

# DP2 Receptor Antagonists: Novel Therapeutic Target for COPD

Karin J. Stebbins, Jilly F. Evans and Daniel S. Lorrain

*Amira Pharmaceuticals San Diego, California*

PharmSight on Stebbins KJ et al., Pharmacological blockade of the DP2 receptor inhibits cigarette smoke-induced inflammation, mucus cell metaplasia, and epithelial hyperplasia in the mouse lung. *J Pharm Exp Ther* 2010;332:764-75.

## Abstract

Chronic obstructive pulmonary disease (COPD) is a chronic and progressive disease of the small airways and lung parenchyma that is characterized by chronic airway inflammation, airway remodeling and parenchymal destruction. Current treatment options for patients with COPD consist primarily of long acting bronchodilators. Inhaled corticosteroids provide benefit for exacerbations but have little impact on the persistent inflammation. At present, there are no approved drugs that are disease modifying to treat the underlying causes of COPD. One pharmacological mechanism we and others are exploring as a therapeutic target for COPD is antagonism of the DP2 receptor (also known as chemoattractant receptor-homologous molecule expressed on Th2 cells, CRTh2). Since cigarette smoking is a risk factor for COPD, we used mouse models of acute and subchronic cigarette smoke exposure to explore the role of DP2 in COPD. We observed an increase in prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), the natural ligand for DP2, in the mouse lungs following subchronic smoke exposure. We demonstrated that DP2 antagonism alleviated much of the cigarette smoke-induced lung phenotype, including cellular inflammation, mucus cell metaplasia and epithelial hyperplasia. Although the role of PGD<sub>2</sub> and DP2 in COPD is yet to be fully elucidated, our results support the development of DP2 antagonists clinically as a novel pharmacological mechanism to treat COPD.

**Keywords:** DP2; CRTh2; COPD; Cigarette smoke

Chronic obstructive pulmonary disease (COPD) is a chronic and progressive disease of the small airways and lung parenchyma that is characterized by airflow obstruction which is not fully reversible. Chronic airway inflammation, airway remodeling and parenchymal destruction contribute to the airflow limitation which is usually progressive and a result of an abnormal response to noxious particles such as air pollution and cigarette smoke. The pathobiology of the disease is complex in nature, involving the innate (macrophages, neutrophils) and adaptive (CD8<sup>+</sup> T cells) immune systems, elevated inflammatory mediators (IL-8, MCP-1, IL-1 $\beta$ ), oxidative stress (reactive oxygen species) and proteases (Figure 1) (1-13).

Current treatment options for patients with COPD consist primarily of long acting anticholinergic bronchodilators such as Spiriva®. Inhaled corticosteroids are often prescribed despite being ineffective against the neutrophilic inflammation associated with the disease. However, corticosteroids do provide some benefit for the inflammation associated with frequent exacerbations due to bacterial or viral infections. At present, there are no approved drugs that are disease modifying to treat the underlying causes of this complex disease. Numerous pharmacological mechanisms are being explored and developed which target the underlying pathology, namely inflammation, mucus hypersecretion and matrix breakdown. Much research and drug development effort has focused on mechanisms such as PDE<sub>4</sub> inhibition, CXCR2 antagonism, MAPK p38 inhibition, PI3 kinase inhibition, antioxidant therapy, mucolytics and more recently, HDAC activation. Of these, Daxas®, an oral phosphodiesterase 4 inhibitor, is the furthest along in development; however, the FDA advisory panel recently voted against recommending approval of

Received 05/05/10; accepted 06/10/10

Correspondence: Dr. Karin Stebbins, Amira Pharmaceuticals, 9535 Waples St. Ste. 100, San Diego, CA 92121, USA. Tel. 858-875-4810, Fax. 858-875-4910. email: karin.stebbins@amirapharm.com

