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., $PM_{2.5}$) 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-cells via 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

294 (soluble humanised IL-4 inhibitor) blocked airway eosinophil infiltration and mucus
295 hypersecretion in allergen-challenged mice (Henderson et al. 2000). It is safe in moderate
296 asthma patients and reduces inflammation (Borish et al. 2001; Borish et al. 1999). However,
297 again the respiratory function of asthma patients was not improved (Borish et al. 2001; Borish
298 et al. 1999). Further studies are warranted to improve the anti-IL-4 medications for asthma,
299 but it is likely more effective as a preventative rather than a treatment.
300
301 Anti-IL-5
302 IL-5 has important roles in allergen-induced asthma as a mediator of the activation,
303 proliferation, and maturation of eosinophils (Stirling et al. 2001). Animal studies show that
304 anti-IL-5 mAb, TRFK-5, reduced eosinophil influx into mouse airways after allergen challenge
305 (Garlisi et al. 1999), and suppressed AHR in mouse models of asthma (Mauser et al. 1995).
306 Early clinical trials in mild and chronic asthma with a similar anti-IL-5 mAb, mepolizumab
307 showed that it is safe (Holgate 2008; Leckie et al. 2000; Tanaka et al. 2004), but therapeutic
308 efficacy was inconsistent (Leckie et al. 2000; Mauser et al. 1995; Tanaka et al. 2004). Some
309 patients responded well, those with elevated IL-5/eosinophils, and the levels of eosinophils
310 were significantly reduced, but likely not sufficiently so, and overall it did not improve
311 functional endpoints, such as lung function and asthma symptoms. Interestingly, in a phase II
312 trial (NCT00292877) intravenous administration of mepolizumab to chronic corticosteroid-
313 resistant asthma patients demonstrated clinically reduced blood and sputum levels of
314 eosinophils, and improved asthma symptoms (Haldar et al. 2009; Nair et al. 2009). A later
315 phase III clinical trial (NCT01000506) in patients with severe, uncontrolled asthma with
316 eosinophilic inflammation, mepolizumab met its primary and secondary endpoints by reducing
317 the number of exacerbations, increasing the time to first exacerbation, and improving FEV_1

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 FEV_1 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 FEV_1 (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 via αvβ3-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

572 The application of novel drug delivery systems is gaining popularity for the treatment of
573 various chronic lung diseases, including asthma (Mehta et al. 2020a; Mehta et al. 2020b;
574 Prasher et al. 2020). These include nanoparticle-based drug delivery, dry powder inhalers,
575 micelle pharmacosomes, liposomes, dendrimers, and antibody-mediated drug delivery systems
576 (Lanza et al. 2017).
577 *Nanoparticles:* A recent study evaluated the *in vivo* efficacy of biocompatible nanoparticles
578 targeting IL-4Rα. These particles have enhanced permeability, and reduced lung inflammation
579 and improved the immunosuppressive effects of anti-IL4Rα in ovalbumin-sensitised mice
580 (Halwani et al. 2016; Maret et al. 2007). Other studies employed anti-IL-4Rα-blocking
581 antibodies bound to superparamagnetic iron oxide nanoparticles, using polyethylene glycol
582 polymers. These nanocarriers have improved targeting effects on various inflammatory cells
583 (Al Faraj et al. 2016). Another study developed strontium-doped hydroxyapatite porous
584 spheres (SHAS), an adjuvant and carrier in allergen-specific immunotherapy, where they have
585 showed that the subcutaneous injection of allergen (OVA) stimulates both CD4+ and CD8+.
586 The treatment of SHAS-OVA has proven better in efficacy as compared to soluble OVA alone
587 with no necrotic or apoptotic effects (Garbani et al. 2016) .
588 One of the latest advances are protein corona (the outer layer of proteins adsorbed onto the
589 nanoparticles), which are combined with inhaled nanoparticles to facilitate their movement
590 through the respiratory tract, particularly the lining fluid. The corona contains various innate
591 immune proteins like surfactant protein A, napsin A and complement (C1q, C3) (Shahabi et al.
592 2015). Inhaled nanoparticles often acquire a layer of protein corona as they pass through the
593 respiratory tract. The identification of individual components of protein corona would improve
594 their use with inhaled nanoparticles in therapeutics. Investigations are underway to identify
595 types of proteins and the mechanisms involved. A recent attempt undertook proteomic and

596 lipidomic analysis to define the composition of the surfactant corona on inhaled nanoparticles
597 (Raesch et al. 2015).
598 *Liposomes:* Alternative drug delivery modes include liposomes, which are spherical vesicles
599 of lipid bilayers. Maret *et al.*, used all-trans retinoic acid encapsulated liposomes in a mouse
600 model of ovalbumin-induced allergic airways disease, which reduced the synthesis of IgE and
601 airway inflammation (Maret et al. 2007). Similarly, the efficacy of budesonide in stealth
602 liposomal formulations is greater than the drug alone at reducing lung inflammation (Konduri
603 et al. 2003). Liposomal formulations encapsulated with procaterol hydrochloride have
604 sustained release and potent pharmacological effects on pulmonary administration (Tahara et
605 al. 2016). Also, various liposomes can combat the problem of bacterial biofilms in asthma
606 (Bandara et al. 2016; Liu and Post 2009). Other studies used liposomal formulations with
607 various other therapeutic moieties, including amphotericin B, ciprofloxacin, topotecan, and
608 calcifediol against different infections including Aspergillosis and Pseudomonas infection
609 (Adhikari et al. 2015; Castoldi et al. 2016; Saraf et al. 2016). Blom *et al.*, developed a triple
610 co-culture model of epithelial cells, macrophages, and DCs to mimic the human respiratory
611 tract to better understand the immuno-modulatory effects of novel drug delivery systems, such
612 as liposomes and virosomes. These advanced drug delivery modes have proven as a great
613 antigen carriers demonstrating lesser inflammation and controlling the mucosal immune
614 responses (Blom et al. 2016).
615 *Other drug delivery systems:* Mucoadhesion of drugs is an important aspect of drug delivery
616 in airway diseases, particularly asthma. Co-adhesive microspheres of levosalbutamol sulphate
617 were prepared using spray drying techniques. Microspheres demonstrated sustained release of
618 Levosalbutamol Sulphate because of their particle size, swell-ability, and increased
619 mucoadhesion features (Patel et al. 2012). Similarly, chitosan-based microspheres containing
620 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.

Acknowledgments

The authors acknowledge the support of fellowships from the University of Tasmania to MS, and the National health and Medical Research Council of Australia and Gladys Brawn/Faculty

672 of Health and Medicine (1079187), The University of Newcastle and the Rainbow
673 Foundation/Hunter Medical Research Institute to PMH.

