of events leading to airway inflammation and asthma pathogenesis. Immunogenic antigens in the air, such as viruses, microbes, and allergens trigger inflammatory cascades. Activated inflammatory cells, including mast cells, eosinophils and neutrophils subsequently release a plethora of inflammatory mediators. These mediators drive airway tissue remodelling and asthma pathogenesis.

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