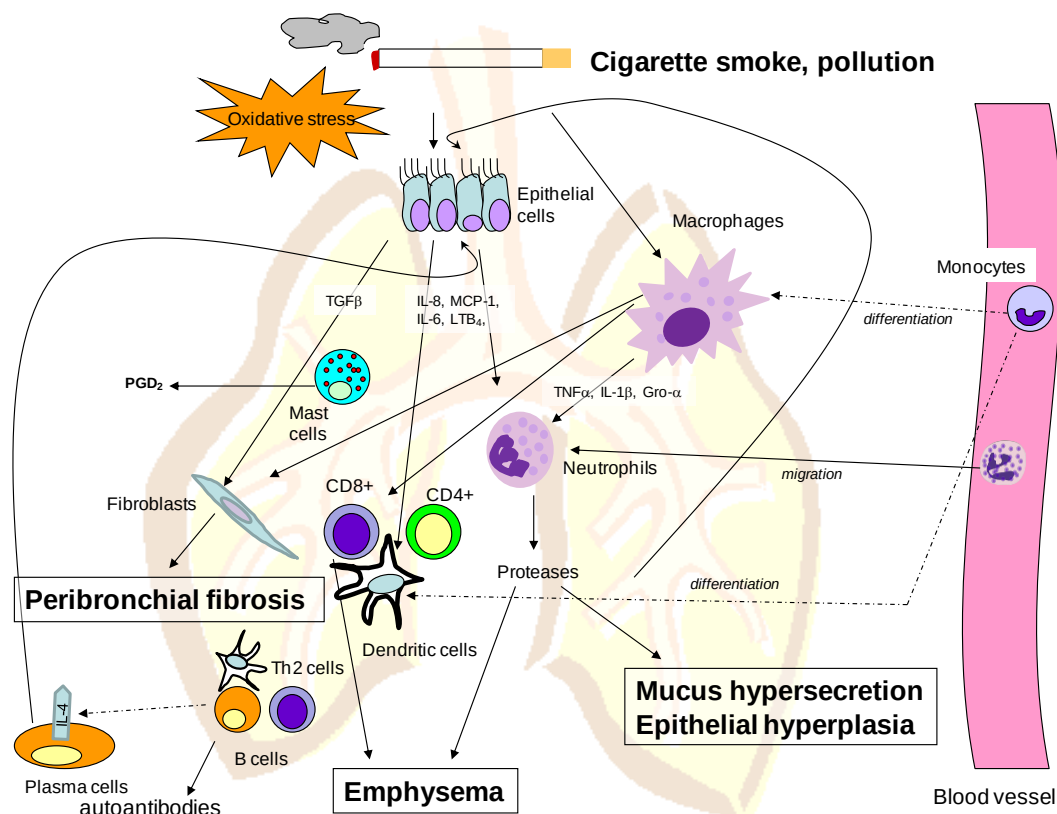


**Figure 1. Inflammatory pathways involved in COPD pathogenesis.** COPD is a complex disease involving cells of both the innate and adaptive immune system. Cigarette smoke contains oxidants which result in the release of pro-inflammatory cytokines and chemokines from the airway epithelial cells and resident alveolar macrophages. Amplification of the inflammatory process continues with the recruitment and activation of additional cell types (eg. neutrophils, dendritic cells, T lymphocytes). When the process remains unchecked, as in COPD, mucus hypersecretion, epithelial hyperplasia, emphysema and peribronchial fibrosis can manifest.

Daxas® citing minimal benefits and numerous potential side effects (14). In contrast, the European Medicines Agency recommended approval of Daxas® (15). Regardless of ultimate approval status of Daxas®, there remains a large unmet medical need for novel therapeutics aimed at treating COPD. One pharmacological mechanism we and others are exploring for COPD is antagonism of the DP2 receptor.

