Novel Drug Targets for Asthma and COPD: Lessons Learned from in vitro and in vivo Models

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Abstract

Asthma and chronic obstructive pulmonary disease (COPD) are highly prevalent respiratory diseases characterized by airway inflammation, airway obstruction and airway hyperresponsiveness. Whilst current therapies, such as β-agonists and glucocorticoids, may be effective at reducing symptoms, they do not reduce disease progression. Thus, there is a need to identify new therapeutic targets. In this review, we summarize the potential of novel targets or tools, including anti-inflammatories, phosphodiesterase inhibitors, kinase inhibitors, transient receptor potential channels, vitamin D and protease inhibitors, for the treatment of asthma and COPD.
Introduction

Obstructive airway diseases, including asthma and chronic obstructive pulmonary disease (COPD), represent a major health problem worldwide. According to the World Health Organization (WHO), asthma is the most common chronic disease among children (WHO 2013), and it is predicted that COPD will become the third leading cause of death worldwide by 2030 (Mannino and Buist 2007; Miles et al. 2012).

The common pathophysiological components of asthma and COPD are airway inflammation, airway obstruction and airway hyperresponsiveness (AHR); the differences are mainly related to the cellular and molecular features of inflammation and the reversibility of airflow obstruction. Airway inflammation in allergic asthma is usually eosinophilic, whereas COPD is associated with predominantly neutrophilic inflammation. However, some non-allergic endotypes of asthma, such as exercise-induced, aspirin-sensitive and infection-induced asthma, may present with little to no eosinophilic inflammation. Severe, steroid-resistant and obesity related asthma endotypes are frequently associated with a neutrophilic inflammatory profile [reviewed in (Lötvall et al. 2011)]. Physiologically, asthma is typically characterized by reversible AHR, whereas COPD is characterized by airflow limitation that is not fully reversible and is usually progressive.

Whilst current asthma therapies, namely β-agonists and glucocorticoids, reduce airway inflammation, reverse bronchoconstriction and improve quality of life in the majority of patients, these treatments have little or no effect on the structural alterations associated with the disease. Furthermore, there remains a considerable population of people for whom these treatments are ineffective and thus their asthma is poorly controlled. Similarly, current treatments for COPD have limited efficacy in terms of inhibiting chronic inflammation and do not reverse the disease process or prevent its progression. Hence, there is a clear need to increase our understanding of disease processes, identify novel targets and develop new therapies. In this review, we provide an update on existing treatments and highlight novel emerging targets and treatments for asthma and COPD.

Novel Anti-Inflammatory Drugs for the Treatment of Allergic Asthma

Anti-inflammatory therapies for allergic asthma were introduced in the mid-20th century, and inhaled corticosteroids have been the primary therapy for asthma for the past 35 years (Chu and Drazen 2005). In recent decades, research has focused on more specific targeting of the asthmatic inflammatory response, with the advent of anti-leukotriene and anti-IgE drugs. Ongoing studies in this area have provided promising results with regard to additional anti-inflammatory interventions.
Anti-cytokine therapies

The inflammatory response to respiratory allergen exposure is characterized by eosinophilic infiltration of the airways driven by increased expression of Th2 cytokines [interleukin (IL)-4, IL-5, IL-13 and in more severe cases IL-17 and IL-22] (Chu and Drazen 2005). In the lung, eosinophils release a potent combination of inflammatory mediators, cytokines, chemokines and basic proteins that are thought to not only exacerbate the inflammatory response to allergen, but also contribute to the structural changes to the airway wall known as airway remodeling. These processes in combination lead to the lung dysfunction that defines allergic asthma (Efraim and Levi-Schaffer 2008). It is well-established that IL-5 promotes the maturation and release of eosinophils from the bone marrow, as well as the secretion of mediators and survival in the tissue (Garcia et al. 2013). Although early efforts to target IL-5 met with only limited success (Kips et al. 2003; O’Byrne 2007; Garcia et al. 2013), more recent clinical trials on the anti-IL-5 antibody mesolizumab showed a positive effect of this intervention, as mesolizumab treatment significantly reduced asthma exacerbations in patients with severe eosinophilic asthma (Nair et al. 2009; Pavord et al. 2012).

Other Th2 cytokines have recently been targeted as novel anti-inflammatory therapies for allergic asthma. IL-4 and IL-13 are typical Th2 cytokines that play important roles in the B cell switch to IgE synthesis, eosinophil accumulation in the lung, goblet cell hyperplasia and enhanced airway smooth muscle (ASM) contractility (Walsh 2013), and are therefore promising targets for therapeutic intervention. Dupilumab (SAR231893/REGN668) is a human monoclonal antibody targeting the IL-4Rα/IL-13Rα receptor complex; a randomized, double-blind, placebo-controlled, parallel-group phase 2A study was recently completed to assess the impact of this therapy in moderate-to-severe asthmatics. Subcutaneous injections of dupilumab for 12 weeks led to significant improvements in asthma control and lung function, as well as a reduction in Th2 inflammatory markers (Wenzel et al. 2013). Targeting IL-13 signaling alone, however, may be less promising. An early study using lebrikizumab, a humanized anti-IL-13 antibody, showed significantly improved lung function in patients with high pretreatment levels of serum periostin (Zeskind 2011), which is a systemic indicator of Th2/IL-13 activity and airway eosinophilia in asthmatics (Jia et al. 2012). However, a follow-up study did not find significant improvements in lung function in treated asthmatics compared to controls, even when patients were stratified according to serum periostin levels (Noonan et al. 2013). This lack of consensus may have been due to a briefer treatment period (12 weeks vs 6 months of treatment) and continued corticosteroid use by patients in the latter study. Further investigations are certainly warranted to clarify the role of anti-inflammatory therapies for patients with the allergic endotypes of asthma.
**Statins**

Statins are a family of 3 hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors that have been used clinically as cholesterol-lowering drugs; however, recent evidence from *in vitro* studies (Wang et al. 2011b; Zeki et al. 2012) and in mouse models of allergic airway disease (Huang et al. 2013a) has demonstrated that these compounds also have potent anti-inflammatory effects. It has been suggested that the addition of a statin to maintenance corticosteroid therapy in asthmatics might be beneficial, but human studies have provided mixed results (Walker and Edwards 2013). Encouragingly, recently reported clinical trials focusing on severe asthmatics showed that the addition of a statin to standard inhaler controller therapy led to improved asthma symptoms, fewer exacerbations and reduced corticosteroid use (Zeki et al. 2013; Tse et al. 2013). Further investigations into the mechanism of action of the corticosteroid/statin combination revealed that this treatment increases the induction of T regulatory cells in asthmatics, suggesting an enhanced ability to suppress airway inflammation in these patients (Maneechotesuwan et al. 2013).

**Macrolides**

Macrolides represent another drug class with a long history of use that has only recently been explored as a novel asthma therapy. Macrolides are antibiotics used to treat infections caused by Gram-positive bacteria and act by inhibiting protein synthesis in target microorganisms. When used in a mouse model of allergic airways disease, the macrolide azithromycin attenuated expression of IL-13 and IL-5 and reduced mucus production in the lung (Beigelman et al. 2009). This success has been translated to the clinic with the publication of a number of clinical trials showing that short-term (three weeks) macrolide therapy improved some aspects of lung function, symptoms and quality of life in asthmatic patients (Reiter et al. 2013). When given for a longer period of time along with corticosteroids, azithromycin treatment did not lead to a significant improvement in lung function in the entire cohort, but patients with non-eosinophilic asthma saw better outcomes with this therapy, suggesting that macrolides may be of interest in treating this particular asthma phenotype (Brusselle et al. 2013).

**Novel Anti-Inflammatory Drugs for the Treatment of COPD**

Inflammation in COPD is characteristically distinct from other lung diseases and is less responsive to inhaled glucocorticoids compared to asthma. In acute exacerbations of COPD (AECOPD), which can be triggered by bacterial or viral infections, lung inflammation is dramatically increased (Bathoorn et al. 2008). It is crucial to understand the underlying contributions of different cell types, for example increased neutrophils and macrophages, and how these contribute to complicated cytokine and chemokine profiles seen in patients
with COPD and commonly occurring AECOPD. Given the complexity of the inflammatory response in COPD, there have been two main approaches to improving patient quality of life. The first is to adopt a broad anti-inflammatory approach and the second is to specifically target individual components of the inflammatory cascade which differ in specific COPD patients. Patient biopsies and sputum samples have identified increased numbers of many inflammatory cells, including macrophages, neutrophils, T and B cells. Activation of these cell types contributes to increased levels of cytokines and chemokines which are detected in the sputum, bronchoalveolar lavage fluid (BALF) and systemically in blood samples; these include IL-1α and IL-1β (Botelho et al. 2011), tumor necrosis factor (TNF)α (Keatings et al. 1996), IL-5 (Schild et al. 2011) and (Molfino et al. 2012), IL-6 (Agustí et al. 2012) and (Celli et al. 2012), IL-8 (Keatings et al. 1996), IL-17 (Zhang et al. 2013), IL-18 (Kawayama et al. 2012) and granulocyte macrophage-colony stimulating factor (GM-CSF) (Saha et al. 2009). In addition to these cytokines and chemokines, there is reduced expression of histone deacetylase 2 (HDAC2), which in normal patients maintains steroid sensitivity and in COPD patients is believed to be a key component in steroid insensitivity (Ito et al. 2005). There is also evidence of increased expression of epidermal growth factor receptors (Anagnostis et al. 2013) and leukotriene B4 (LTB4) (Keatings et al. 1996). Given these diverse factors and their potential complex interplay, it is perhaps not surprising that it is difficult to achieve a therapeutic benefit regarding inflammation in COPD.