674 **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)

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677 **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 exacerbations Increases 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 Volume Reduces 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 rate Significant adverse effects, including aplastic anaemia and eosinophilia	Phase III	(Hanania et al. 2016; Scheerens et al. 2014)

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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.

Reference list

Abdel-Rahman AM, El-Sahrigy SA, Bakr SI (2006) A comparative study of two angiogenic factors: vascular endothelial growth factor and angiogenin in induced sputum from asthmatic children in acute attack. Chest 129:266-271

Adcock IM, Ford PA, Bhavsar P, Ahmad T, Chung KF (2008) Steroid resistance in asthma: mechanisms and treatment options. Curr Allergy Asthma Rep 8:171

Adhikari K, Buatong W, Thawithong E, Suwandecha T, Srichana T (2015) Factors Affecting Enhanced Permeation of Amphotericin B Across Cell Membranes and Safety of Formulation. AAPS PharmSciTech:1-9

Ahmed T, Mirbahar KB, Oliver Jr W, Eyre P, Wanner A (1982) Characterization of H1-and H2-receptor function in pulmonary and systemic circulations of sheep. J Appl Physiol 53:175-184

Al Faraj A, Shaik AS, Afzal S, Al-Muhsen S, Halwani R (2016) Specific targeting and noninvasive magnetic resonance imaging of an asthma biomarker in the lung using polyethylene glycol functionalized magnetic nanocarriers. Contrast Media Mol Imaging 11:172-183

Asai K, Kanazawa H, Kamoi H, Shiraishi S, Hirata K, Yoshikawa J (2003) Increased levels of vascular endothelial growth factor in induced sputum in asthmatic patients. Clin Exp Allergy 33:595-599

Asher MI, Ellwood P (2014) The global asthma report 2014. Global Asthma Network. 2020

Asher MI et al. (2006) Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 368:733-743

Bakakos P, Patentalakis G, Papi A (2016) Vascular biomarkers in asthma and COPD. Curr Top Med Chem 16:1599-1609

Baluk P, Lee CG, Link H, Ator E, Haskell A, Elias JA, McDonald DM (2004) Regulated angiogenesis and vascular regression in mice overexpressing vascular endothelial growth factor in airways. Am J Pathol 165:1071-1085

Bandara H, Herpin M, Kolacny Jr D, Harb A, Romanovicz D, Smyth H (2016) Incorporation of farnesol significantly increases the efficacy of liposomal ciprofloxacin against Pseudomonas aeruginosa biofilms in vitro. Mol Pharm 13:2760-2770

Barnes PJ (2001) Th2 cytokines and asthma: an introduction. Respir Res 2:64-65

Barnes PJ (2006) Drugs for asthma. Br J Pharmacol 147:S297-303 doi:10.1038/sj.bjp.0706437

Barnes PJ (2008) The cytokine network in asthma and chronic obstructive pulmonary disease. J Clin Invest 118:3546-3556

Barnes PJ, Adcock IM (2009) Glucocorticoid resistance in inflammatory diseases. The Lancet 373:1905-1917

Bateman ED et al. (2006) Efficacy and safety of roflumilast in the treatment of asthma. Ann Allergy, Asthma Immunol 96:679-686

Beasley R (1998) Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 351:1225-1232

Beck KM, Koo J (2019) Brodalumab for the treatment of plaque psoriasis: up-to-date. Expert Opin Biol Ther 19:287-292

Benayoun L, Druilhe A, Dombret M-C, Aubier M, Pretolani M (2003) Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med 167:1360-1368

741 Black PN et al. (2001) Trial of roxithromycin in subjects with asthma and serological
742 evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med
743 164:536-541
744 Blanco FJ et al. (2017) Secukinumab in active rheumatoid arthritis: a phase III randomized,
745 double-blind, active comparator–and placebo-controlled study. Arthritis &
746 rheumatology 69:1144-1153
747 Bleecker ER et al. (2016) Efficacy and safety of benralizumab for patients with severe
748 asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-
749 agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial.
750 Lancet 388:2115-2127
751 Blom RA et al. (2016) A Triple Co-Culture Model of the Human Respiratory Tract to Study
752 Immune-Modulatory Effects of Liposomes and Virosomes. PLoS ONE 11:e0163539
753 Borish LC, Nelson HS, Corren J, Bensch G, Busse WW, Whitmore JB, Agosti JM (2001)
754 Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. J Allergy
755 Clin Immunol 107:963-970
756 Borish LC, Nelson HS, Lanz MJ, Claussen L, Whitmore JB, Agosti JM, Garrison L (1999)
757 Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-
758 controlled trial. Am J Respir Crit Care Med 160:1816-1823
759 Bousquet J et al. (2006) Comparison of roflumilast, an oral anti-inflammatory, with
760 beclomethasone dipropionate in the treatment of persistent asthma. Allergy 61:72-78
761 Brusselle GG, Maes T, Bracke KR (2013) Eosinophils in the spotlight: eosinophilic airway
762 inflammation in nonallergic asthma. Nat Med 19:977-979
763 Bundschuh DS, Eltze M, Barsig J, Wollin L, Hatzelmann A, Beume R (2001) In vivo
764 efficacy in airway disease models of roflumilast, a novel orally active PDE4 inhibitor.
765 J Pharmacol Exp Ther 297:280-290
766 Burgess JK et al. (2010) Reduction of tumstatin in asthmatic airways contributes to
767 angiogenesis, inflammation, and hyperresponsiveness. Am J Respir Crit Care Med
768 181:106-115
769 Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, Lin S-L (2013) Randomized,
770 double-blind, placebo-controlled study of brodalumab, a human anti–IL-17 receptor
771 monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med
772 188:1294-1302
773 Cahill KN et al. (2017) KIT inhibition by imatinib in patients with severe refractory asthma.
774 N Engl J Med 376:1911-1920
775 Calverley PM, Rabe KF, Goehring U-M, Kristiansen S, Fabbri LM, Martinez FJ (2009)
776 Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised
777 clinical trials. The Lancet 374:685-694
778 Camargo LdN et al. (2018) Effects of anti-IL-17 on inflammation, remodeling, and oxidative
779 stress in an experimental model of asthma exacerbated by LPS. Frontiers in
780 immunology 8:1835
781 Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6:389-396
782 Carmeliet P (2005) Angiogenesis in life, disease and medicine. Nature 438:932-936
783 Castoldi A et al. (2016) Calcifediol-loaded liposomes for local treatment of pulmonary
784 bacterial infections. Eur J Pharm Biopharm 118:62-67
785 Castro M et al. (2018) Dupilumab efficacy and safety in moderate-to-severe uncontrolled
786 asthma. N Engl J Med 378:2486-2296
787 Chakir J et al. (2003) Airway remodeling-associated mediators in moderate to severe asthma:
788 effect of steroids on TGF-β, IL-11, IL-17, and type I and type III collagen expression.
789 J Allergy Clin Immunol 111:1293-1298