PGD<sub>2</sub> is one of a family of biologically active lipids which is derived from arachidonic acid via the actions of COX-1 or COX-2 and PGD<sub>2</sub> synthase. Mast cells are the primary source of PGD<sub>2</sub> but antigen-presenting cells and Th2 cells also produce PGD<sub>2</sub> (16-18). PGD<sub>2</sub> is rapidly metabolized in vivo to six primary metabolites (13,14-dihydro-15-keto-PGD<sub>2</sub>, Δ<sup>12</sup>PGD<sub>2</sub>, Δ<sup>12</sup>PGJ<sub>2</sub>, 15-deoxy-Δ<sup>12,14</sup>PGD<sub>2</sub>, 15-deoxy-Δ<sup>12,14</sup>PGJ<sub>2</sub>, and 9α<sup>11</sup>PGF<sub>2</sub>) which are themselves biologically active. PGD<sub>2</sub> and its metabolites, with the exception of 9α<sup>11</sup>PGF<sub>2</sub>, bind primarily to two GPCRs, DP1 and DP2. In addition, there are other non-PGD<sub>2</sub>-derived products of the

arachidonic acid cascade (11-dehydro-thromboxane B<sub>2</sub>, 9α<sup>11</sup>PGF<sub>2</sub>, 15R-PGD<sub>2</sub>) which have DP2 agonist activity (19).

DP2 is expressed predominantly on eosinophils, Th2 lymphocytes and basophils. DP2 is also expressed, although to a lesser extent, on monocytes (20), mast cells (21), fibroblasts (22-23) and lung epithelial cells (23-25). Interaction of PGD<sub>2</sub> and its biologically active metabolites with DP2 leads to increased intracellular calcium and decreased cAMP via activation of Gαi proteins. In lymphocytes, and presumably other DP2-expressing cells, downstream events are mediated via PI3 kinase-dependent phosphorylation of Akt and nuclear translocation of NFAT (nuclear factor of activated T cells) (26). The biological consequences of DP2 activation include eosinophil shape change, basophil degranulation, Th2 cytokine production, upregulation of adhesion molecules, and chemotaxis of eosinophils, basophils and Th2 lymphocytes.

Cell surface expression of DP2 may be influenced by the local environment in specific disease states.

In cystic fibrosis, for example, sputum neutrophils but not peripheral blood neutrophils express high levels of DP2 receptor (27). Similarly, DP2 expression is higher in the nasal mucosa of individuals with allergic rhinitis versus normal controls (21). The percentage of DP2 positive eosinophils, macrophages, mast cells and T cells was increased in allergic mucosa relative to non-allergic mucosa without a concomitant change in surface expression patterns on circulating peripheral blood leukocytes. The *in vitro* culture conditions may also influence DP2 expression. Human lung fibroblasts (HFL-1), for example, show increased DP2 expression when cultured in the presence of Th2 cytokines and TGF $\beta$ ; expression of which could be detected both as mRNA and by flow cytometry (22). Additionally, bronchial fibroblasts obtained from asthmatics but not healthy airways revealed increased DP2 expression when cultured in the presence of IL-13 or TGF $\beta$  (23). Furthermore, LPS altered DP2 expression in a concentration-dependent fashion when added to cultured asthmatic primary bronchial epithelial cells (23). From these examples it is reasonable to speculate that cell surface expression of DP2 may be altered locally in COPD tissue and contribute to amplification of the disease process. However, to our knowledge, DP2 expression has not been profiled in COPD patients and remains an area for further exploration.

There are several notable differences in DP2 expression patterns between mouse and human that need to be considered when extrapolating data generated from mouse models to human disease. Like human DP2, mouse DP2 is expressed on eosinophils, basophils, lymphocytes and mast cells. However, mouse DP2 is expressed equally on Th1 and Th2 cells (28-29). In contrast to human, DP2 is expressed on neutrophils under basal conditions (30). Furthermore, mouse DP2 is expressed on B cells and monocytes (28). From a functional signaling perspective, activation of mouse DP2, like human DP2, involves G $_i$  and PI3-kinase dependent signaling resulting in a reduction in intracellular cAMP (31). Likewise, transfected cells stably expressing mouse DP2 chemotax toward PGD $_2$  (31).