Cigarette smoke is a major contributor to COPD and has been shown to skew the immune response in patients with COPD [reviewed in (Stämpfli and Anderson 2009)]. Cigarette smoke exposure in animals, primarily rodents, can cause inflammation directly and/or indirectly by inducing oxidative stress [reviewed in (Vlahos and Bozinovski 2014)]. Short-term exposure models induce glucocorticoid-resistant inflammation whereas long-term models induce emphysematous changes. It is worth noting that minor changes in the smoke exposure protocol can influence the outcome and may coincide with the heterogeneity of inflammation in COPD. In the following sections, we review IL-8 and IL-1β inhibitors as potential novel anti-inflammatory treatments for COPD.

**Interleukin-8 antibodies – from promising pre-clinical assessments to failed clinical trials**

IL-8 is a chemotactic mediator involved in recruiting neutrophils to the lung. Concentrations of IL-8 are increased in both sputum and BALF from COPD patients (Keatings et al. 1996). The development of a monoclonal antibody recognizing IL-8 was characterized in vitro in human neutrophils by blocking IL-8 binding (Yang et al. 1999). This was also confirmed in an in vivo model of IL-8 driven skin inflammation in rabbits, whereby administration of the IL-8 antibody decreased neutrophil recruitment and subsequent inflammation (Yang et al. 1999). Despite promising pre-clinical evidence, a phase 2 clinical trial assessing the
effects of the monoclonal antibody recognizing IL-8 (ABX-IL8) showed very limited clinical improvements in patients symptoms with COPD (Mahler et al. 2004).

**Interleukin 1α antibodies – promising pre-clinical evidence**

In some patients, IL-1α and IL-1β are increased in sputum and BAL samples. Pre-clinical evidence using cigarette smoke exposed mouse models has shown a critical role of IL-1α in the initiation of neutrophilic inflammation and that signaling through IL-1R1 is a key driver in the production of other pro-inflammatory cytokines such as TNFα and matrix metalloproteinase-9 (MMP-9) (Botelho et al. 2011). In IL-1α knockout mice and in naïve mice treated with short-term cigarette smoke and an IL-1α antibody, this neutrophilic inflammation was decreased. This is promising pre-clinical evidence; however it is crucial that clinical trials involving these IL-1α targeting antibodies are given to patients with increased levels of IL-1α.

Given the complexity and heterogeneity of inflammation in COPD patients, and the relatively limited success with broad spectrum anti-inflammatory agents, the overarching goal for treating this underlying inflammation is to endotype/phenotype patients to ensure that anti-inflammatory drugs will specifically target patient needs.

**G-Protein-Coupled Receptors in Asthma and COPD**

Currently, β₂-adrenoreceptor agonists and muscarinic receptor antagonists are the two main types of bronchodilators that provide effective symptomatic relief for the treatment of airway constriction (Meurs et al. 2013). These receptors are members of the G-protein coupled receptor (GPCR) family. GPCRs are a large family of cell surface receptors, characterized by the presence of seven membrane-spanning peptide chains. Agonist binding induces a conformational change and subsequently dissociation of the α subunit from the βγ dimer. The modulation of downstream effector proteins is dependent on the alpha subunit type: Gₐs, Gₐi, Gₐq or Gₐ12/13. Upon activation, Gₛ-protein coupled β₂-receptors activate the enzyme adenylyl cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). This in turn activates protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac) (Schmidt et al. 2013). Via this signaling cascade, several intracellular proteins become phosphorylated, leading to the relaxation of ASM, increased mucociliary clearance, decreased microvascular leakage and inhibition of mediator release from mast cells and basophils (Nelson 1995). When the Gₛ-coupled muscarinic M3 receptor (M3R) is triggered, phospholipase C (PLC) is subsequently activated. PLC can release inositol 1,4,5-triphosphate (IP₃) and thereby increase the levels of intracellular calcium, as shown in Figure 1. Thus, by antagonizing M3R, the contraction of the ASM is prevented.
Figure 1. Muscarinic M3 receptor – β2-adrenoceptor cross-talk in airway smooth muscle. Activation of muscarinic M3-receptors by acetylcholine causes protein kinase C (PKC)-mediated β2-adrenoceptor uncoupling, thereby attenuating β-agonist-induced relaxation of airway smooth muscle, presumably by phosphorylation of the third intracellular loop of the receptor. AC = adenyllylcyclase, IP3 = inositol 1,4,5-trisphosphate, PLC = phospholipase C.

Therefore, it is not surprising that β2-adrenoreceptor agonists and muscarinic receptor antagonists are the mainstay therapies for COPD patients. In asthma, however, muscarinic receptor antagonists are less effective and therapeutic management relies mainly on treatment with β2-adrenoreceptor agonists in combination with inhaled glucocorticoids (Dekkers et al. 2013). The next section will provide an overview of new developments in targeting GPCRs for the treatment of asthma and COPD.

New developments for once-daily treatment

The pharmaceutical industry has shown considerable interest in the development of inhaled bronchodilators with a long duration of action, i.e. >24 h, to strive for a once-daily treatment regime. This is an important development, as inadequate adherence to inhaled therapy is a major cause of poor clinical outcomes in the treatment of asthma and COPD (Tamura and Ohta 2007; Cazzola and Matera 2009). Several long-acting β2-adrenoceptor agonists (LABA) are being studied, including carmoterol, indacaterol and vilanterol (for a comprehensive review see (Cazzola et al. 2011)).

Olodaterol (Boehringer Ingelheim) was pharmacologically characterized in preclinical models in 2010 (Bouyssou et al. 2010a). Two 48-week Phase III studies demonstrated that olodaterol, in addition to standard therapy, provides a statistically significant improvement in lung function vs. standard therapy alone in COPD patients. These outcomes led to olodaterol becoming the first once-daily LABA approved for maintenance therapy in COPD.
patients (Gibb and Yang 2013). Whilst studies to unravel the effectiveness of olodaterol in asthmatic patients are ongoing, its bronchoprotective effects have been demonstrated in a guinea pig model of allergic asthma (Smit et al. 2014).

Tiotropium is a long-acting muscarinic receptor antagonist (LAMA) that has been available for over a decade, and is widely accepted for the treatment of COPD (Yohannes et al. 2013). Glycopyrronium was recently approved as once-daily maintenance treatment for patients with moderate-to-severe COPD. Treatment with this LAMA leads to fast and sustained improvements in lung function, health status and exercise endurance, as well as reduced risk of exacerbations, to a comparable level as seen with tiotropium (van Noord et al. 2010; Yu et al. 2011). Although anticholinergics are not a mainstay therapy for asthma, a recent publication by Kerstjens et al. (Kerstjens et al. 2011) demonstrated improved lung function in patients with severe uncontrolled asthma.

### A-kinase anchoring proteins as modulators of GPCR downstream signaling

Whilst activation of GPCRs can trigger a cascade of signaling events via the second messenger cAMP, transmission of this signal is conveyed by effector proteins such as Epac and PKA. The communication between receptors, cAMP effectors, and other downstream targets are coordinated by A-kinase anchoring proteins (AKAPs) (Figure 2) (Wong and Scott 2004; Dekkers et al. 2013). AKAPs are scaffolding proteins known to associate with PKA via a short α-helical structure (Kritzer et al. 2012). AKAPs act as targeting devices that assemble a large variety of structural and signaling molecules and thereby support their targeting to different microdomains in cells (Wong and Scott 2004). Dysfunction of AKAP complexes has been implicated in a wide variety of diseases, such as cancer (Troger et al. 2012; Scott et al. 2013). The following sections briefly describe how AKAPs modulate the downstream signaling of GPCRs in ASM cells.

In particular, in the cardiac system, it has been reported that PDE4 can bind to mAKAP, AKAP5 and AKAP9 (Dodge et al. 2001; Taskén et al. 2001; Lynch et al. 2005). As ASM seems to express several AKAPs (Horvat et al. 2012), recruitment of PDE, PKA and Epac to a distinct subset of AKAPs might bear the potential to control the cAMP pathway in the lung as well. Elevation of cAMP leads to the activation of mAKAP-bound PKA and subsequent phosphorylation and activation of PDE (Dodge-Kafka et al. 2005). Activated PDE4 then hydrolyzes local cAMP and thereby de-activates the mAKAP-bound PKA. As a result of locally decreased cAMP levels, the inhibitory effect of Epac on ERK5 is attenuated. Activated ERK5 then phosphorylates PDE4 and decreases its activity, allowing the local accumulation of cAMP. This action loop creates a unique negative feedback control to spatio-temporally compartmentalize cAMP signaling (Dodge et al. 2001; Dodge-Kafka et al. 2005).
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Figure 2. Compartmentalization of cAMP signaling by A-kinase anchoring proteins (AKAPs).
After the activation of β2-adrenoceptors by β2-agonists, adenylyl cyclase (AC) generates cAMP and thereby produce localized cAMP pools. AKAPs organize the anchoring of PKA in the vicinity of such cAMP pools, allowing for the control of its maintenance in space and time by the counterbalancing activities of AC and phosphodiesterases (PDEs).