790 Chang Y et al. (2012) Th17-associated cytokines promote human airway smooth muscle cell
791 proliferation. The FASEB Journal 26:5152-5160
792 Chen M et al. (2017) MiR-23b controls TGF-beta1 induced airway smooth muscle cell
793 proliferation via direct targeting of Smad3. Pulm Pharmacol Ther 42:33-42
794 doi:10.1016/j.pupt.2017.01.001
795 Chesné J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A (2014) IL-17 in severe
796 asthma. Where do we stand? Am J Respir Crit Care Med 190:1094-1101
797 Chetta A et al. (2003) Vascular component of airway remodeling in asthma is reduced by
798 high dose of fluticasone. Am J Respir Crit Care Med 167:751-757
799 Chung AS, Ferrara N (2011) Developmental and pathological angiogenesis. Annu Rev Cell
800 Dev Biol 27:563-584
801 ClinicalTrial (2015) Safety, Tolerability, and Efficacy of AIN457 in Patients With
802 Uncontrolled Asthma (NCT01478360). US National Library of Medicine,
803 Corren J et al. (2018) Tezepelumab demonstrates clinically meaningful improvements in
804 asthma control (ACQ-6) in patients with uncontrolled asthma: results from a phase 2b
805 clinical trial. J Allergy Clin Immunol 141:AB80
806 Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, van der Merwe R (2017)
807 Tezepelumab in adults with uncontrolled asthma. N Engl J Med 377:936-946
808 Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP,
809 Locksley RM (1996) Interleukin 4, but not interleukin 5 or eosinophils, is required in
810 a murine model of acute airway hyperreactivity. J Exp Med 183:109-117
811 Curry JJ (1946) The effect of antihistamine substances and other drugs on histamine
812 bronchoconstriction in asthmatic subjects. The Journal of clinical investigation
813 25:792-799
814 Di Gioia S et al. (2015) Nanocomplexes for gene therapy of respiratory diseases: Targeting
815 and overcoming the mucus barrier. Pulm Pharmacol Ther 34:8-24
816 Djukanovic R (2002) Airway inflammation in asthma and its consequences: implications for
817 treatment in children and adults. J Allergy Clin Immunol 109:S539-S548
818 Doucet C, Brouty-Boyé D, Pottin-Clémenceau C, Canonica GW, Jasmin C, Azzarone B
819 (1998) Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in
820 asthma. The Journal of clinical investigation 101:2129-2139
821 Douwes J, Gibson P, Pekkanen J, Pearce N (2002) Non-eosinophilic asthma: importance and
822 possible mechanisms. Thorax 57:643-648
823 Dua K et al. (2017a) Chitosan and Its Derivatives in Nanocarrier Based Pulmonary Drug
824 Delivery Systems. Pharm Nanotechnol 5:243-249
825 Dua K, Hansbro NG, Foster PS, Hansbro PM (2017b) MicroRNAs as therapeutics for future
826 drug delivery systems in treatment of lung diseases. Drug Deliv Transl Res:1-11
827 Elbehidy RM, Youssef DM, El-Shal AS, Shalaby SM, Sherbiny HS, Sherief LM, Akeel NE
828 (2016) MicroRNA-21 as a novel biomarker in diagnosis and response to therapy in
829 asthmatic children. Mol Immunol 71:107-114 doi:10.1016/j.molimm.2015.12.015
830 Ennis M (2003) Neutrophils in asthma pathophysiology. Curr Allergy Asthma Rep 3:159-165
831 Esposito S, Blasi F, Bosis F Efficacy of clarithromycin for the treatment of acute episodes of
832 bronchospasm in children with a history of recurrent wheezing. In: 22nd annual
833 meeting of European Society for Pediatric Infectious Diseases (ESPID). Tampere,
834 Finland, 2004.
835 Essilfie A-T et al. (2015) Macrolide therapy suppresses key features of experimental steroid-
836 sensitive and steroid-insensitive asthma. Thorax 70:458-467 doi:10.1136/thoraxjnl-
837 2014-206067
838 Essilfie A-T et al. (2011) Haemophilus influenzae infection drives IL-17-mediated
839 neutrophilic allergic airways disease. PLoS Pathog 7:e1002244

840 Essilfie AT et al. (2012) Combined Haemophilus influenzae respiratory infection and allergic
841 airways disease drives chronic infection and features of neutrophilic asthma. Thorax
842 67:588-599 doi:10.1136/thoraxjnl-2011-200160
843 Esteban-Gorgojo I, Antolín-Amérigo D, Domínguez-Ortega J, Quirce S (2018) Non-
844 eosinophilic asthma: current perspectives. J Asthma Allergy 11:267-281 doi:
845 10.2147/JAA.S153097
846 Fan L et al. (2016) MicroRNA-145 influences the balance of Th1/Th2 via regulating RUNX3
847 in asthma patients. Exp Lung Res 42:417-424 doi:10.1080/01902148.2016.1256452
848 Ferguson GT et al. (2018) Assessment of an accessorized pre-filled syringe for home-
849 administered benralizumab in severe asthma. J Asthma Allergy 11:63-72
850 Ferrara N (2007) Vascular endothelial growth factor: pathophysiology and clinical
851 implications. Angiogenesis From basic science to clinical applications:1-36
852 Fireman P Understanding asthma pathophysiology. In: Allergy and Asthma Proceedings,
853 2003. vol 2. OceanSide Publications, Inc, pp 79-83
854 Fogli LK et al. (2013) T cell–derived IL-17 mediates epithelial changes in the airway and
855 drives pulmonary neutrophilia. The Journal of Immunology 191:3100-3111
856 Foster PS et al. (2013) The emerging role of microRNAs in regulating immune and
857 inflammatory responses in the lung. Immunol Rev 253:198-215
858 Frieri M Asthma concepts in the new millennium: update in asthma pathophysiology. In:
859 Allergy and Asthma Proceedings, 2005. vol 2. OceanSide Publications, Inc, pp 83-88
860 Galli SJ (2017) Mast Cells and KIT as Potential Therapeutic Targets in Severe Asthma. N
861 Engl J Med 376:1983-1984
862 Galvão I, Kim RY, Shen S, Budden KF, Vieira AT, Hansbro PM (2020) Emerging
863 therapeutic targets and preclinical models for severe asthma. Expert Opin Ther
864 Targets 7:1-13
865 Gao H, Ying S, Dai Y (2017) Pathological roles of neutrophil-mediated inflammation in
866 asthma and its potential for therapy as a target. Journal of Immunology Research 2017
867 doi:10.1155/2017/3743048
868 Garbani M et al. (2016) Allergen-loaded strontium-doped hydroxyapatite spheres improve
869 allergen-specific immunotherapy in mice. Allergy 72:570-578
870 Garlisi CG et al. (1999) Effects of chronic anti-interleukin-5 monoclonal antibody treatment
871 in a murine model of pulmonary inflammation. Am J Respir Cell Mol Biol 20:248-
872 255
873 Gauvreau GM et al. (2014) Effects of an anti-TSLP antibody on allergen-induced asthmatic
874 responses. N Engl J Med 370:2102-2110
875 Genuneit J et al. (2017) The state of asthma epidemiology: an overview of systematic reviews
876 and their quality. Clin Transl Allergy 7:1-9
877 Gibson PG et al. (2017) Effect of azithromycin on asthma exacerbations and quality of life in
878 adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind,
879 placebo-controlled trial. Lancet 390:659-668
880 Grafton KT, Moir LM, Black JL, Hansbro NG, Hansbro PM, Burgess JK, Oliver BG (2014)
881 LF-15 & T7, synthetic peptides derived from tumstatin, attenuate aspects of airway
882 remodelling in a murine model of chronic OVA-induced allergic airway disease.
883 PLoS ONE 9:e85655
884 Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID (2002) Analysis
885 of induced sputum in adults with asthma: identification of subgroup with isolated
886 sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 57:875-879
887 Greene CM, Gaughan KP (2013) microRNAs in asthma: potential therapeutic targets. Curr
888 Opin Pulm Med 19:66-72