Cigarette smoke is a risk factor for COPD and therefore smoke exposure models are useful tools for studying the disease. The mouse model of cigarette smoke exposure produces steroid-resistant pulmonary inflammation and is widely accepted as an experimental model of COPD. The role of PGD $_2$  and DP2 in COPD is an exciting area of new

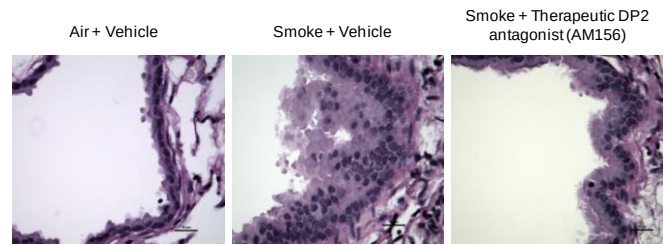


**Figure 2. Effect of cigarette smoke exposure on epithelial thickening and inhibition following therapeutic treatment with DP2 antagonist (400x magnification).** Mice were exposed to cigarette smoke for 1.75 hours per day on days 0-1, 4-8, and 11-12. AM156 was dosed daily on days 5-12. On day 13, following bronchoalveolar lavage, lungs were inflated with formalin, excised and fixated in formalin. Paraffin-embedded sections were stained with Periodic Acid Schiff for detection of mucins. Scale bar, 10  $\mu$ m.

research. Theoretically, DP2 antagonism could impact several aspects of COPD pathobiology (Figure 3), each of which will be described in more detail below. We sought to explore the role of DP2 in COPD using mouse models of acute and subchronic smoke exposure (32). Acute (<4 days) smoke exposure leads to lung inflammation that is predominantly neutrophilic (32-35), whereas subchronic exposure (10-30 days) causes a more complex inflammatory process and includes mucus hypersecretion and epithelial hyperplasia (32,36). Subchronic exposure additionally leads to an increase in lung PGD $_2$  (32). Chronic smoke exposure (3 to 6 months) is characterized by emphysema, goblet cell metaplasia as well as increases in cells of both the innate and adaptive immune systems (37-39). To our knowledge, PGD $_2$  concentrations have not been determined in these chronic models.

Blocking DP2 by oral administration of our novel, potent small molecule DP2 receptor antagonists, AM156 and AM206, ameliorated much of the cigarette smoke-induced lung phenotype in acute and subchronic smoke exposure models. Importantly, these receptor antagonists had a significant impact on cigarette smoke-induced epithelial pathology (Figure 2) (32). In a subchronic smoke model, epithelial thickness was increased and this was attenuated by both prophylactic (dosed during the entire duration of the experiment) and therapeutic (administered on days 5-13) treatment with oral DP2 antagonist. In addition to these epithelial changes, cigarette smoke exposure resulted in increased mucin in the BAL, as measured by enzyme-linked lectin assay (ELLA), and mucus cell metaplasia in the large airways, detected histologically as increased Periodic Acid Schiff staining. AM156 and AM206 also reduced both secreted mucin in the BAL and stored mucin