The importance of cellular cAMP homeostasis in ASM has been shown in studies on the “β-agonist paradox” by breaking the spatio-temporal compartmentalization of cAMP. Instead of relaxing ASM, overexpression of the β2-adrenoceptor (McGraw et al. 2003) or adenylyl cyclase (AC) subtype 5 (Wang et al. 2011a) leads to AHR. It has become clear that the intracellular cAMP gradient in human ASM is spatially controlled by PDEs via cAMP hydrolyzation (Billington and Hall 2012). Although, being a minor component of the tissue PDE pool, using fluorescence resonance energy transfer (FRET) technique, others found that inhibition of PDE4 by rolipram augmented isoproterenol-induced cAMP production, suggesting that PDE4D5 plays a key role as a physiological regulator of β2AR-induced cAMP signaling within human ASM (Billington et al. 2008). Furthermore, research has shown that PDE4D deficient mice are neither responsive to cholinergic stimulation nor develop AHR after exposure to antigen, a process believed to relate to an inability to decrease-cellular cAMP in PDE4D deficient mice (Hansen et al. 2000). Due to the property of plasticity, ASM cells not only contract but also have the ability to produce inflammatory cytokines and chemokines, which can serve as an immune-modulator in the airway (Halayko and Amrani 2003; Wright et al. 2013). It has been shown that cAMP-mobilizing agents including β-agonists and PGE2 reduce TNFα-induced expression of eotaxin and RANTES (Pang and Knox 2000; Ammit et al. 2000). Thus, subtle alterations on the level of cellular cAMP might profoundly change cellular responses of ASM. It is tempting to speculate that AKAP
might contribute to the distinct functional responses of ASM. Indeed, a recent study by Penn and colleagues demonstrated that the AKAP-PKA complex disrupting peptides did not change global cAMP production in ASM but cAMP elevations close to the membrane compartments (Horvat et al. 2012). All this evidence implies that AKAPs might function as potential regulators during inflammatory responses of ASM by cAMP compartmentalization. In this sense, distinct AKAP complexes in ASM cells might rely on various molecular mechanisms to modulate GPCR downstream signaling pathways, and may be exploited as novel therapeutic targets for obstructive lung diseases.

**Phosphodiesterase Inhibitors**

Phosphodiesterases are a superfamily of enzymes involved in the regulation of cellular functions. Theophylline, a non-specific PDE inhibitor, has been used in the treatment of asthma and COPD for over 75 years as it promotes bronchodilation and inhibits inflammation. Despite its high oral bioavailability and anti-inflammatory, anti-proliferative and bronchodilator effects, its use has been hampered by its adverse effects. However, recent advancements in our understanding of PDE isoenzymes have led to renewed interest in PDE inhibitors as potential treatments for asthma and COPD. Both physiological and pharmacological studies have highlighted the important role of PDEs in the control of airway inflammation, airway function and remodeling. PDEs consist of 11 subfamilies (PDE1-11) capable of hydrolyzing the second messenger molecules cAMP and cyclic guanosine monophosphate (cGMP) into their inactive forms, 5-AMP and 5-GMP, respectively, thus terminating their downstream activity. PDE-1, -2, -3, -10 and -11 hydrolyze both cAMP and cGMP, whereas PDE-4, -7 and -8 are specific for cAMP and PDE-5, -6, -9 are specific for cGMP (Bingham et al. 2006). Since PDEs regulate the breakdown of cAMP and cGMP, the inhibition of PDEs results in an elevation in cAMP and cGMP and thus regulates ASM relaxation, proliferation and immunomodulatory functions. Whilst many of the PDE isoforms are expressed in the lung, PDE4 is the major therapeutic target in respiratory diseases (Méhats et al. 2003). Increased understanding of the PDE superfamily has allowed the development of more targeted approaches, including rolipram, cilomilast and roflumilast (PDE4 inhibitors) and RPL554 (a dual PDE3/PDE4 inhibitor).

**Targeting phosphodiesterases as a treatment for asthma**

PDEs have been implicated in the pathogenesis of asthma as they can regulate airway tone, airway hyperplasia and airway remodeling (Torphy et al. 1993; Burgess et al. 2006). Recently, *in vitro* studies have demonstrated deregulation of the cAMP-PDE pathway in asthma with elevated PDE activity and lower cAMP levels in ASM cells from asthmatic subjects compared with cells from non-asthmatic subjects (Trian et al. 2011). Furthermore, PDE4D, the dominant
PDE4 expressed on ASM cells (Méhats et al. 2003; Niimi et al. 2012), has been implicated as an asthma susceptibility gene (Himes et al. 2009). Recently, both preclinical and clinical studies have examined the potential of PDE inhibitors for the treatment of asthma. Preclinical studies have highlighted the potential for isoenzyme-specific PDE inhibitors for the treatment of bronchoconstriction in both rodent and human airways. For example, the PDE4 inhibitor roflumilast decreases ovalbumin (OVA)-induced contraction in the guinea pig trachea as well as large and small airway contraction in ventilated rats and guinea pigs (Bundschuh et al. 2001). Similarly, inhibition of both PDE3 and PDE4 decreases leukotriene C4-induced contraction in human airways (Rabe et al. 1993; Schmidt et al. 2000; Bundschuh et al. 2001). Thus, targeting PDEs may be beneficial to reduce the increased bronchomotor tone associated with asthma. In addition, in vitro studies have highlighted the potential of PDE inhibitors to reduce ASM proliferation, migration and extracellular matrix deposition as well as inflammatory mediators (Goncharova et al. 2003; Burgess et al. 2006; Burgess et al. 2006). Recently, Mata and colleagues reported that the PDE4 inhibitor roflumilast decreases respiratory syncytial virus infection in bronchial epithelial cells and reduces the expression of both the mucin gene MUC5AC and inflammatory mediators (Mata et al. 2013). Similarly, Kobayashi and colleagues reported that ASP3258 (3-[4-(3-chlorophenyl)-1-ethyl-7-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-3-yl] propanoic acid), a PDE4 inhibitor, reduces eosinophilia in a chronic OVA-induced murine model of asthma (Kobayashi et al. 2012).

Clinical studies have shown promising effects of PDE4 inhibitors for the treatment of asthma, such as improvements in forced expiratory volume in 1 s (FEV1), inhibition of the late phase response and a reduction in inflammatory cell numbers. Gauvreau and colleagues recently showed that roflumilast inhibits the allergen-induced late phase response, eosinophilia and neutrophilia in a trial that included 25 subjects with mild allergic asthma (Gauvreau et al. 2011). However, despite some promising results, PDE4 inhibitors also exhibit unwanted adverse effects, including nausea, diarrhea and headaches and thus they are not currently approved for the clinical treatment of asthma.

**Targeting phosphodiesterases as a treatment for COPD**

The selective PDE4 inhibitor cilomilast has been shown to have anti-inflammatory properties and some clinical efficacy, including the prevention of exacerbations in COPD patients (Rennard et al. 2008). However, despite showing some beneficial effects, its use has been limited due to severe adverse effects. Recently, the PDE4 inhibitor roflumilast was approved for use in the treatment of COPD by both the European Medicines Agency and the US Food and Drugs Administration (FDA). In vitro studies showed promising results for the use of roflumilast in the treatment of COPD. Roflumilast reduces cytokine release from a range of inflammatory cells in vitro, suggesting that it may be beneficial in targeting the influx of inflammatory cells associated with COPD. Furthermore, in animal models of COPD,
roflumilast reduced smoke-induced emphysema (Martorana et al. 2005) as well as the influx of neutrophils in the BALF (Martorana et al. 2008). In addition, roflumilast decreased the expression of MUC5AC in cultured epithelial cells (Mata et al. 2013) and improved cilia motility in vivo (Milara et al. 2012), suggesting that it may improve mucociliary clearance. The first large randomized clinical trial of roflumilast in COPD studied 1411 patients treated with either roflumilast (250 μg or 500 μg) or placebo for 24 weeks showed that roflumilast improved post-bronchodilator FEV1 and quality of life and decreased exacerbations (Rabe et al. 2005). However, while more recent studies have also shown roflumilast to reduce the frequency of exacerbations in patients with COPD (Chong et al. 2011; Yu et al. 2014), the effects on quality of life and FEV1 have been inconclusive. A Cochrane review of 29 randomized control trials reported that PDE4 inhibitors reduced the frequency of exacerbations; however, they also reported little effect on quality of life or symptom scores (Chong et al. 2011). Whilst further long term studies are required to fully investigate the effects of PDE4 inhibitors on lung function, issues regarding safety have been raised with many patients experiencing adverse effects including gastrointestinal and psychiatric effects. However, there is ongoing development of more potent PDE4 inhibitors such as GSK256066, which may have a reduced likelihood of causing vomiting (Nials et al. 2011).