889 Grigoras A, Caruntu I-D, Grigoras C, Mihaescu T, Amalinei C (2012) Relationship between
890 immunohistochemical assessment of bronchial mucosa microvascularization and
891 clinical stage in asthma. Rom J Morphol Embryol 53:485-490
892 Grünig G et al. (1998) Requirement for IL-13 independently of IL-4 in experimental asthma.
893 Science 282:2261-2263
894 Haj-Salem I et al. (2015) MicroRNA-19a enhances proliferation of bronchial epithelial cells
895 by targeting TGFbetaR2 gene in severe asthma. Allergy 70:212-219
896 doi:10.1111/all.12551
897 Haldar P et al. (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. N
898 Engl J Med 360:973-984
899 Hallstrand TS, Hackett TL, Altemeier WA, Matute-Bello G, Hansbro PM, Knight DA (2014)
900 Airway epithelial regulation of pulmonary immune homeostasis and inflammation.
901 Clin Immunol 151:1-15
902 Halwani R, Shaik AS, Ratemi E, Afzal S, Kenana R, Al-Muhsen S, Al Faraj A (2016) A
903 novel anti-IL4Rα nanoparticle efficiently controls lung inflammation during asthma.
904 Exp Mol Med 48:e262
905 Hanania NA et al. (2016) Efficacy and safety of lebrikizumab in patients with uncontrolled
906 asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-
907 blind, placebo-controlled trials. Lancet Respir Med 4:781-796
908 Hanania NA, Rosén K, Griffin NM, Trzaskoma BL, Haselkorn T, Chipps BE, Casale TB
909 (2018) Response to Omalizumab Observed Over Wide Range of Blood Eosinophil
910 Levels. J Allergy Clin Immunol 141:AB15
911 Hansbro PM, Kaiko GE, Foster PS (2011) Cytokine/anti-cytokine therapy–novel treatments
912 for asthma? Br J Pharmacol 163:81-95
913 Hansbro PM et al. (2017) Mechanisms and treatments for severe, steroid-resistant allergic
914 airway disease and asthma. Immunol Rev 278:41-62
915 Hansbro PM et al. (2013) Th2 cytokine antagonists: potential treatments for severe asthma.
916 Expert Opin Investig Drugs 22:49-69
917 Harkness LM, Ashton AW, Burgess JK (2015) Asthma is not only an airway disease, but also
918 a vascular disease. Pharmacol Ther 148:17-33
919 Hashimoto M, Tanaka H, Abe S (2005) Quantitative analysis of bronchial wall vascularity in
920 the medium and small airways of patients with asthma and COPD. Chest 127:965-972
921 Hellström M, Gerhardt H, Kalén M, Li X, Eriksson U, Wolburg H, Betsholtz C (2001) Lack
922 of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J
923 Cell Biol 153:543-554
924 Henderson WR, Chi EY, Maliszewski CR (2000) Soluble IL-4 receptor inhibits airway
925 inflammation following allergen challenge in a mouse model of asthma. J Immunol
926 164:1086-1095
927 Herbert C, Sebesfi M, Zeng QX, Oliver BG, Foster PS, Kumar RK (2015) Using multiple
928 online databases to help identify microRNAs regulating the airway epithelial cell
929 response to a virus-like stimulus. Respirology 20:1206-1212
930 Ho CY, Lu CC, Weng CJ, Yen GC (2016) Protective Effects of Diallyl Sulfide on
931 Ovalbumin-Induced Pulmonary Inflammation of Allergic Asthma Mice by
932 MicroRNA-144, -34a, and -34b/c-Modulated Nrf2 Activation. J Agric Food Chem
933 64:151-160 doi:10.1021/acs.jafc.5b04861
934 Holgate S (2000) The role of mast cells and basophils in inflammation. Clin Exp Allergy
935 30:28-32
936 Holgate ST (2008) Pathogenesis of asthma. Clin Exp Allergy 38:872-897
937 Horvat JC et al. (2010a) Chlamydial respiratory infection during allergen sensitization drives
938 neutrophilic allergic airways disease. The Journal of Immunology 184:4159-4169