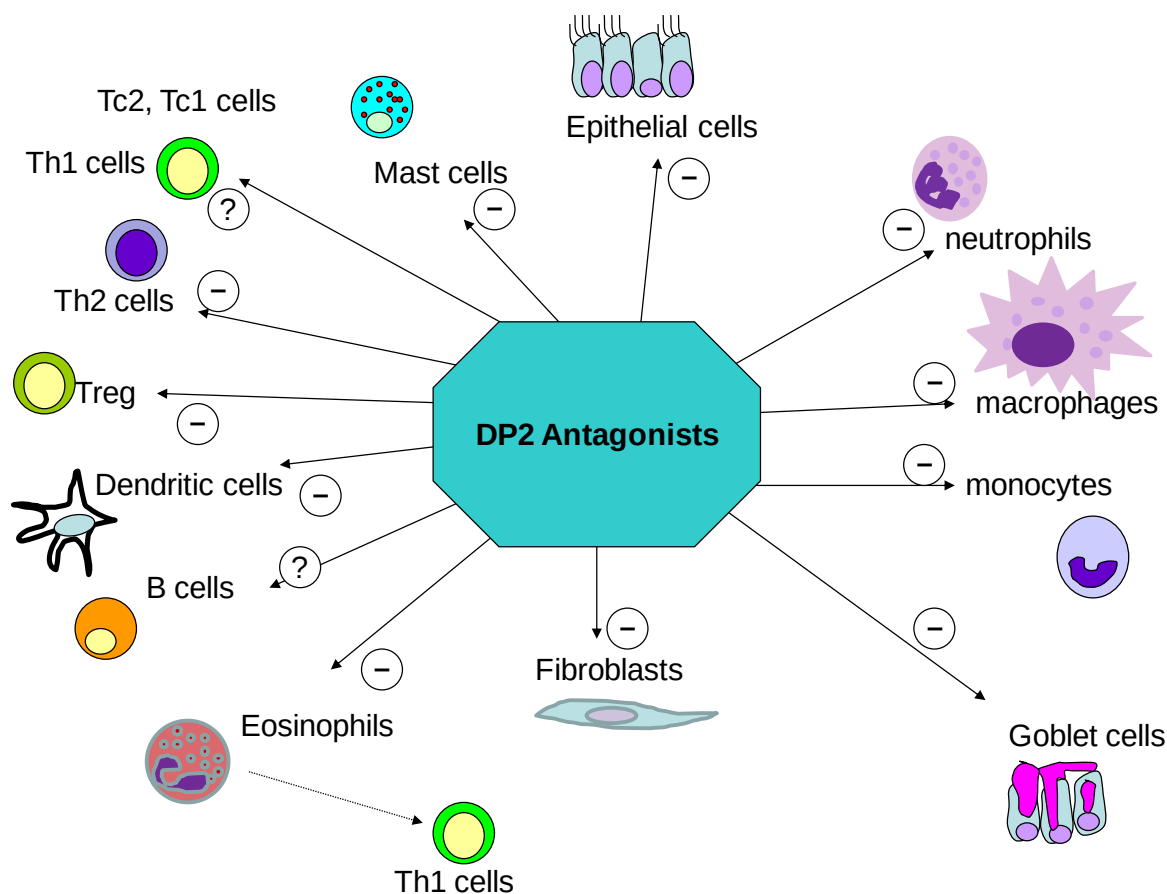


**Figure 3. Effect of DP2 antagonism on COPD-relevant cells.** DP2 antagonists have the potential to be a disease modifying therapy for COPD.

the airways. Moreover, therapeutically administered DP2 antagonist was as effective as prophylactically administered DP2 antagonist. Epithelial cell injury plays a pivotal role in the pathogenesis of COPD (40). In addition to direct damage to the epithelial cells, exposure of the airways to agents such as cigarette smoke or pollutants can initiate an inflammatory cascade within the airways. As a result of increases in inflammatory mediators such as IL-13 and neutrophil elastase, mucus hypersecretion and mucus cell metaplasia occur in the chronic bronchitic lung. Our results suggest that treatment with a DP2 antagonist may reduce the mucus hypersecretion associated with the productive cough of chronic bronchitis and may thereby improve patients' quality of life. Furthermore, blocking the DP2 receptor decreases epithelial hyperplasia should, therefore, slow disease progression by halting downstream inflammatory events and structural changes which occur as a result of airway epithelial damage.

Both AM156 and AM206 reduced pulmonary neutrophilia in acute and subchronic cigarette

smoke mouse models after either prophylactic or therapeutic administration (32). Neutrophils play a key role in the pathogenesis of COPD and have been implicated in the both the initiation and progression of the disease (9). Neutrophils, arriving in response to stimuli such as cigarette smoke, release mediators and enzymes such as neutrophil elastase causing the release of LTB<sub>4</sub> from macrophages and resulting in further amplification of the inflammation. Neutrophil elastase and other proteases break down alveolar tissue, leading to emphysema. We suspect that human neutrophils in the COPD lung may have altered DP2 expression patterns, as observed in cystic fibrosis (27). Therefore, oral DP2 antagonists could prevent the development of the chronic inflammatory milieu which contributes to disease progression. In our study, we did not measure neutrophil elastase or myeloperoxidase, however we expect that these may be reduced by DP2 antagonist treatment. Therefore, DP2 antagonists may directly impact mucus hypersecretion and parenchymal destruction via a reduction in neutrophil elastase.