**Targeting TRP Channels**

The transient receptor potential (TRP) family of ion channels are cation-selective transmembrane proteins which show a preference for Ca2+ ions (Caterina et al. 1997; Ramsey et al. 2006). The TRP family of proteins consists of 28 members in six subfamilies (TRPC, TRPV, TRPM, TRPA, TRPP and TRPML) based on sequence homology (Clapham 2003). They are known as cellular sensors as they respond to changes in the local environment (Clapham 2003). Whilst expression of TRP channels has been mainly demonstrated in sensory nerve cells, they have also been identified in a range of cell types associated with respiratory diseases (Peier et al. 2002; Story et al. 2003; Bandell et al. 2004; Jia et al. 2004; Kunert-Keil et al. 2006; Bautista et al. 2006; Yang et al. 2006; Alvarez et al. 2006; Dietrich et al. 2006; Grant et al. 2007; Grant et al. 2007; Nassenstein et al. 2008; Prasad et al. 2008; Banner et al. 2011; Li et al. 2011; Jang et al. 2012). TRPA1, TRPV1, TRPC6 and TRPV4 are expressed on ASM (Jia et al. 2004; Kunert-Keil et al. 2006; Banner et al. 2011; Jang et al. 2012) and inflammatory cells, including neutrophils (Heiner et al. 2003; Li et al. 2003; McMeekin et al. 2006; Banner et al. 2011), CD8+ T cells (Prasad et al. 2008; Banner et al. 2011) and airway macrophages (Liedtke et al. 2000; Finney-Hayward et al. 2010; Banner et al. 2011). Therefore, they represent novel therapeutic targets for the treatment of asthma and COPD.
**TRP channels as targets for asthma**

TRP channels are increasingly being linked to key features of the asthma phenotype. Here, we summarize the emerging roles of two members of the TRP family (TRPA1 and TRPV1) in bronchoconstriction, allergic airway inflammation and AHR.

TRPA1 has been reported to be expressed in asthma-relevant cell types such as CD4+ T cells, CD8+ T cells, B cells and mast cells (Prasad et al. 2008; Banner et al. 2011); activation of these receptors has been linked to the symptoms of asthma. For example, studies have shown that environmental irritants that usually cause asthma-like symptoms also activate TRPA1 in the airways (Deering-Rice et al. 2011; Shapiro et al. 2013).

TRPA1 has been associated with the characteristic bronchoconstriction of the late asthmatic response (LAR). Raemdonck et al. demonstrated that administration of a TRPA1 antagonist in a rodent model of allergic asthma attenuated the LAR. In this same study, the authors also deduced a role for airway sensory nerves, a central reflex component and a parasympathetic cholinergic constrictor response. Following from this, it could be concluded that allergen challenge activates TRPA1 channels present on airway sensory nerves which triggers a neuronal response, resulting in bronchoconstriction (Raemdonck et al. 2012). Other studies have also indicated a role for TRPA1 in the control of airway tone. Andre et al. reported that activation of TRPA1 channels on isolated guinea pig bronchus results in constriction, which is secondary to the release of neuropeptides via local axons (Andrè et al. 2008). Hox et al. investigated the increased incidence of AHR in swimmers using a mouse model of hypochlorite exposure. Exposure to hypochlorite induced AHR, which was abolished in TRPA1 deficient mice (Hox et al. 2013). Similarly, TRPA1 deficient mice have reduced inflammation and AHR when exposed to OVA (Caceres et al. 2009). Conversely, Spiess et al. have recently shown that exposure to acrolein (a known TRPA1 agonist) resulted in a decreased inflammatory response in a murine allergic asthma model (Spiess et al. 2013).

Studies exploring the role of TRPV1 in AHR and allergic inflammation have produced conflicting results. Contraction of isolated guinea pig ASM by TRPV1 agonists was demonstrated to involve the release of sensory neuropeptides from finite stores in nerve endings which consequently act upon neurokinin receptors on ASM, resulting in contraction (Belvisi et al. 1992). Rehman et al. reported that TRPV1 modulation inhibits inflammation, AHR and airway remodeling in their IL-13 driven murine model (Rehman et al. 2013). However, other studies have indicated a protective role for TRPV1 in allergic inflammation (Mori et al. 2011) and others report no part played by TRPV1 in features of allergic asthma (Caceres et al. 2009; Raemdonck et al. 2012). However, it is currently unclear whether TRPV1 is involved in bronchospasm in humans and if its action is altered in diseases such as asthma.
In conclusion, the roles of TRP channels in asthma continue to be identified (briefly summarized in Figure 3). Further study into their mechanisms of action within this disease will hopefully result in therapeutic targeting of these channels and the development of novel treatments for asthma.

Figure 3. The role of TRP channels in Asthma.

**TRP channels as targets for COPD**

Many direct activators of TRP channels, including arachidonic acid derivatives, lowered airway pH, increased temperature and altered airway osmolarity are present in the diseased airway, which makes them promising targets for the treatment of COPD. Seven genetic variants of the TRPV4 gene are associated with developing COPD (Zhu et al. 2009), and expression of the TRPV1 channel is upregulated following exposure to inhaled pollutants in the rat bronchus (Costa et al. 2010), suggesting that TRP proteins may play an important role in the pathogenesis and susceptibility to COPD. The roles of four TRP channels (TRPA1, TRPV1, TRPC6 and TRPV4) in lung inflammation, bronchoconstriction and cough associated with COPD are outlined in this section.

A defining feature of COPD is lung inflammation, caused by increased numbers of macrophages, neutrophils and CD8+ T lymphocytes (Keatings et al. 1996; Keatings et al. 1997). It has recently been demonstrated that TRPC6 plays a modulatory role in neutrophil migration as migration to CXCL2 in bone-marrow derived neutrophils is attenuated in TRPC6−/− mice (Damann et al. 2009). Cigarette smoke, the primary causative agent of COPD (Sethi and Rochester 2000), contains many stimuli that activate the TRPA1 ion
channel (Gylling et al. 1991; Bautista et al. 2006; Facchinetti et al. 2007; Andrè et al. 2008; Lin et al. 2010), and TRPA1 plays a major role in the early phase of bronchial inflammation to cigarette smoke (Bautista et al. 2006). A further role for TRPA1 in COPD has also been found in human fibroblasts epithelial and smooth muscle cells where cigarette smoke induces cells to release IL-8, and, in mice, KC (IL-8 murine mimetic) in a non-sensory nerve driven mechanism (Nassini et al. 2010). TRPV1 has also been implicated in the inflammatory processes associated with COPD, where attenuation of neutrophil influx and cytokine release was achieved by pretreatment with a TRPV1 agonist, through desensitization of the receptor (Tsuji et al. 2010). In addition, airway neutrophilia was significantly reduced following exposure to cigarette smoke in TRPV1−/− mice compared to wild type controls (Baxter et al. 2012). However, there is no evidence as of yet to implicate TRPV4 in COPD-associated inflammation as relatively little is known and further investigation is required.

COPD is characterized by progressive, irreversible airway obstruction, and a number of TRP channels have been associated with bronchoconstriction. TRPV1 agonists are known to cause bronchoconstriction in humans, probably through a central reflex as bronchoconstriction is inhibited by ipratropium bromide (Fuller et al. 1985), as well as in animals (Lalloo et al. 1995). In animals, this has been caused by neurogenic inflammation and activation of a central reflex. The guinea pig trachea contracts in response to TRPV1 through the release of the neuropeptides substance P and neurokinin A, which activate neurokinin receptors on ASM (Belvisi et al. 1992). TRPA1 agonists have also been found to cause bronchoconstriction secondary to release of neuropeptides (Andrè et al. 2008). In contrast, TRPV4 agonists have been shown to cause direct activation of ASM (Jia et al. 2004). It has been shown that a selective TRPV4 agonist causes ASM contraction in the absence of a reflex arc (Bonvini et al. 2013).

Cough is a troublesome symptom and is currently the most common complaint for which patients visit a doctor in the UK (Barnes 2000). Cough is normally a protective reflex which clears the lungs of harmful particles (Nasra and Belvisi 2009) and during disease the cough response can become excessive leading to chronic cough (cough which lasts over 8 weeks) because of hypersensitivity of the neuronal pathways responsible (Young and Smith 2011). This is a problem, lowering the quality of life for sufferers [149] and causing damage to the airway mucosa (Canning 2006). Chronic non-productive cough can affect up to 7% of the population at any one time (Ford et al. 2006) and is a characteristic symptom in patients with COPD, caused by activation of airway sensory nerves (Nasra and Belvisi 2009). The role of TRPV1 has been extensively characterized in sensory nerves, and TRPV1-expressing nerves innervate the entire respiratory tract (Watanabe et al. 2006). TRPV1 agonists are well-documented to cause cough, and in COPD there is increased cough sensitivity to capsaicin (O’Connell et al. 1996; Doherty et al. 2000; Higenbottam 2002). Animal models have shown that exposure to cigarette smoke causes hypersensitivity to TRPV1 agonist
inhalation (Karlsson et al. 1991; Lewis et al. 2007; Maher and Belvisi 2010; Grace and Belvisi 2011). Similarly, TRPA1 channels have been shown to cause cough in both guinea pigs and humans (Birrell et al. 2009), and recently it has been shown that TRPV4 agonists can also activate airway sensory nerves directly to cause cough (Belvisi et al. 2013).