939 Horvat JC et al. (2010b) Early-life chlamydial lung infection enhances allergic airways
940 disease through age-dependent differences in immunopathology. J Allergy Clin
941 Immunol 125:617-625. e616
942 Hoshino M, Nakamura Y, Hamid QA (2001a) Gene expression of vascular endothelial
943 growth factor and its receptors and angiogenesis in bronchial asthma. J Allergy Clin
944 Immunol 107:1034-1038
945 Hoshino M, Takahashi M, Takai Y, Sim J, Aoike N (2001b) Inhaled corticosteroids decrease
946 vascularity of the bronchial mucosa in patients with asthma. Clin Exp Allergy 31:722-
947 730
948 Hu R et al. (2014) MicroRNA-10a controls airway smooth muscle cell proliferation via direct
949 targeting of the PI3 kinase pathway. FASEB J 28:2347-2357 doi:10.1096/fj.13-
950 247247
951 Huang M, Liu X, Du Q, Yao X, Yin K-S (2009) Inhibitory effects of sunitinib on ovalbumin-
952 induced chronic experimental asthma in mice. Chin Med J (Engl) 122:1061-1066
953 Huang YJ, Nariya S, Harris JM, Lynch SV, Choy DF, Arron JR, Boushey H (2015) The
954 airway microbiome in patients with severe asthma: associations with disease features
955 and severity. J Allergy Clin Immunol 136:874-884
956 Humbert M, Corrigan CJ, Kimmitt P, Till SJ, BARRY KAY A, Durham SR (1997)
957 Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic
958 asthma. Am J Respir Crit Care Med 156:704-708
959 Huo X et al. (2016) Decreased epithelial and plasma miR-181b-5p expression associates with
960 airway eosinophilic inflammation in asthma. Clin Exp Allergy 46:1281-1290
961 doi:10.1111/cea.12754
962 Hussein MH, Toraih EA, Aly NM, Riad E, Fawzy MS (2016) A passenger strand variant in
963 miR-196a2 contributes to asthma severity in children and adolescents: A preliminary
964 study. Biochem Cell Biol 94:347-357 doi:10.1139/bcb-2016-0010
965 Hutchings H, Ortega N, Plouët J (2003) Extracellular matrix-bound vascular endothelial
966 growth factor promotes endothelial cell adhesion, migration, and survival through
967 integrin ligation. FASEB J 17:1520-1522
968 Ichinose M, Barnes PJ (1989) Inhibitory histamine H3-receptors on cholinergic nerves in
969 human airways. Eur J Pharmacol 163:383-386
970 Ishizaka K, Ishizaka T (1967) Identification of γE-antibodies as a carrier of reaginic activity.
971 J Immunol 99:1187-1198
972 Janson C et al. (2001) The European Community Respiratory Health Survey: what are the
973 main results so far? Eur Respir J 18:598-611
974 Jayan SC, Sandeep A, Rifash M, Mareema C, Shamseera S (2009) Design and in-vitro
975 evaluation of gelatin microspheres of salbutamol sulphate. Hygeia 1:17-20
976 Kaiko GE, Horvat JC, Beagley KW, Hansbro PM (2008a) Immunological decision-making:
977 how does the immune system decide to mount a helper T-cell response? Immunology
978 123:326-338
979 Kaiko GE, Phipps S, Hickey DK, Lam CE, Hansbro PM, Foster PS, Beagley KW (2008b)
980 Chlamydia muridarum infection subverts dendritic cell function to promote Th2
981 immunity and airways hyperreactivity. J Immunol 180:2225-2232
982 Kanazawa H, Nomura S, Asai K (2007) Roles of angiopoietin-1 and angiopoietin-2 on
983 airway microvascular permeability in asthmatic patients. Chest 131:1035-1041
984 Kanazawa H, Nomura S, Yoshikawa J (2004) Role of microvascular permeability on
985 physiologic differences in asthma and eosinophilic bronchitis. Am J Respir Crit Care
986 Med 169:1125-1130
987 Karish SB, Gagnon JM (2006) The potential role of roflumilast: the new phosphodiesterase-4
988 inhibitor. Ann Pharmacother 40:1096-1104

Kaur R, Chupp G (2019) Phenotypes and endotypes of adult asthma: Moving toward precision medicine. J Allergy Clin Immunol 144:1-12
Kawauchi H, Yanai K, Wang D-Y, Itahashi K, Okubo K (2019) Antihistamines for allergic rhinitis treatment from the viewpoint of nonsedative properties. Int J Mol Sci 20:213
Kay LJ, Suvarna SK, Peachell PT (2018) Histamine H4 receptor mediates chemotaxis of human lung mast cells. Eur J Pharmacol 837:38-44
Khokhlovich E et al. (2017) Late Breaking Abstract-The biological pathways underlying response to anti-IL-17A (AIN457; secukinumab) therapy differ across severe asthmatic patients. Eur Respiratory Soc,
Kim J-H (2017) Serum vascular endothelial growth factor as a marker of asthma exacerbation. Korean J Intern Med 32:258-260
Kim RY et al. (2017a) MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase–mediated suppression of histone deacetylase 2. J Allergy Clin Immunol 139:519-532
Kim RY et al. (2017b) Role for NLRP3 inflammasome–mediated, IL-1β–dependent responses in severe, steroid-resistant asthma. Am J Respir Crit Care Med 196:283-297
Kim RY, Pinkerton JW, Gibson PG, Cooper MA, Horvat JC, Hansbro PM (2015) Inflammasomes in COPD and neutrophilic asthma. Thorax 70:1199-1201
Konduri KS, Nandedkar S, Düzgünes N, Suzara V, Artwohl J, Bunte R, Gangadharam PR (2003) Efficacy of liposomal budesonide in experimental asthma. J Allergy Clin Immunol 111:321-327
Korenblat P et al. (2018) Efficacy and safety of lebrikizumab in adult patients with mild-to-moderate asthma not receiving inhaled corticosteroids. Respir Med 134:143-149
Kotsimbos TC, Ernst P, Hamid QA (1996) Interleukin-13 and interleukin-4 are coexpressed in atopic asthma. Proc Assoc Am Physicians 108:368-373
Kraft M, Cassell GH, Pak J, Martin RJ (2002) Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest 121:1782-1788
Krishnan V, Diette GB, Rand CS, Bilderback AL, Merriman B, Hansel NN, Krishnan JA (2006) Mortality in patients hospitalized for asthma exacerbations in the United States. Am J Respir Crit Care Med 174:633-638
Kuruvilla ME, Lee FE-H, Lee GB (2019) Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clin Rev Allergy Immunol 56:219-233 doi:10.1007/s12016-018-8712-1
Langley RG et al. (2014) Secukinumab in plaque psoriasis—results of two phase 3 trials. N Engl J Med 371:326-338
Lanza GM et al. (2017) Anti-angiogenic nanotherapy inhibits airway remodeling and hyper-responsiveness of dust mite triggered asthma in the Brown Norway rat. Theranostics 7:377-389 doi:10.7150/thno.16627
Larché M, Robinson DS, Kay AB (2003) The role of T lymphocytes in the pathogenesis of asthma. J Allergy Clin Immunol 111:450-463
Lau JY et al. (2010) Fibulin-1 is increased in asthma–a novel mediator of airway remodeling? PLoS ONE 5:e13360
Leckie MJ et al. (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 356:2144-2148
Lee SY et al. (2006) Immunostimulatory DNA inhibits allergen-induced peribronchial angiogenesis in mice. J Allergy Clin Immunol 117:597-603
Lee YC, Kwak Y-G, Song CH (2002) Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. J Immunol 168:3595-3600