We demonstrated that our DP2 antagonists reduced influx of lymphocytes to the airways that occurs in response to cigarette smoke exposure (32). In the mouse, DP2 is equally expressed on Th1 and Th2 cells (28-29). CD8<sup>+</sup> and, to a lesser extent, CD4<sup>+</sup> and T<sub>reg</sub>, lymphocytes are elevated in COPD lungs (2,41-42). The link between Th2 cells and COPD has been controversial. Clinical studies have produced conflicting results, demonstrating both increased and decreased IL-13 in COPD (43-44). IL-13 increases goblet cell density in cultured human bronchial epithelial cells (45) and overexpression of IL-13 in the mouse lung results in mucus cell metaplasia (46). Pre-clinically, we demonstrated that AM156 reduced lung IL-13 in a murine model of allergic pulmonary inflammation (47). Thus, if IL-13 is indeed clinically relevant for COPD, oral DP2 antagonists should reduce this Th2 cytokine and impact mucus hypersecretion and goblet cell metaplasia in the COPD lung.

Our results in the acute cigarette smoke mouse model demonstrate a role for DP2 in macrophages influx, however this effect was not observed in the subchronic smoke model (32). The lack of effect may in part be due to the relatively modest increase in macrophages we observed following smoke exposure. Subchronic smoke exposure has been reported in the literature to result in larger increases in macrophages (36,39). Our relatively modest increase may be due to choice of mouse strain (BALB/c vs. A/J or C57Bl/6). Macrophages are implicated in the pathogenesis of COPD and are increased in COPD lungs (48). From our current experiments we cannot conclude the effect of DP2 antagonism on pulmonary macrophages, however literature reports suggest that DP2 may be important. Mouse peritoneal macrophages migrate toward DK-PGD<sub>2</sub>, a selective DP2 agonist; however migration toward MCP-1 is not DP2-dependent (49). Therefore, DP2 is likely involved in macrophage migration toward some but not all, chemotactic agents. We are unaware of any reports characterizing the expression of DP2 on human alveolar macrophages either in normal or COPD lung tissue. In allergic rhinitic nasal mucosa, on the other hand, the DP2 receptor expression has been shown to be increased on macrophages (21). This raises the possibility that DP2 expression may also be altered on alveolar macrophages resident to the COPD lung. Furthermore, human blood monocytes, which are precursors of macrophages, do express low levels of DP2, as determined by RT-PCR and by FACS analysis, and do chemotax toward DK-PGD<sub>2</sub> (20). Therefore, DP2 antagonists may reduce

the number of alveolar macrophages in the COPD lung which may, in turn, reduce the recruitment and activation of other cells (eg. neutrophils, CD8<sup>+</sup> T cells, fibroblasts) and diminish the resulting pathobiological consequences (e.g., mucus hypersecretion and airspace enlargement due to released proteases).

In our experimental model of COPD, we found that the mast cell product PGD<sub>2</sub> was elevated in tissue homogenates prepared from lungs of smoke-exposed mice (32). Mast cells express DP2 (21) and mast cell numbers increase in the airway epithelium of smokers with COPD (50). Csansky et al. demonstrated a correlation between BALF PGD<sub>2</sub> concentrations and COPD disease severity (51). Experimentally, mast cells chemotax toward DK-PGD<sub>2</sub> and this effect could be inhibited by a selective DP2 antagonist (52). Together these findings suggest that DP2 antagonists may result in a decrease in mast cells in COPD lungs; thereby reducing lung PGD<sub>2</sub> concentrations and the ensuing biological events.