To conclude, a role for a number of TRP channels has been outlined regarding the inflammation, bronchoconstriction and chronic cough associated with the pathogenesis of COPD (briefly summarized in Figure 4). Taken together with the fact that many direct activators of TRP channels are present in the diseased airways, we suggest that TRP channels are emerging as promising new targets for the treatment of COPD.

**Figure 4. The role of TRP channels in COPD.**

**Kinases**

Kinases are key regulators of normal cellular functions, through the site-specific phosphorylation of a downstream substrate. As kinases are involved in diverse regulations of various cellular functions, they have become an obvious target for rational drug design for diseases such as asthma and COPD. In this section, we will briefly discuss the roles of various kinases that have been linked to the pathogenesis of asthma and COPD, including mitogen activated protein kinase (MAPK; e.g. extra cellular signal-regulated kinase/ERK, c-Jun N-terminal kinase/JNK and p38), tyrosine kinases, Ras human ortholog (Rho)/Rho kinase (ROCK), protein kinase A (PKA), protein kinase C (PKC), phosphoinositide 3 kinase (PI3K) (Blease 2005).
Kinases in asthma

Activation of the MAPK signaling cascade is associated with differentiation, proliferation, activation, degranulation and migration of various cell types such as immune cells, airway epithelial cells and ASM (Duan and Wong 2006), thus making MAPK an important therapeutic target for asthma. To support this, in the in vitro setting, SB239063, a p38 MAPK inhibitor, has been found to induce apoptosis in eosinophils isolated and cultured from the BALF of guinea pigs. In addition, in in vivo models, SB239063 inhibits OVA- or leukotriene D4-induced eosinophilia (Underwood et al. 2000) as well as airway inflammation and remodeling in OVA-sensitized and ozone challenged mice (Liang et al. 2013). ERK1/2 has been found to be elevated in asthmatic mice in comparison to naïve control mice. U0126, a specific ERK1/2 inhibitor, significantly inhibits the infiltration of inflammatory cells and various cytokines in the BALF of OVA-challenged mice. In addition, U0126 treatment also inhibits eosinophils, goblet cell hyperplasia and vascular cell adhesion molecules (VCAM-1) and reduces AHR to methacholine challenge (Duan et al. 2004).

Another MAPK implicated in asthma is JNK, as inhibition of JNK with SP600125 has been found to reduce CXCL10 mRNA expression (Alrashdan et al. 2012). CXCL10/IP10 is responsible for mast cell migration toward ASM cells. In vivo studies in Brown Norway rats has shown that SP600125 inhibits the allergen-induced infiltration of inflammatory cells, including macrophages, lymphocytes, neutrophils and eosinophils; however, it did not affect eosinophils or T cell accumulation in lung tissue, nor did it reduce the mRNA expression of various allergen-induced cytokines such as IL-1β, IL-4 and IL-5. Furthermore, this intervention did not affect allergen-induced AHR. In light of these effects, the utility of JNK inhibitors in the treatment of asthma is limited (Eynott et al. 2004).

Phosphatidylinositol 3-kinase (PI3K) activation leads to various cellular functions such as cell growth, proliferation, survival and migration. Recent insight into the PI3K pathway has revealed that it is associated with numerous inflammatory processes such as activation and recruitment of inflammatory mediators and cells along with airway remodeling and steroid insensitivity (Finan and Thomas 2004; Ito et al. 2007). This has led to the evaluation of various PI3K inhibitors as potential therapeutic options in asthma. Wortmannin, a PI3K inhibitor, significantly reduces the in vitro release of eosinophil cationic protein and eosinophil peroxidase from eosinophils and myeloperoxidase from neutrophils in asthmatics, suggesting a role in eosinophil and neutrophil degranulation (Kämpe et al. 2012). LY294002 and TG100-115, additional PI3K inhibitors, significantly inhibit inflammatory cell infiltration and the expression of inflammatory mediators (IL-5, 13 and eotaxin) in the BALF of OVA-challenged Balb/C mice, as well as eosinophilia, goblet cell hyperplasia and AHR (Duan et al. 2005). Furthermore, the PI3K isoforms p110α, p110β and p110δ are present in ASM cells (Krymskaya et al. 1999; Moir et al. 2011; Ge et al. 2012), and both wortmannin and LY294002 have been found to decrease AHR in a murine model of OVA-induced asthma (Duan et al. 2004).
2005). Taken together, these data provide promising evidence that PI3K inhibitors could emerge as potential therapeutic options for asthma.

Rho associated coiled coil-containing protein kinase (ROCK) is one of the most studied downstream signaling molecules of the monomeric GTP-binding protein RhoA. The major physiological functions of the Rho/ROCK axis include contraction, migration and proliferation; this pathway has been implicated in the pathogenesis of asthma (Wettschureck and Offermanns 2002; Kume 2008; Schaafsma et al. 2008). For example, targeting the Rho/ROCK axis inhibits inflammation and airway contraction. Y-27632, a ROCK inhibitor, suppresses the release of inflammatory cytokines from activated T cells (Aihara et al. 2004) and reverses the carbachol-mediated contraction of rabbit tracheal and human bronchial smooth muscle cells via the inhibition of calcium sensitization \textit{ex vivo} (Yoshii et al. 1999). Furthermore, in an \textit{in vivo} model of allergic asthma, Y-27632 improved AHR to contractile stimuli in response to allergen or viral challenge and also reduced airway eosinophilia (Hashimoto et al. 2002; Henry et al. 2005; Schaafsma et al. 2006). Similarly, another ROCK inhibitor, fasudil (HA-1077), has been shown to inhibit allergen-induced airway inflammation, hyperreactivity and hyperresponsiveness in an OVA-driven murine model of allergic asthma (Taki et al. 2007; Wu et al. 2009).

**Kinases in COPD**

Current forms of therapy for COPD are relatively ineffective, however, in more recently kinase inhibitors have been suggested as potential treatments. This is because a variety of extracellular stimuli such as Toll receptor ligands (e.g. lipopolysaccharide) and cytokines, which are thought to contribute to the progression of COPD, activate kinase pathways such as p38 MAPks, PI3K, Janus kinase/signal transducer and activator of transcription (JAK/STAT) and Rho kinase, resulting in downstream activation of transcription factors such as NF-κB and increasing pro-inflammatory mediators. In recent years drugs which target specific isoforms of these kinases have been developed and their use in \textit{in vitro} and \textit{in vivo} models has enhanced our understanding of the roles of these kinases in COPD.

The p38 MAPK family (α, β, γ, δ) is activated by cellular stress, regulates the expression of inflammatory cytokines and has been implicated in the induction and maintenance of airway inflammation in COPD. For example, levels of phosphorylated (active) p38 are greater in alveolar macrophages from COPD lungs compared with those from non-COPD control smokers and non-smokers (Renda et al. 2008). In addition, phosphorylated p38 is also increased in sputum from COPD patients and correlates with both CXCL8 and decreased lung function (Huang et al. 2013b). Further support for the role of p38 was recently shown in an \textit{in vivo} transgenic mouse model study where it accelerated the rate of LPS + cigarette smoke induced emphysema (Amano et al. 2014). Similarly, in another murine inflammatory model of COPD inhibition of p38 using the α-isoform selective inhibitor SD-282 reduced...
the increased number of tobacco smoke-induced macrophages and neutrophils back to baseline (Medicherla et al. 2008). Thus, p38 appears to play a pivotal role in the airway inflammation associated with COPD, hence making it a potential target for therapeutic intervention.

Several oral p38 inhibitors, such as losmapimod and PH797804, have undergone phase II clinical trials for the treatment of COPD; however, their findings are variable. Singh and colleagues reported that orally administered SB681323 reduced p38 MAPK signaling and suppressed TNFα production in a small clinical pharmacological study of 17 COPD patients (Singh et al. 2010). In a study by Lomas et al., oral administration of the p38 inhibitors losmapimod (GW856553) for 12-weeks significantly reduced plasma fibrinogen levels [a biomarker for COPD (Duvoix et al. 2013)] but had no effect on sputum neutrophil levels or lung function (Lomas et al. 2012). Similarly, in a recent multicentre clinical trial study Watz and colleagues reported that despite being well tolerated losmapimod (2.5, 7.5 or 15 mg) treatment for 24 weeks had no effect on exercise tolerance in patients with COPD as demonstrated using the 6-min walk test (Watz et al. 2014). In contrast, in a 6-week clinical trial PH797804 improved dyspnea and lung function in moderate to severe COPD patients (MacNee et al. 2013), thus PH797804 shows great promise. However, whilst there may be some beneficial effects of p38 inhibitors further longer term-studies are required. One of the major limiting problems reported in clinical studies using oral p38 inhibitors is adverse effects therefore drugs which are directly delivered to lung via inhalation, such as PF-03715455 may prove beneficial (Millan et al. 2011).