Liang L, Hur J, Kang JY, Rhee CK, Kim YK, Lee SY (2018) Effect of the anti-IL-17 antibody on allergic inflammation in an obesity-related asthma model. The Korean journal of internal medicine 33:1210

Liu G et al. (2016) Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases. J Clin Invest Insight 1:e86380

Liu G et al. (2017) Airway remodelling and inflammation in asthma are dependent on the extracellular matrix protein fibulin-1c. J Pathol 243:510-523

Liu Y-CC, Post JC (2009) Biofilms in pediatric respiratory and related infections. Curr Allergy Asthma Rep 9:449-455

Londei M, Kenney B, Los G, Marino MH (2017) A Phase 1 Study of ANB020, an anti-IL-33 monoclonal Antibody in Healthy Volunteers. J Allergy Clin Immunol 139:AB73

Long CM, Lukomska E, Marshall NB, Nayak A, Anderson SE (2016) Potential Inhibitory Influence of miRNA 210 on Regulatory T Cells during Epicutaneous Chemical Sensitization. Genes 8:1-9

Lötvall J et al. (2011) Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 127:355-360

Louw C, Williams Z, Venter L, Leichtl S, Schmid-Wirlitsch C, Bredenbröker D, Bardin P (2007) Roflumilast, a phosphodiesterase 4 inhibitor, reduces airway hyperresponsiveness after allergen challenge. Respiration 74:411-417

Lovato P, Norsgaard H, Tokura Y, Røpke MA (2016) Calcipotriol and betamethasone dipropionate exert additive inhibitory effects on the cytokine expression of inflammatory dendritic cell–Th17 cell axis in psoriasis. J Dermatol Sci 81:153-164

Luo J, Wang K, Liu D, Liang B-M, Liu C-T (2016) Can roflumilast, a phosphodiesterase-4 inhibitor, improve clinical outcomes in patients with moderate-to-severe chronic obstructive pulmonary disease? A meta-analysis. Respir Res 17:18

Maret M et al. (2007) Liposomal retinoic acids modulate asthma manifestations in mice. J Nutr 137:2730-2736

Matsukura S et al. (2016) Overexpression of microRNA-155 suppresses chemokine expression induced by Interleukin-13 in BEAS-2B human bronchial epithelial cells. Allergol Int 65 Suppl:S17-23 doi:10.1016/j.alit.2016.04.018

Mauser PJ et al. (1995) Effects of an antibody to interleukin-5 in a monkey model of asthma. Am J Respir Crit Care Med 152:467-472

McInnes IB et al. (2015) Secukinumab, a human anti-interleukin-17A monoclonal antibody, in patients with psoriatic arthritis (FUTURE 2): a randomised, double-blind, placebo-controlled, phase 3 trial. The Lancet 386:1137-1146

Mehta M, Chellappan DK, Wich PR, Hansbro NG, Hansbro PM, Dua K (2020a) miRNA nanotherapeutics: potential and challenges in respiratory disorders. Future Med Chem 12:987-990

Mehta M et al. (2020b) Cellular signalling pathways mediating the pathogenesis of chronic inflammatory respiratory diseases: an update. Inflammopharmacology:1-23

Meyer N, Akdis CA (2013) Vascular endothelial growth factor as a key inducer of angiogenesis in the asthmatic airways. Curr Allergy Asthma Rep 13:1-9

Miossec P, Korn T, Kuchroo VK (2009) Interleukin-17 and type 17 helper T cells. N Engl J Med 361:888-898

Mitchell PD, O'byrne PM (2017) Epithelial-derived cytokines in asthma. Chest 151:1338-1344

Mizutani N, Nabe T, Yoshino S (2014) IL-17A promotes the exacerbation of IL-33–induced airway hyperresponsiveness by enhancing neutrophilic inflammation via CXCR2 signaling in mice. The Journal of Immunology 192:1372-1384

Müller T et al. (2006) Functional characterization of histamine receptor subtypes in a human bronchial epithelial cell line. Int J Mol Med 18:925-931
Murad HA, Habib HS, Rafeeq MM, Sulaiman MI, Abdulrahman AS, Khabaz MN (2017) Co-inhalation of roflumilast, rather than formoterol, with fluticasone more effectively improves asthma in asthmatic mice. Exp Biol Med 242:516-526
Nadeem A, Al-Harbi NO, Alfardan AS, Ahmad SF, AlAsmari AF, Al-Harbi MM (2018) IL-17A-induced neutrophilic airway inflammation is mediated by oxidant-antioxidant imbalance and inflammatory cytokines in mice. Biomedicine & Pharmacotherapy 107:1196-1204
Nair P et al. (2009) Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 360:985-993
Nakae S et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 17:375-387
Narayanan V, Baglole C, Eidelman DH, Hussain S, Martin JG, Hamid Q, Panariti A (2016) Increased Vascularity Of The Bronchial Mucosa In Patients With Severe Asthma And The Role Of IL-17A In Angiogenesis And Vascular Remodeling. In: Novel Mechanisms of Allergy and Airway Inflammation. American Thoracic Society, p A6680
Nasr M, Najlah M, D'Emanuele A, Elhissi A (2014) PAMAM dendrimers as aerosol drug nanocarriers for pulmonary delivery via nebulization. Int J Pharm 461:242-250
Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M (2003) Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. Am J Respir Crit Care Med 168:983-988
Nixon J, Newbold P, Mustelin T, Anderson GP, Kolbeck R (2017) Monoclonal antibody therapy for the treatment of asthma and chronic obstructive pulmonary disease with eosinophilic inflammation. Pharmacol Ther 169:57-77
Novak N, Tepel C, Koch S, Brix K (2003) Evidence for a differential expression of the FcεRIγ chain in dendritic cells of atopic and nonatopic donors. J Clin Invest 111:1047-1056
Odajima H et al. (2017) Long-term safety, efficacy, pharmacokinetics and pharmacodynamics of omalizumab in children with severe uncontrolled asthma. Allergol Int 66:106-115
Okubo K et al. (2020) Japanese guidelines for allergic rhinitis 2020. Allergol Int 69:331-345
Pachuau L, Sarkar S, Mazumder B (2008) Formulation and evaluation of matrix microspheres for simultaneous delivery of salbutamol sulphate and theophylline. Trop J Pharm Res 7:995-1002
Page C, O'Shaughnessy B, Barnes P (2017) Pathogenesis of COPD and Asthma. Pharmacol Ther:1-21
Panchal R, Patel H, Patel V, Joshi P, Parikh A (2012) Formulation and evalution of montelukast sodium-chitosan based spray dried microspheres for pulmonary drug delivery. J Pharm Bioallied Sci 4:S110-S111
Panettieri RA et al. (2018) Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. Lancet Respir Med 6:511-525
Patel DD, Patel VN, Thakkar TV, Gandhi RT (2012) Preparation and evaluation of Levosalbutamol sulphate chitosan microsphere for the treatment of asthma. J Pharm Bioallied Sci 4:S46-S47
Pavord ID et al. (2012) Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 380:651-659