Eosinophils, which highly express DP2, are generally associated with allergic diseases such as asthma. The exact role of eosinophils in COPD is not entirely clear however, evidence implicates eosinophils in both acute exacerbations and progressive decline in lung function in advancing disease (53). Eosinophils are elevated in both sputum and bronchial biopsies from COPD patients (54). Bronchial biopsies obtained from patients with acute exacerbations show a further increase in eosinophils (55). We and others have demonstrated PGD<sub>2</sub> stimulates eosinophil shape change and chemotaxis and that this effect can be blocked by selective antagonists of DP2 (32,56). Small molecule DP2 antagonists inhibit pulmonary eosinophilia in allergic models (47, 57-58), therefore, DP2 antagonism could limit, if not prevent, acute exacerbations of COPD.

While we did not examine dendritic cells in our model, the number of dendritic cells are reported to be increased in both the BAL fluid and lung parenchyma (small and large airways) in response to cigarette smoke exposure (39). This increase was evident after 3 days of smoke exposure and persisted for at least 24 weeks. Bronchial epithelial cells of mice treated intranasally with cigarette smoke extract exhibit a significant increase in thymic stromal lymphopoietin (TSLP) expression (59). This effect on TSLP was observed in human airway smooth muscle after chronic but not acute exposure to cigarette smoke extract (60). Studies examining

dendritic cells in the lungs of smokers or COPD patients have produced conflicting results, seemingly varying with the dendritic cell markers utilized (e.g., immature vs. mature) and disease stage. In our cigarette smoke experiment we did not examine TSLP concentration in either the BALF or the lung; however, DP2 antagonists have been shown to impact dendritic cells in other animal models. In models of cutaneous inflammation, selective DP2 antagonists reduced TSLP, prevented dendritic cell activation and reduced migration of dendritic cells from the site of injury to the draining lymph nodes, thereby downregulating the T cell mediated immune response (61-62). Therefore, DP2 antagonism could have a significant impact on the role of dendritic cells in COPD.

The role of fibroblasts in COPD has received little research attention despite the presence of peribronchial fibrosis in some patient populations. Cigarette smoke releases TGF $\beta$  and other profibrotic mediators stimulating fibroblast proliferation and collagen synthesis, leading to fibrosis (63-65). In vitro, macrophage-derived TGF $\beta$  induces DP2 expression on cultured fibroblasts (23); therefore TGF $\beta$ , in response to cigarette smoke, may upregulate DP2 on the resident pulmonary fibroblasts. Preclinical fibrosis models under taken using DP2  $-/-$  mice suggest that DP2 plays a protective role in fibrosis (66). Interestingly, in this bleomycin model of pulmonary fibrosis genetically deleting the DP2 receptor did not result in any inhibitory effect on inflammation which is in contrast to results obtained in other disease models using genetic knockouts and selective pharmacological agents (32, 57-58). Therefore, the implication of DP2 antagonism for peribronchial fibrosis is an area which needs further exploration, both preclinically and clinically.

To summarize, we observed an increase in PGD<sub>2</sub> in lungs following subchronic smoke exposure and demonstrated that DP2 antagonism ameliorated much of the cigarette smoke-induced lung phenotype in acute and subchronic smoke exposure models, including cellular inflammation, mucus cell metaplasia and epithelial hyperplasia. Although the role of PGD<sub>2</sub> and DP2 in COPD remains to be fully elucidated, our results demonstrating beneficial effects in a widely accepted mouse model of COPD strongly supports the development of DP2 antagonists clinically as a novel therapy to treat COPD. Indeed, at least one DP2 antagonist (AZD1981) has completed phase II clinical trials in patients with COPD. While results of these trials

have not yet been published, DP2 antagonism represents a potential new therapy for the largely unmet clinical need in COPD.

## Acknowledgements

We thank all our colleagues at Amira Pharmaceuticals for their support of the DP2 program.

## Conflicts of Interest

No potential conflicts of interest to disclose.

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