Another kinase to be considered is JAK. In COPD several cytokines and inflammatory mediators signal via the JAK/STAT pathway and therefore inhibition of JAK may provide another therapeutic target. Whilst oral JAK inhibitors such as tofacitinib have shown promise in other diseases, such as ulcerative colitis and rheumatoid arthritis (Fleischmann et al. 2012a; Fleischmann et al. 2012b; Sandborn et al. 2012; Kawalec et al. 2013), their effects on COPD have not been investigated.

PI3K is another important kinase in COPD as PI3Ks generate second messengers that regulate several cellular events involved in inflammation and PI3K activity is increased in the peripheral lungs of people with COPD (To et al. 2010). PI3K-δ and -γ are likely to be the most important isoforms as they play an important role in inflammation associated with COPD. PI3K-δ is increased in macrophages from people with COPD compared with smokers with normal lung function (Marwick et al. 2010). In addition, p110δ isoform expression is higher on ASM from the lungs of patients with COPD compared with those without COPD (Ge et al. 2012). The PI3K-γ isoform is expressed predominantly on leukocytes and knockout of the PI3K-γ gene inhibits neutrophil migration in mice (Medina-Tato et al. 2007). Whilst non-selective PI3K inhibitors have a high toxicity and therefore have had limited success, there are several novel PI3K isoform specific inhibitors in development. Although none of
these are currently undergoing clinical trial for COPD they are beginning to emerge in *in vitro* and *in vivo* studies. Aerosol administration of the dual PI3K-γ/δ inhibitor TG100-115 has been used in a preclinical mouse model study of LPS- and smoke-induced pulmonary inflammation. In this study by Doukas et al., TG100-115 reduced pulmonary neutrophilia and restored corticosteroid insensitivity in mice (Doukas et al. 2009). Theophylline has recently re-emerged into the arena as a treatment for COPD as when it is given at a low-dose it acts as an inhibitor of PI3Kδ (To et al. 2010). Currently, clinical trials examining the effectiveness of low-dose theophylline are under way.

**Kinase inhibitors in asthma & COPD – adverse effects**

On a cautionary note kinases modulate a number of key cellular functions, thus a global inhibition of a particular kinase via systemic delivery is likely to produce adverse events, for example various protein kinase inhibitors currently used as anticancer agents are associated with several major adverse events such as hypertension, anemia and other hematological disorders (Sodergren et al. 2014). Since kinases are inhibitors of enzymes belonging to cytochrome P450 superfamily in particular CYP1A2, 2C9, 2D6, and 3A4, their systemic use may result into the potential interaction with concomitant medication and may produce unwanted effects (Wang et al. 2014). In order to bypass this issue a localized delivery of these agents at a very low dose should be considered to target patients with asthma or COPD. Inhalation therapy rather than oral administration should also be considered. Nevertheless the positive impact of kinase therapy in *in vitro* and *in vivo* studies has led to the development of various molecules that are currently undergoing clinical trials for their efficacy in human asthma and COPD. Some of these molecules and their stages of development are shown in Table 1.

**Vitamin D**

Whilst vitamin D and its receptors are known for their role in bone mineralization and calcium homeostasis, there is growing evidence that vitamin D deficiency may contribute to respiratory diseases. Here, we discuss the role of vitamin D in asthma and COPD.

**Vitamin D in asthma**

Recently, it has been suggested that vitamin D deficiency has contributed to the rise in asthma and allergy. The current hypothesis is that Westernization has led to human populations spending more time indoors, resulting in less sun exposure and hence vitamin D deficiency (Litonjua and Weiss 2007). Epidemiological studies show that low serum vitamin D levels are associated with adverse asthma outcomes, including worse asthma control (Chinellato et al. 2011), increased corticosteroid use (Brehm et al. 2009; Gupta et
Chapter 2

al. 2011) and increased asthma exacerbations (Brehm et al. 2009; Brehm et al. 2010). Much of the data from these observational studies support the hypothesis that higher vitamin D levels lead to better asthma outcomes.

Table 1. Clinical development of kinase inhibitors for the treatment of asthma and COPD. (Source: ClinicalTrials.gov).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Kinase</th>
<th>Drug name</th>
<th>Sponsor</th>
<th>Trial phase</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>p38 MAPK</td>
<td>–</td>
<td>Imperial College London</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PI3K</td>
<td>IPI-145</td>
<td>Infinity Pharmaceuticals</td>
<td>2</td>
<td>Phase 2a, Efficacy and Safety Study of IP-145 in Mild Asthmatic Subjects</td>
</tr>
<tr>
<td>Tyrosine kinase inhibitor</td>
<td>AB1010/ Mastinib</td>
<td>AB Science</td>
<td>2</td>
<td>Efficacy of Oral AB1010 in Adult Patients with Severe Persistent Corticosteroid Dependent Asthma</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>p38 MAPK</td>
<td>Losmapimod</td>
<td>Cambridge University Hospitals NHS Foundation Trust</td>
<td>2</td>
<td>Losmapimod in Chronic Obstructive Pulmonary Disease Patients Stratified by Fibrinogen. (EVOLUTION)</td>
</tr>
<tr>
<td></td>
<td>PH797804</td>
<td>Pfizer</td>
<td>2</td>
<td>A Phase II, study to evaluate the efficacy and safety of PH-797804 in adults with moderate to severe Chronic Obstructive Pulmonary Disease (COPD) (study completed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PF03715455</td>
<td>Pfizer</td>
<td>1</td>
<td>Single Dose Lipopolysaccharide (LPS) Study In Healthy Volunteers</td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td>Low-dose theophylline</td>
<td>Hospital Son Espases</td>
<td>3</td>
<td>Low-dose Theophylline as anti-inflammatory enhancer in severe chronic obstructive pulmonary disease (ASSET)</td>
<td></td>
</tr>
</tbody>
</table>

Birth cohort studies investigating the associations between vitamin D status and asthma and allergy outcomes in children have however revealed conflicting results. While there have been studies showing that a lower dietary intake of vitamin D during pregnancy leads to an increased risk of wheeze (Camargo et al. 2007; Devereux et al. 2007) and asthma development in children (Erkkola et al. 2009), a Finnish study has also demonstrated an increased prevalence of asthma and atopy in adults who had received vitamin D supplementation during the first year of life (Hyppönen et al. 2004). Vitamin D status was assessed using food questionnaires rather than directly measuring serum 25(OH) D concentrations and may not provide an accurate representation of vitamin D status since this excludes vitamin D synthesized from sun exposure, a major determinant of vitamin D status. Studies that directly measure maternal serum vitamin D status are conflicting with
one study showing no association with risk of childhood asthma, wheeze and atopy (Pike et al. 2012), and another showing an increased risk of asthma in children at 9 years of age (Gale et al. 2008). As such, there has yet to be convincing data that vitamin D influences asthma and allergy outcomes. However, vitamin D deficiency in humans is often an indirect marker of confounding factors such as nutritional status and physical fitness, making it difficult to determine a causal association between vitamin D status and chronic lung disease. Therefore, in order to study the causal pathways between vitamin D and disease outcomes, in vitro and in vivo models are invaluable tools.

In vitro studies using human ASM cells obtained from asthmatic and normal subjects have demonstrated that 1,25-dihydroxy vitamin D, the active metabolite of vitamin D, inhibits ASM cell proliferation by preventing cell cycle progression (Damera et al. 2009). These findings support a study showing increased ASM mass in vitamin D-deficient children with severe asthma (Gupta et al. 2011), and suggests that vitamin D may have a role in airway remodeling. However, this has yet to be demonstrated in in vivo studies. The immunomodulatory functions of vitamin D have, however, been well-studied using mouse models of allergic asthma. One study found that vitamin D deficiency enhanced the capacity of airway draining lymph nodes to secrete Th2 cytokines in both male and female mice. Eosinophil and neutrophil numbers, as well as bacterial levels, were increased in the BALF of male mice, and vitamin D supplementation reversed these effects (Gorman et al. 2013). Other mouse studies have shown that 1,25-dihydroxy vitamin D supplementation inhibits eosinophil and lymphocyte recruitment into the airway lumen, reduces IL-4 production from T cells and impairs T cell migration, thus damping the inflammatory response (Topilski et al. 2004). It has also been found that early treatment with 1,25-dihydroxy vitamin D results in enhanced Th2 cytokine (IL-4 and IL-13) and IgE production, but IL-5 release and eosinophilia are inhibited when 1,25-dihydroxy vitamin D is administered at a later stage (Matheu et al. 2003). Despite different findings regarding T cell responses in different experimental protocols, the data from mouse models of asthma suggest that vitamin D supplementation may be beneficial for the treatment of established disease. Whether vitamin D supplementation can prevent disease onset still needs to be determined.