1138 Pelaia G, Renda T, Romeo P, Busceti MT, Maselli R (2008) Review: Omalizumab in the
1139 treatment of severe asthma: efficacy and current problems. Ther Adv Respir Dis
1140 2:409-421
1141 Pelaia G, Vatrella A, Maselli R (2012) The potential of biologics for the treatment of asthma.
1142 Nat Rev Drug Discov 11:958-972
1143 Peng X et al. (2015) Targeting Mast Cells and Basophils with Anti-FcεRIα Fab-Conjugated
1144 Celastrol-Loaded Micelles Suppresses Allergic Inflammation. J Biomed Nanotechnol
1145 11:2286-2299
1146 Piper E et al. (2013) A phase II placebo-controlled study of tralokinumab in moderate-to-
1147 severe asthma. Eur Respir J 41:330-338
1148 Plank MW, Maltby S, Tay HL, Stewart J, Eyers F, Hansbro PM, Foster PS (2015)
1149 MicroRNA expression is altered in an ovalbumin-induced asthma model and targeting
1150 miR-155 with antagomirs reveals cellular specificity. PLoS ONE 10:e0144810
1151 Polosa R, Thomson NC (2013) Smoking and asthma: dangerous liaisons. Eur Respir J
1152 41:716-726 doi:10.1183/09031936.00073312
1153 Prasher P et al. (2020) Plants derived therapeutic strategies targeting chronic respiratory
1154 diseases: Chemical and immunological perspective. Chem Biol Interact:109125
1155 Presta LG, Lahr S, Shields R, Porter J, Gorman C, Fendly B, Jardieu P (1993) Humanization
1156 of an antibody directed against IgE. J Immunol 151:2623-2632
1157 Qureshi J, Amir M, Ahuja A, Baboota S, Ali J (2008) Chronomodulated drug delivery system
1158 of salbutamol sulphate for the treatment of nocturnal asthma. Indian J Pharm Sci
1159 70:351-356
1160 Rabe KF et al. (2018) Efficacy and safety of dupilumab in glucocorticoid-dependent severe
1161 asthma. N Engl J Med 378:2475-2485 doi:10.1056/NEJMoa1804093
1162 Raesch SS et al. (2015) Proteomic and lipidomic analysis of nanoparticle corona upon contact
1163 with lung surfactant reveals differences in protein, but not lipid composition. ACS
1164 Nano 9:11872-11885
1165 Romagnani S (2000) The role of lymphocytes in allergic disease. J Allergy Clin Immunol
1166 105:399-408
1167 Salomonsson M, Malinovschi A, Kalm-Stephens P, Dahlin JS, Janson C, Alving K, Hallgren
1168 J (2019) Circulating mast cell progenitors correlate with reduced lung function in
1169 allergic asthma. Clinical & Experimental Allergy 49:874-882
1170 Salvato G (2001) Quantitative and morphological analysis of the vascular bed in bronchial
1171 biopsy specimens from asthmatic and non-asthmatic subjects. Thorax 56:902-906
1172 Saraf S, Jain A, Hurkat P, Jain SK (2016) Topotecan Liposomes: A Visit from a Molecular to
1173 a Therapeutic Platform. Crit Rev Ther Drug Carrier Syst 33:401-432
1174 Scheerens H et al. (2014) The effects of lebrikizumab in patients with mild asthma following
1175 whole lung allergen challenge. Clin Exp Allergy 44:38-46
1176 Schipf A, Heilmann A, Boue L, Mossmann H, Brocker T, Röcken M (2003) Th2 cells shape
1177 the differentiation of developing T cell responses during interactions with dendritic
1178 cells in vivo. Eur J Immunol 33:1697-1706
1179 Shah A, Church M, Holgate S (1995) Tumour necrosis factor alpha: a potential mediator of
1180 asthma. Clinical and experimental allergy (Print) 25:1038-1044
1181 Shahabi S, Treccani L, Dringen R, Rezwan K (2015) Utilizing the protein corona around
1182 silica nanoparticles for dual drug loading and release. Nanoscale 7:16251-16265
1183 Sharma A, Kumar M, Ahmad T, Mabalirajan U, Aich J, Agrawal A, Ghosh B (2012)
1184 Antagonism of mmu-mir-106a attenuates asthma features in allergic murine model. J
1185 Appl Physiol 113:459-464 doi:10.1152/japplphysiol.00001.2012

Shastri MD, Peterson GM, Stewart N, Sohal SS, Patel RP (2014) Non-anticoagulant derivatives of heparin for the management of asthma: distant dream or close reality? Expert Opin Investig Drugs 23:357-373
Shastri MD et al. (2015a) Opposing effects of low molecular weight heparins on the release of inflammatory cytokines from peripheral blood mononuclear cells of asthmatics. PLoS ONE 10:e0118798
Shastri MD et al. (2015b) Non-anticoagulant fractions of enoxaparin suppress inflammatory cytokine release from peripheral blood mononuclear cells of allergic asthmatic individuals. PLoS ONE 10:e0128803
Shields R et al. (1995) Inhibition of allergic reactions with antibodies to IgE. Int Arch Allergy Immunol 107:308-312
Shukla SD et al. (2019) Microbiome-focused asthma management strategies. Curr Opin Pharmacol 46:143-149
Silva R, D'Amico G, Hodivala-Dilke KM, Reynolds LE (2008) Integrins The keys to unlocking angiogenesis. Arterioscler Thromb Vasc Biol 28:1703-1713
Simpson JL, Scott R, Boyle MJ, Gibson PG (2006) Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology 11:54-61
Singh D, Kane B, Molfino NA, Faggioni R, Roskos L, Woodcock A (2010) A phase 1 study evaluating the pharmacokinetics, safety and tolerability of repeat dosing with a human IL-13 antibody (CAT-354) in subjects with asthma. BMC Pulm Med 10:1-8
Spahn JD, Fost DA, Covar R, Martin RJ, Brown EE, Szefler SJ, Leung DY (2001) Clarithromycin potentiates glucocorticoid responsiveness in patients with asthma: results of a pilot study. Ann Allergy, Asthma Immunol 87:501-505
Starkey M et al. (2013a) Constitutive production of IL-13 promotes early-life Chlamydia respiratory infection and allergic airway disease. Mucosal Immunol 6:569-579
Starkey MR et al. (2013b) Murine models of infectious exacerbations of airway inflammation. Curr Opin Pharmacol 13:337-344
Starkey MR, McKenzie ANJ, Belz GT, Hansbro PM (2019) Pulmonary group 2 innate lymphoid cells: Surprises and challenges. Mucosal Immunol 12:299-311
Stirling RG, Van Rensen EL, Barnes PJ, Fan Chung K (2001) Interleukin-5 induces CD34+ eosinophil progenitor mobilization and eosinophil CCR3 expression in asthma. Am J Respir Crit Care Med 164:1403-1409
Suzaki Y et al. (2005) A potent antiangiogenic factor, endostatin prevents the development of asthma in a murine model. J Allergy Clin Immunol 116:1220-1227
Szefler S, Kattan M, Ortiz B, Trzaskoma B, Haselkorn T, Iqbal A, Busse W (2018) Greater Treatment Benefit with Omalizumab in Children with Increased Asthma Severity: Exploratory Analyses from the Preventative Omalizumab or Step-up Therapy for Fall Exacerbations (PROSE) Study. In: Pediatric Severe Asthma and Phenotyping. American Thoracic Society, pp A1157-A1157
Tahara K et al. (2016) Pulmonary liposomal formulations encapsulated procaterol hydrochloride by a remote loading method achieve sustained release and extended pharmacological effects. Int J Pharm 505:139-146
Tanaka H et al. (2004) Role of interleukin-5 and eosinophils in allergen-induced airway remodeling in mice. Am J Respir Cell Mol Biol 31:62-68
Tang GN et al. (2016) MicroRNAs Involved in Asthma After Mesenchymal Stem Cells Treatment. Stem Cells Dev 25:883-896 doi:10.1089/scd.2015.0339
Thangam EB et al. (2018) The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. Frontiers in immunology 9:1873