Vitamin D can also act as an adjuvant for other therapies. Administration of 1,25-dihydroxy vitamin D with allergen immunotherapy enhanced the beneficial effects of immunotherapy in a mouse model of asthma (Taher et al. 2008). Xystrakis et al. demonstrated that the addition of vitamin D and dexamethasone to CD4+ regulatory T cell cultures from steroid-resistant asthmatic patients enhanced IL-10 synthesis to levels comparable to steroid-sensitive patients treated with dexamethasone alone. Furthermore, vitamin D overcame dexamethasone-induced downregulation of glucocorticoid receptor expression by CD4+ T cells (Xystrakis et al. 2006). In a separate study, addition of vitamin D to an experimental in vitro model of steroid resistance resulted in the suppression of T cell proliferation when
dexamethasone on its own did not inhibit cell proliferation (Searing et al. 2010). Together, these data provide evidence that vitamin D could enhance the anti-inflammatory effects of glucocorticoids and potentially be used as a therapy in severe asthma for patients who are steroid-insensitive.

Vitamin D and COPD

Vitamin D deficiency is also common in patients with COPD (Janssens et al. 2010; Persson et al. 2012). In a study by Janssens et al., serum 25-hydroxy vitamin D, the circulating form of vitamin D, correlated with lung function in patients with COPD. Certain genetic variants of the vitamin D binding protein (VDBP), the major carrier protein for vitamin D that binds circulating 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D with high affinity, are risk factors for COPD (Janssens et al. 2010). Recent studies investigating the effects of vitamin D supplementation on COPD patients have primarily focused on the role of vitamin D in improving muscle strength in COPD patients. While a Belgian study demonstrated improvements in inspiratory muscle strength and maximal oxygen uptake following vitamin D supplementation (Hornikx et al. 2012), another study which supplemented patients with higher doses of vitamin D showed no effect in physical performance (Bjerk et al. 2013). A positive association between dietary vitamin D intake and FEV1, FEV1/FVC and a negative association with COPD incidence has also been shown (Shaheen et al. 2011). In the same study serum 25(OH) D levels were positively associated with COPD prevalence but not FEV1 and FVC. These data further confirm that analysis of vitamin D intake from the diet is not an ideal indication of vitamin D status and that associations should be interpreted with caution in COPD due to the link between vitamin D and musculoskeletal abnormalities in COPD. Despite an association between vitamin D and COPD studies investigating the role of vitamin D and COPD using in vitro and in vivo models are lacking, and the majority of the available data are from cross-sectional human studies.

Alveolar macrophages obtained from a population with a high risk of COPD showed increased macrophage activation and higher levels of VDBP in the airways (Wood et al. 2011). In this population, the GC2 variant of VDBP, which is less able to activate macrophages, was protective against COPD. Macrophage accumulation and activation in the lung causes the release of neutrophil chemoattractants which may contribute to lung damage in COPD. Another variant of the VDBP gene, rs7041, was predictive of vitamin D deficiency, which in turn was associated with reduced lung function (Janssens et al. 2010; Wood et al. 2011). Vitamin D levels have been shown to correlate with lung function in a large population based study (Black and Scragg 2005); this was also demonstrated in a mouse model of vitamin D deficiency (Zosky et al. 2011). In this study, vitamin D-deficient juvenile mice not only had reduced lung function, but also had altered lung structure and smaller lungs (Zosky et al. 2011). Reduced lung function has also been reported in a vitamin D receptor (VDR)
knockout mouse model (Sundar et al. 2011). VDR deletion also led to chronic inflammation and immune dysregulation, which ultimately resulted in a COPD phenotype in this model. Given these data, it is tempting to speculate that vitamin D deficiency may be a risk factor for COPD. The strong evidence for a genetic susceptibility to COPD with variants of the VDBP genes warrants further investigation into the functional mechanisms of VDBP. *In vitro* and *in vivo* studies will certainly be useful for determining how VDBP and vitamin D contribute to inflammation and altered lung structure and, ultimately, if vitamin D supplementation will be beneficial.

**Matrix Metalloproteinases as Targets in Asthma and COPD**

Matrix metalloproteinases (MMPs) are a family of zinc endopeptidases capable of degrading most components of the extracellular matrix. They exist in balance with their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs). A disruption of this balance is a key event in the development of pulmonary diseases such as asthma and COPD where elevated levels of MMPs have been reported. Thus, targeting the MMPs may be an alternative therapeutic strategy.

**MMPs and asthma**

MMPs have been implicated in asthma, and recent studies have highlighted polymorphisms in MMP genes which may contribute to a predisposition to asthma (Jiménez-Morales et al. 2013). Altered levels of MMPs have also been reported; for example, MMP-9 is increased in the lung tissues as well as sputum, BALF and serum from asthmatic subjects when compared with healthy subjects (Hoshino et al. 1998; Cataldo et al. 2000; Mattos et al. 2002; Ko et al. 2005; Hong et al. 2012). Other MMPs, including MMPs -1, -2, -7, -10, -12 and -19, have also been implicated in asthma. There is strong evidence for a role for MMP-12 in asthma as mice deficient in MMP-12 exhibit a reduction in inflammation following allergen challenge. Interestingly, MMP-12 regulates airway remodeling through its capacity to degrade a wide range of ECM proteins including elastin, type IV collagen, fibronectin and gelatin. Similarly, MMP-10 has also been implicated in asthma exacerbations as it is induced by respiratory syncytial virus in human nasal epithelial cells (Hirakawa et al. 2013). Thus, there is evidence of elevated MMP levels in asthma which may contribute to airway remodeling.

Broad spectrum MMP inhibitors such as tetracyclines have been investigated as potential treatments for asthma, although the results are not clear-cut. Tetracyclines work by directly binding to the Ca$^{2+}$ and Zn$^{2+}$ ions in the active site of MMPs, thus rendering the MMP inactive. Doxycycline, a broad spectrum MMP inhibitor, has been found to decrease allergen-induced eosinophilic inflammation and AHR in an OVA-driven mouse model of asthma by reducing the proteolytic activity of MMP-9 when administered by aerosol (Gueders et al. 2008). In
addition, this intervention reduced MMP-9 mRNA as well as airway inflammation and AHR in a mouse model of toluene diisocyanate-induced asthma (Lee et al. 2004). In contrast, doxycycline had no effect in a cat model of Ascaris suum-induced asthma (Leemans et al. 2012). In addition, the broad spectrum MMP inhibitor R94138 reduced the development of allergic inflammation in a mouse model, and inhibition of MMP-12 led to a significant reduction in the early and late airway responses in a sheep model of asthma (Li et al. 2009; Mukhopadhyay et al. 2010). It has been suggested that other MMPs may have a protective role in asthma, since MMP-8 deficiency promotes allergen-induced airway inflammation and MMP-8 deficient mice exhibit AHR (Gueders et al. 2005).

Whilst in vivo studies have found that broad spectrum inhibitors may be beneficial, their clinical use remains unclear. Clinical trials with MMP inhibitors for other diseases have been disappointing, partially due to the off-target effects observed with broad spectrum inhibition. More targeted approaches may provide therapeutic benefit with reduced adverse effects.

**MMPs and COPD**

Initially, emphysema was believed to be driven by neutrophil elastase; however, it is now accepted that MMPs, including -1, -2, -7, -9 and -12, produced by both inflammatory and structural cells in the lung, also play a significant role in the alveolar destruction. Studies have shown elevated levels of MMPs -1, -2, -9, and -12 in COPD (Segura-Valdez et al. 2000; Culpitt et al. 2005; Demedts et al. 2006; D'Armiento et al. 2013), with sputum and exhaled breath condensate levels of MMP-9 further increased during exacerbations (Mercer et al. 2005; Kwiatkowska et al. 2012). MMP-12 has been implicated in the pathogenesis of COPD with expression of the common serine variant at codon 357 of the MMP-12 gene associated with clinical manifestations of the disease (Mukhopadhyay et al. 2010). Furthermore, elevated levels of MMP-12 have been reported in many (Demedts et al. 2006; Ilumets et al. 2007) but not all (Imai et al. 2001) studies of COPD.

There is increasing evidence that elevated MMP levels may cause alveolar destruction and inflammation and that inhibition may be an effective strategy to treat COPD. Chronic exposure of mice to cigarette smoke induces alveolar airspace enlargement and alveolar destruction as well as expression of MMPs, including MMP-1, MMP-9 and MMP-12 (Lavigne and Eppihimer 2005; Vlahos et al. 2006; Churg et al. 2007a; Churg et al. 2007b; Xu et al. 2011; Bezerra et al. 2011; Geraghty et al. 2011). Overexpression of collagenase or MMP-9 induces emphysema (D'Armiento et al. 1992; Foronjy et al. 2008), whereas in mouse models of smoke-induced emphysema, treatment with the broad spectrum metalloproteinase inhibitors RS113456 or PKF242-484 prevented neutrophil infiltration (Churg et al. 2001; Morris et al. 2008). Recent studies have investigated the roles of specific MMPs. Elevated MMP-1 has been implicated in the alveolar disruption associated with COPD, as expression of human
MMP-1 in a transgenic mouse model caused disruption of alveolar walls, coalescence of alveolar spaces and pulmonary emphysema in mice (Shiomi et al. 2003). MMP-12 induces alveolar destruction and degradation of elastin in COPD and MMP-12 deletion or inhibition (via MMP408) prevents inflammation and emphysema in mouse models of COPD (Li et al. 2009). Thus, evidence from animal models suggests that targeting MMPs may be beneficial for the treatment of COPD.