Tomkinson A et al. (2001) A murine IL-4 receptor antagonist that inhibits IL-4-and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness. J Immunol 166:5792-5800

Tucker A, Weir EK, Reeves J, Grover R (1975) Histamine H1-and H2-receptors in pulmonary and systemic vasculature of the dog. American Journal of Physiology-Legacy Content 229:1008-1013

Vazquez-Tello A, Halwani R, Hamid Q, Al-Muhsen S (2013) Glucocorticoid receptor-beta up-regulation and steroid resistance induction by IL-17 and IL-23 cytokine stimulation in peripheral mononuclear cells. J Clin Immunol 33:466-478

Vazquez-Tello A, Semlali A, Chakir J, Martin J, Leung D, Eidelman D, Hamid Q (2010) Induction of glucocorticoid receptor-β expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines. Clinical & Experimental Allergy 40:1312-1322

Wakashin H et al. (2008) IL-23 and Th17 cells enhance Th2-cell–mediated eosinophilic airway inflammation in mice. Am J Respir Crit Care Med 178:1023-1032

Wang SY et al. (2017) The lncRNAs involved in mouse airway allergic inflammation following induced pluripotent stem cell-mesenchymal stem cell treatment. Stem Cell Res Ther 8:2 doi:10.1186/s13287-016-0456-3

Wang Y, Mao G, Lv Y, Huang Q, Wang G (2015) MicroRNA-181b stimulates inflammation via the nuclear factor-kappaB signaling pathway in vitro. Exp Ther Med 10:1584-1590 doi:10.3892/etm.2015.2702

Wark P, Nichol K, Dorahy D, Collison A, Mattes J (2018) The Effect of Treatment with Omalizumab on Anti-Viral Responses in Adults with Severe Allergic Asthma. In: Immunotherapy In Lung Disease. American Thoracic Society, pp A6166-A6166

Wenzel S et al. (2016) Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. Lancet 388:31-44

Wenzel SE (2012) Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 18:716-725

Wenzel SE (2013) Complex phenotypes in asthma: current definitions. Pulm Pharmacol Ther 26:710-715

Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, Chu HW (1999) Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 160:1001-1008

Wisnivesky JP et al. (2017) Impact Of Exacerbations On Lung Function In Patients With Asthma. In: Improving Asthma Management: Research At The Forefront. American Thoracic Society, pp A2632-A2632

Xepapadaki P, Koutsoumpari I, Papaevagelou V, Karagianni C, Papadopoulos NG (2008) Atypical bacteria and macrolides in asthma. Allergy, Asthma & Clinical Immunology 4:1-6

Xiang Y, Eyers F, Herbert C, Tay HL, Foster PS, Yang M (2016) MicroRNA-487b is a negative regulator of macrophage activation by targeting IL-33 production. J Immunol 196:3421-3428

Yamauchi K, Ogasawara M (2019) The role of histamine in the pathophysiology of asthma and the clinical efficacy of antihistamines in asthma therapy. Int J Mol Sci 20:1733

Yamauchi K et al. (1994) Structure and function of human histamine N-methyltransferase: critical enzyme in histamine metabolism in airway. American Journal of Physiology-Lung Cellular and Molecular Physiology 267:L342-L349

Yang M, Kumar RK, Hansbro PM, Foster PS (2012) Emerging roles of pulmonary macrophages in driving the development of severe asthma. J Leukoc Biol 91:557-569

Yhee JY, Im J, Nho RS (2016) Advanced therapeutic strategies for chronic lung disease using nanoparticle-based drug delivery. J Clin Med 5:1-13

Yildiz-Pekoz A, Ozsoy Y (2017) Inhaled Heparin: Therapeutic Efficacy and Recent Formulations. J Aerosol Med Pulm Drug Deliv 30:143-156

Yoo SY, Kwon SM (2013) Angiogenesis and its therapeutic opportunities. Mediators Inflamm 2013:1-11

Yuksel H et al. (2013) Role of vascular endothelial growth factor antagonism on airway remodeling in asthma. Ann Allergy, Asthma Immunol 110:150-155

Zanini A, Chetta A, Imperatori AS, Spanevello A, Olivieri D (2010) The role of the bronchial microvasculature in the airway remodelling in asthma and COPD. Respir Res 11:1-11

Zhang XY et al. (2016) LncRNAs BCYRN1 promoted the proliferation and migration of rat airway smooth muscle cells in asthma via upregulating the expression of transient receptor potential 1. Am J Transl Res 8:3409-3418

Zhou C-Y, Crocker IC, Koenig G, Romero FA, Townley RG (1997) Anti-interleukin-4 inhibits immunoglobulin E production in a murine model of atopic asthma. J Asthma 34:195-201

Zhou H, Li J, Gao P, Wang Q, Zhang J (2016) miR-155: A Novel Target in Allergic Asthma. Int J Mol Sci 17:1-11 doi:10.3390/ijms17101773

Zock J-P et al. (2006) Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 118:682-690