The effectiveness of MMP inhibitors as a treatment for COPD in humans remains unknown as few clinical trials have been conducted. In a recent clinical trial of 55 patients with stable moderate-to-severe COPD, treatment with AZD1236, a selective MMP-9/MMP-12 inhibitor was examined. Whilst treatment for 6 weeks was well-tolerated, there was little effect on clinical outcomes (Dahl et al. 2012); thus, further studies are required.

Overall, given the body of evidence supporting a role for MMPs in emphysema, further research into MMP inhibitors as a possible treatment for COPD is justified.

Combination Therapies in the Treatment of Asthma and COPD

New developments for combination therapy

The bronchodilating effectiveness of β2-adrenoreceptor agonists is influenced by functional antagonism by bronchoconstricting agents. Thus, studies in human (Raffestin et al. 1985; Van Amsterdam et al. 1990) and animal (Torphy et al. 1985; Van Amsterdam et al. 1989) ASM preparations have demonstrated that the potency and efficacy of β2-adrenoreceptor agonists are gradually reduced in the presence of increasing concentrations of contractile stimuli, including muscarinic receptor antagonists and histamine. This reduced β2-adrenergic responsiveness may be due to cross-talk between Gq-coupled muscarinic M3 or histamine H1 receptors and Gs-coupled β2-adrenoceptors (Figure 1). This provides a strong rationale for combination treatment with β2-adrenoreceptor agonists and muscarinic receptor antagonists, as muscarinic receptor antagonists both attenuate bronchoconstriction and potentiate β2-adrenoceptor agonist-induced bronchodilation by relieving the cholinergic restraint on β2-adrenoceptor function. Therefore, several long acting LABA + LAMA combinations are under development, as well as bi-functional molecules that link a muscarinic receptor antagonist and β2-adrenoreceptor agonist (MABA).

Several studies have demonstrated its safety and tolerability profile (Dahl et al. 2013b; Dahl et al. 2013a) as well as a significant improvements in dyspnea and health status (Mahler et al. 2014). Furthermore, a once-daily fixed-dose combination of olodaterol and tiotropium is being studied in the TOviTO Phase III clinical trial program (Boehringer Ingelheim 2013). In preliminary studies, this combination has already demonstrated synergistic effects on bronchodilation (Bouyssou et al. 2010b), as well as anti-proliferative effects (Costa et al. 2013) and anti-inflammatory effects (Profita et al. 2012; Costa et al. 2012).
Another approach to combining β₂-adrenoreceptor agonists and muscarinic receptor antagonists can be found in single molecules, which possess activity at both muscarinic receptors and β₂-adrenergic receptors. GlaxoSmithKline and Theravance have synthesized GSK961081, a MABA representing the combination of tiotropium and salmeterol. In a 4-week phase IIb clinical trial, GSK961081 appeared to be well-tolerated and bronchodilator efficacy was comparable to tiotropium + salmeterol (Bateman et al. 2013; Wielders et al. 2013). Taken together, this evidence suggests that MABA molecules are a potential new therapeutic approach for the treatment of COPD.

A novel combined corticosteroid/bronchodilator

Combined formulations of LABAs and inhaled corticosteroids (ICS) in a single inhaler have been put forward in the last decade as an improved therapy for allergic asthma since this drug combination has been found to confer synergistic effects in terms of controlling airway inflammation and improving lung function. Importantly, providing therapy in a single inhaler has also been shown to improve patient compliance (Cates and Karner 2013). Recently, a new combined formulation has been developed that allows for once-daily dosing, combining the corticosteroid fluticasone furoate (FF) and the long-acting β-agonist vilanterol (VI). A one-year safety study of FF/VI in asthma patients showed that this intervention is well-tolerated (Busse et al. 2013). In another study in moderate-to-severe asthmatics, FF/VI significantly improved lung function compared to treatment with the single drugs alone (O’Byrne et al. 2014). FF/VI was also found to be effective in inhibiting the early and late asthmatic responses to allergen challenge (Oliver et al. 2013), supporting the use of this therapy for the treatment of allergic asthma. Additionally, this drug was approved by the FDA in May 2013 for the treatment of COPD. Clinical trials on the use of FF/VI for the treatment of COPD showed a reduced incidence of exacerbations with long-term treatment (Bollmeier and Prosser 2014).

Anti-leukotrienes as add-on therapy for asthma

Several recent studies have assessed targeting leukotrienes, which are well-known lipid mediators of allergic inflammation, as further add-on therapy. Approaches have included adding a novel 5-lipoxygenase-activating protein inhibitor (GSK2190915) or an established leukotriene receptor antagonist (montelukast) to either inhaled corticosteroids alone or a combination corticosteroid/long-acting β-agonist (Snowise et al. 2013). Although these two studies did not find a statistically significant benefit of inhibiting leukotrienes in terms of the primary outcome (improvement in FEV1), targeting this pathway did have a positive effect on asthma symptoms and reduced the need for short-acting β-agonist rescue therapy. A recent study by Gao et al. investigated the impact of anti-leukotriene add-
on therapy on lung structure in moderate-to-severe asthmatics using high-resolution CT scans after 24 weeks of treatment with salmeterol/fluticasone (SFC) plus montelukast (SFC + M) or SFC plus placebo. Although the addition of montelukast treatment did not have a beneficial effect in terms of reducing airway wall thickness, SFC + M triple combination therapy did reduce air trapping, suggesting that interfering with leukotriene signaling may have a beneficial effect on the physiology of the small airways (Gao et al. 2013).

**Anticholinergic drugs as add-on therapy for COPD**

Combined therapy with an anticholinergic agent such as tiotropium and either a long-acting β-agonist alone or along with combined corticosteroid/long-acting β-agonist therapy has been recently evaluated for the treatment of COPD. In a study by Hoshino et al., tiotropium + salmeterol + fluticasone propionate treatment for 16 weeks was associated with reduced airway wall thickening, improved lung function and reduced symptoms compared to treatment with tiotropium, salmeterol or salmeterol + fluticasone propionate (Hoshino and Ohtawa 2013). These results were corroborated by Maltais et al. who found significant improvements in lung function in COPD patients after four weeks of treatment; however, there was no significant improvement in exercise endurance with the addition of tiotropium to salmeterol + fluticasone propionate (Maltais et al. 2013).

**Targeting the small airways**

Small airway dysfunction is a feature of both allergic asthma and COPD; however, few inhaled drug formulations are designed to reach the distal parts of the lung. However, with the advent of extrafine particle corticosteroids, inflammation in these areas of the lung can now be addressed. Asthma patients who were switched from conventional combined corticosteroid/long-acting β-agonist therapy to an extrafine formulation of beclomethasone/formoterol showed improvements in asthma symptom scores and blood eosinophils as well as a reduction in air trapping, indicating a positive effect on the small airways with the extrafine formulation (Popov et al. 2013). In another study on patients with stable asthma, extrafine ciclesonide given as an add-on therapy led to a reduction in indices of peripheral lung inflammation and significantly improved symptom scores (Nakaji et al. 2013). Targeting the small airways in COPD has also led to improved symptom scores and small airways function, despite no change in FEV1 (Timmins et al. 2014).

**Future Directions**

In this review, we have highlighted some of the potential targets that are emerging for the treatment of asthma and COPD. However, due to space limitations, other potential candidates for future treatments such as Toll-like receptors, bitter taste receptors and the
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Receptor for advanced glycation end products (RAGE) should also be considered [reviewed in (Bezemer et al. 2012; Sukkar et al. 2012; Drake et al. 2012; Liggett 2013)]. Whilst significant advances in our understanding of the cellular and molecular mechanisms involved in the pathogenesis of asthma and COPD have allowed for the identification of novel therapeutic targets, few new pharmaceutical agents have been developed for clinical treatment. Asthma and COPD are multifaceted diseases and, therefore, it is unlikely that a single treatment will be optimal; thus, further investigations into potential combination therapies are needed. In order to develop new therapeutic interventions, we not only need to fully understand the multifaceted action of potential drug targets, but also improve drug delivery systems such that therapeutic agents are delivered to these targets. One potential delivery system is the use of nanotechnology and nanoparticles [refer to (Vij 2011)]. Whilst the use of nanoparticles in the treatment of lung health may be controversial, with arguments that they may aggravate pulmonary diseases, there may also be advantages. Nanotechnology allows for more targeted drug delivery and controllable release of the drug by protecting the drug from inactivation or degradation upon administration, thus potentially minimizing adverse reactions (Da Silva et al. 2013). Experimental models of asthma have shown greater treatment responses with drugs encapsulated in nanoparticles when compared to the drug alone (John et al. 2003; Matsuo et al. 2009; Kenyon et al. 2013). Similarly, nanocarrier drug delivery is also beneficial in the treatment of COPD models (Geiser et al. 2013). However, further studies are required as some reports have suggested nanoparticles themselves may have adverse effects on the lung (Gwinn and Vallyathan 2006; Hussain et al. 2011).

Overall, in vitro and in vivo studies have identified multiple potential drug targets which may lead to the development of the next generation of treatments for asthma and COPD.

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